Electrolyte imbalances with special focus on hypokalemia: cellular pathophysiology and clinical manifestations

From basic science to clinical and epidemiological studies

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Kiarash Tazmini, MD

Department of Internal Medicine,

Diakonhjemmet Hospital

and

Institute for Experimental Medical Research,

Oslo University Hospital, Ullevål

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”The eyes cannot see what the mind does not know”

-Anonymous

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The story of this project starts in October 2010, when I started working on the project description. From 2012 I started to work part-time with atrial cells alongside my clinical work at Diakonhjemmet Hospital. My ambitions came a step closer realization when I received approval of this project together with a full PhD-grant in 2013. Looking back, the process of isolating atrial cells with all the lab work involved was maybe the toughest and most difficult part of my work. From 2016 I worked full-time with the PhD project until September 2017 when I began working at the Department of Endocrinology. From this time on I worked with my PhD-project in parallel with full-time clinical work. The thesis was finally completed in September 2020.

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Oslo, September 2020

Kiarash Tazmini

LCL

ABBREVIATIONS

AF	Atrial fibrillation
AFl	Atrial flutter
AP	Action potential
APD	Action potential duration
BMI	Body mass index
$[Ca^{2+}]_i$	Intracellular calcium concentration
CaMKII	Ca^{2+}/calmodulin-dependent protein kinase II
DAD	Delayed afterdepolarizations
EAD	Early afterdepolarizations
ECF	Extracellular fluid
ECG	Electrocardiogram
ED	Emergency department
GFR	Glomerular filtration rate
HR	Hazard ratio
I_{Ca}	Calcium current
ICF	Intracellular fluid
I_{K1}	Inward rectifier potassium channel
I_{Kr}	Delayed rectifier potassium channel
I_{Kur}	Ultrarapid delayed rectifier outward K^+ current
I_{Na}	Sodium current
IQR	Interquartile range
I_{to}	Transient outward potassium current
K^+	Potassium ions
$[K^+]$	Potassium concentration
$[K^+]_e$	Extracellular potassium concentration
$[K^+]_i$	Intracellular potassium concentration
KCl	Potassium chloride
LOS	Hospital length of stay
LTCC	L-type Ca^{2+} channels
Na^+	Sodium ions

$[Na^+]_i$	Intracellular sodium concentration
NCX	Na^+/Ca^{2+}-exchange
NKA	Na^+/K^+-ATPase
OR	Odds ratio
PAF	Paroxysmal atrial fibrillation
PCR	Polymerase chain reaction
RMP	Resting membrane potential
ROAF	Recent-onset atrial fibrillation
RyR	Ryanodine receptor
SERCA	Sarcoplasmic reticulum Ca^{2+}-ATPase
SR	Sarcoplasmic reticulum

LIST OF ARTICLES

I. Electrolyte imbalances in an unselected population in an emergency department: a retrospective cohort study

Kiarash Tazmini, Ståle H. Nymo, William E. Louch, Anette H. Ranhoff, Erik Øie

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II. Potassium infusion increases the likelihood of conversion of recent-onset atrial fibrillation – a single-blinded, randomized clinical trial

Kiarash Tazmini, Mai S. Aa. Fraz, Ståle H. Nymo, Mathis K. Stokke, William E. Louch, Erik Øie

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III. Hypokalemia promotes arrhythmia by distinct mechanisms in atrial and ventricular myocytes

Kiarash Tazmini*, Michael Frisk*, Alexandre Lewalle, Martin Laasmaa, Stefano Morotti, David B. Lipsett, Ornella Manfra, Jonas Skogestad, Jan M. Aronsen, Ole M. Sejersted, Ivar Sjaastad, Andrew G. Edwards, Ele Grandi, Steven A. Niederer, Erik Øie, William E Louch

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SUMMARY

Background
Electrolyte imbalances (EIs) are common in patients and are associated with increased morbidity, mortality, and reduced quality of life. However, there are few studies investigating the frequency and outcomes in an unselected group of adult patients admitted to the emergency department (ED). Among the common EIs, hypokalemia is reported to be one of the most prevalent and is associated with a higher risk of ventricular fibrillation and atrial fibrillation (AF). Although recent-onset atrial fibrillation (ROAF), defined as a sudden onset of symptoms within 48 hours from admission, is prevalent in the ED, there is no clear consensus on treatment strategies. Anti-arrhythmic drugs are not atrial specific, have significant risk of side-effects, and several contraindications. Moreover, direct-current cardioversion requires deep sedation, six hours postprandial period to ensure gastric emptying, and associated with additional costs, resources, and risk. Interestingly, some case studies have illustrated conversion of AF to sinus rhythm during hyperkalemia, suggesting that while lowered plasma-potassium levels may promote AF development, increasing plasma-potassium can have therapeutic potential. Mechanistically, it is presently unclear how hypokalemia promotes triggered activity in atrial cardiomyocytes and whether there are similarities with ventricular cardiomyocytes. Better understanding of this pathophysiology is crucial for improved prevention and treatment of these arrhythmias. The main aim of this thesis was to investigate EIs with special focus on hypokalemia, how it may induce arrhythmia, and whether potassium infusion could be promising in patients with AF or atrial flutter (AFl).

Methods
Article I was a retrospective cohort study where all patients ≥ 18 years referred for any reason to the ED, and who had measured blood electrolytes, were included. During the study period from January 1, 2010 until December 31, 2015, 62 991 ED-visits, involving 31 966 patients were registered. We recorded serum-electrolytes, serum-albumin, and serum-glucose. In addition, we recorded hospital length of stay (LOS), readmission within 30 days post-discharge, in-hospital mortality as well as mortality 30-days and one year after discharge.

Article II was a single center, placebo-controlled, single-blinded study, including patients with ROAF or AFl and plasma-potassium ≤4.0 mmol/L at admission. Patients were randomized to receive either potassium chloride (KCl) infusion or placebo (glucose 50 mg/mL) from April 2013 to November 2017. Patients in the KCl group received infusions at one of three different rates: 9.4 mmol/h, 12 mmol/h, or 15 mmol/h.

In Article III, we applied cellular experiments in isolated rat atrial and ventricular cardiomyocytes exposed to hypokalemia (2,7 mmol/L) and verified our results by mathematical modeling studies.

Results
Most of our patients (65.5%) with EIs visiting the ED were above 60 years. EIs were mostly mild, and the most common EI was hyponatremia [24.6% (glucose-corrected)]. Among the patients who were admitted (70,6%), the median LOS was 3 days, and patients with increasing severity of EI had longer LOS than patients with

normal electrolyte measurements. Among admitted patients, there were 20.5% readmissions, where hyponatremic patients accounted for 23.6% of these. Hypocalcemia and hypomagnesemia were also associated with readmissions. Dysnatremia, dyskalemia, hypercalcemia, hypermagnesemia, and hyperphosphatemia were associated with increased in-hospital mortality, and all EIs except hypophosphatemia were associated with increased 30-day and 1-year mortality compared with patients not having the specific EI.

In article II, we included a total of 113 patients with recent-onset AF of AFl of whom 53 were allocated to the placebo group and 60 to the KCl group. KCl infusion had no significant effect regarding time to or frequency of conversion to sinus rhythm compared with the placebo group when analyzed by *intention-to-treat*. However, ten patients had to prematurely stop the KCl infusion because of pain at the infusion site. After excluding these patients, the *per-protocol* analysis showed that significantly more patients converted to sinus rhythm in the KCl group with the fastest infusion rate (15 mmol/h) compared with the placebo group (82 % vs 52%. *P*=0.018). Furthermore, KCl-infused patients who achieved an above-median hourly increase in plasma-potassium (>0.047 mmol/h) exhibited a significantly higher conversion rate, compared with both the placebo group and the group with below-median change in plasma-potassium. However, there was no difference in time to conversion compared with placebo. Patients that converted to sinus rhythm had a significantly higher change in both serum- and plasma-potassium per hour than those that did not convert to sinus rhythm.

In article III, we observed that all ventricular and a subgroup of atrial cardiomyocytes exhibit t-tubules. When exposed to hypokalemia (2,7 mmol/L), the presence of t-tubules and the co-localization and cooperation of Na^+/K^+-ATPase (NKA) and Na^+/Ca^{2+}-exchanger (NCX) within these structures promoted an increased incidence of spontaneous Ca^{2+} waves and delayed afterdepolarizations (DADs). Ventricular and tubulated atrial cells also showed early afterdepolarizations (EADs) during hypokalemia, which were Ca^{2+}-dependent and occurred during the plateau phase of the prolonged action potential (AP). In contrast, untubulated atrial cells showed no significant increase in DADs. These cells had significantly shorter APs due to larger outward K^+ current, which in turn was linked to the exclusive presence of ultrarapid delayed rectifier outward K^+ current (I_{Kur}). During hypokalemia, this AP configuration predisposed these cells to EADs during late repolarization (phase-3) of the AP, driven by reactivation of Na^+ channels.

Conclusions

EIs are common in patients visiting the ED, and EIs are associated with increased LOS, readmissions, and mortality. In patients with ROAF and plasma-potassium in the lower normal range, KCl infusion with a rapid increase in plasma-potassium may increase the likelihood of conversion to sinus rhythm. Lastly, hypokalemia increases the susceptibility to arrhythmia by distinct mechanisms in atrial and ventricular cardiomyocytes.

NORSK SAMMENDRAG

Bakgrunn

Elektrolyttforstyrrelser (EF) er vanlig hos pasienter og er assosiert med økt morbiditet, mortalitet og redusert livskvalitet. Likevel er det få studier som har undersøkt hyppighet og utfall av EF blant uselekterte voksne pasienter henvist til akuttmottak. Blant vanlige EF er hypokalemi en av de mest prevalente og er assosiert med høyere risiko for ventrikkelflimmer og atrieflimmer (AF). Selv om atrieflimmer med varighet under 48 timer ved innleggelse er utbredt i akuttmottak, er det ingen klar konsensus om behandlingsalternativer. Antiarytmika er ikke atriespesifikke, har signifikant risiko for bivirkninger og flere kontraindikasjoner. Videre krever elektrokonvertering dyp sedasjon, seks timers postprandial faste for å sikre tømning av magesekken og er assosiert med tilleggskostnader og økt risiko. Interessant nok er det noen studier som har vist konvertering av AF til sinusrytme ved hyperkalemi, noe som tyder på at mens fallende plasma-kalium nivåer kan øke risikoen for AF, vil en økning i plasma-kalium ha terapeutisk potensiale. Mekanistisk er det uklart hvordan hypokalemi fører til økt arytmogenisitet i atrieceller og om det er likhetstrekk mellom disse og ventrikkelceller. Bedre forståelse av denne patofysiologien er avgjørende for bedre forebygging og behandling av disse rytmeforstyrrelsene. Hovedhensikten med denne avhandlingen var å undersøke EF med spesielt fokus på hypokalemi, hvordan hypokalemi kan indusere arytmi og om kaliuminfusjon kan være gunstig hos pasienter med AF eller atrieflutter.

Metoder

Artikkel I var en retrospektiv kohortstudie hvor alle pasienter ≥ 18 år henvist til et akuttmottak uansett årsak og som hadde målt elektrolytter i blod, ble inkludert. I løpet av studieperioden fra 01.01.2010 til 31.12.2015 ble det registrert 62 991 besøk til akuttmottaket, noe som utgjorde 31 966 pasienter. Vi registrerte serum-elektrolytter, serum-albumin og serum-glukose. I tillegg registrerte vi lengden på sykehusopphold (LOS), reinnleggelser innen 30 dager etter utskrivelse samt mortalitet under innleggelsen og 30 dager og 1 år etter utskrivelse.

Artikkel II var et singel-senter, placebokontrollert, enkeltblindet studie som inkluderte pasienter med nyoppstått atrieflimmer eller atrieflutter og plasma-kalium ≤ 4,0 mmol/l ved innleggelse. Pasientene ble randomisert til å motta enten kaliumklorid (KCl)-infusjon eller placebo (glukose 50 mg/ml) fra april 2013 til november 2017. Pasienter i KCl-gruppen fikk infusjoner med én av tre forskjellige hastigheter: 9,4 mmol/t, 12 mmol/t eller 15 mmol/t.

I artikkel III gjorde vi cellulære eksperimenter i isolerte atriale og ventrikulære kardiomyocytter eksponert for hypokalemi (2,7 mmol/l) og bekreftet resultatene våre ved matematiske modelleringsstudier.

Resultater

De fleste av våre pasienter (65,5 %) med EF som besøkte akuttmottaket, var over 60 år. EF var stort sett milde, og den vanligste EF var hyponatremi [24,6 % (glukosekorrigert)]. Blant pasienter som ble innlagt (70,6 %), var median LOS 3 dager, og pasienter med økende alvorlighetsgrad av EF hadde lengre LOS enn pasienter med normale elektrolyttmålinger. Blant innlagte pasienter var det 20,5 % reinnleggelser, der hyponatremiske pasienter utgjorde 23,6 % av disse. Hypokalsemi og hypomagnesemi var også assosiert med reinnleggelser. Dysnatremi, dyskalemi,

hyperkalsemi, hypermagnesemi og hyperfosfatemi var assosiert med økt mortalitet på sykehus, og alle EF bortsett fra hypofosfatemi var assosiert med økt 30-dagers og 1-års mortalitet sammenlignet med pasienter som ikke hadde den spesifikke elektrolyttforstyrrelsen.

I artikkel II inkluderte vi totalt 113 pasienter med nyoppstått atrieflimmer eller atrieflutter: 53 i placebogruppen og 60 i KCl-gruppen. KCl-infusjon hadde ingen signifikant effekt med hensyn til tid til eller hyppighet av konvertering til sinusrytme sammenlignet med placebogruppen når det ble gjort *intention-to-treat*-analyse. Ti pasienter måtte imidlertid avbryte KCl-infusjonen på grunn av smerter på infusjonsstedet. Etter å ha ekskludert disse pasientene viste *per protokoll*-analysen at signifikant flere pasienter konverterte til sinusrytme i KCl-gruppen med den raskeste infusjonshastigheten (15 mmol/t) sammenlignet med placebogruppen (82 % mot 52 %, P = 0,018). Videre hadde pasienter som fikk KCl-infusjon og som oppnådde en økning i plasma-kalium per time over medianverdien (> 0,047 mmol/t), en betydelig høyere konverteringsfrekvens sammenlignet med både placebogruppen og gruppen med økning i plasma-kalium per time under medianverdien. Imidlertid var det ingen forskjell i tid til konvertering sammenlignet med placebo. Pasienter som konverterte til sinusrytme, hadde en betydelig større endring per time i både serum- og plasma-kalium sammenlignet med dem som ikke konverterte til sinusrytme.

I artikkel III fant vi at alle ventrikulære og en undergruppe av atriale kardiomyocytter hadde t-tubuli. Når de ble utsatt for hypokalemi (2,7 mmol/l), førte en samlokalisering og samarbeid mellom Na^+/K^+-ATPase (NKA) og Na^+/Ca^{2+}-utveksleren (NCX) i t-tubuli til en økt forekomst av spontane Ca^{2+}-bølger og forsinkede etterdepolarisasjoner (DADer). Ventrikulære og tubulerte atrieceller viste også tidlig etterdepolarisering (EAD) ved hypokalemi, som var Ca^{2+}-avhengige og oppstod under platåfasen av det forlengede aksjonspotensialet (AP). I motsetning viste ikke-tubulerte atrieceller signifikant økning i DADer. Disse cellene hadde betydelig kortere AP på grunn av større utgående kaliumstrøm som igjen var på grunn av eksklusiv tilstedeværelse av ultrarask kaliumstrøm (I_{Kur}). Hypokalemi ved denne AP-konfigurasjonen disponerte disse cellene for EADer ved forsinket repolarisering (fase 3) av AP, drevet av reaktivering av Na^+ kanaler.

Konklusjoner

EF er vanlig blant pasienter i et akuttmottak, og de er assosiert med økt sykehusinnleggelse, reinnleggelser og mortalitet. Hos pasienter med nyoppstått atrieflimmer og plasma-kalium i nedre referanseområde kan KCl-infusjon med påfølgende rask stigning i plasma-kalium øke sannsynligheten for konvertering til sinusrytme. Hypokalemi øker risikoen for arytmi i atrie- og ventrikkelceller ved distinkte mekanismer.

INTRODUCTION

Electrolyte imbalance

Our body contains many different ions or electrolytes, and they account for about 4% of our body weight. They carry out different functions that are essential for normal function of our cells and organs. Among these are conducting electrical impulses along cell membranes in neurons and muscle cells, stabilizing enzyme structures, and releasing hormones from endocrine glands. The concentration of the electrolytes is tightly regulated, and any imbalance can lead to various problems in the body. Even a relatively minor electrolyte imbalance (EI) can lead to severe consequences such as lethargy, drowsiness, confusion, falls, or arrhythmia, especially in older persons due to different factors like polypharmacy, multiple illnesses, impaired organ function, reduced physiological reserves, impaired renal function, and changes in neurohumoral mechanisms (1-3). The most important EIs in clinical practice are dysnatremia, dyskalemia, dyscalcemia, dysmagnesemia, and dysphosphatemia.

Prevalence and mortality

EIs are common in the general population particularly in hospitalized patients, and they are associated with increased morbidity and mortality (4-12). Mild EIs are common in the general population aged 55 years or more (15 %) (6). Hyponatremia is the most prevalent EI in the emergency department (ED) and is reported to range from 2.3-44%. The prevalence of hypernatremia is 1.1-4.4%, hypokalemia 10.2-39%, hyperkalemia 0.8-13%, and albumin-corrected hypercalcemia 0.7-7.5% (4, 5, 9, 13-16).

Hospital length of stay and re-admissions

There is an association between EIs and increased hospital length of stay (LOS) (11, 12, 16-25), and there are studies showing a correlation between hyponatremia and rate of readmission (17-19, 26).

Most studies on EIs have studied one or two specific electrolytes in a selected group of patients with a single disease or in patients in a particular risk group. Few studies have examined the frequency and outcomes in an unselected group of adult patients admitted to the ED. Besides some studies on hyponatremia and readmission (17-19, 26), the rate of readmissions among patients with other EIs is unknown.

Furthermore, relatively little is known regarding outcomes of dyscalcemia, dysmagnesemia, and dysphosphatemia.

Electrolytes and cardiac arrhythmia

EIs may generate or facilitate arrhythmias, even in otherwise healthy cardiac tissue, but particularly in the setting of cardiac disease. Els exert their actions by modulating the conduction of ions across specific cardiac membrane channels and this in turn, can result in antiarrhythmic or proarrhythmic consequences (3).

Electrolytes play a pivotal role in the genesis of action potentials (APs), and disturbances in ion homeostasis are common in patients with arrhythmia. Electrical activity of the heart is composed of transmembrane ionic movement, and EIs can contribute to an increased susceptibility to atrial fibrillation (AF) (27), but also to ventricular tachycardia and fibrillation (3). The most important EIs increasing susceptibility to arrhythmia are dyskalemia and hypomagnesemia.

Potassium

Normal regulation of potassium balance

Potassium is the most abundant intracellular cation, and its concentration in the extracellular space is low due to the action of the Na^+-K^+-ATPase (NKA), which pumps three sodium ions (Na^+) out of the cell in exchange for two potassium ions (K^+). Thus, 98% of total body potassium (~3400 mmol) is found within cells, mainly in muscle, with smaller amounts in red blood cells, liver cells, and the remaining cells of the body. Only 2% (~65 mmol) of total body potassium is found in the extracellular space. Maintenance of potassium balance involves three key elements: transmembrane fluxes, renal excretion, and gastrointestinal loss (28). Of the potassium ingested, 90% is excreted through the kidney, whereas 10% is excreted in the stool (29).

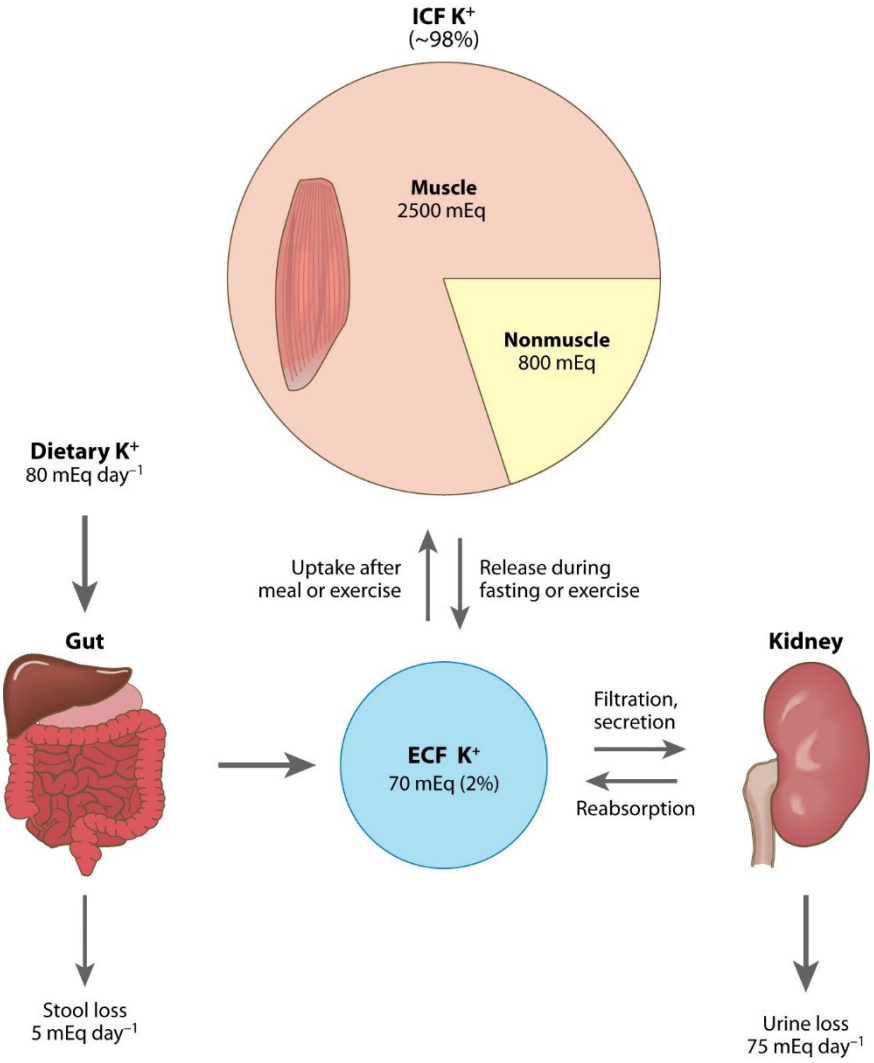

Figure 1. A schematic diagram illustrating daily K^+ fluxes into and out of the extracellular fluid (ECF) pool in an average person. Approximately 98% of the body's K^+ is located in the intracellular fluid (ICF), mainly in muscle, and only approximately 2% is located in the ECF. The ECF pool is regulated by input from the gut, output via the kidney and stools, and redistribution between the ECF and the ICF. 1 mEq = 1 mmol. Figure and figure legend are reused with permission (30).

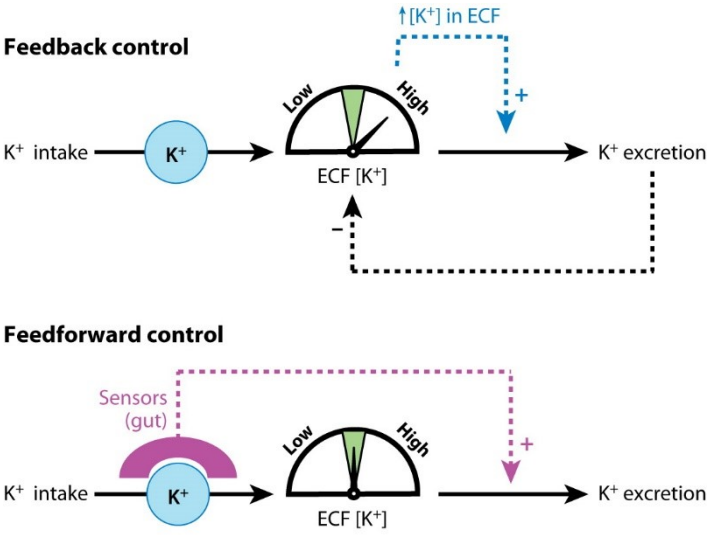

Figure 2. Schematic diagram illustrating control of ECF [K^+] via feedback versus feedforward mechanisms. (Top) In feedback control, a rise in ECF [K^+] is the signal that initiates stimulation of K^+ excretion by the kidney. The increased excretion brings ECF [K^+] back toward the normal value. This process depends on the error signal of elevated ECF [K^+] and stops when ECF [K^+] is returned to the control range. (Bottom) In feedforward control, a local increase in [K^+] in the gut is sensed during K^+ intake and initiates stimulation of K^+ excretion by the kidney independently of (i.e., before) a rise in ECF [K^+], which helps to prevent a rise in ECF [K^+]. Figure and figure legend are reused with permission (30).

The kidneys are mainly responsible for maintaining total body potassium constant by matching potassium intake with potassium excretion. The healthy kidney has a robust capacity to excrete potassium. Under normal conditions, most persons can ingest enormous quantities of potassium (400 mmol per day or more) without development of clinically significant hyperkalemia (31). Thus, most cases of hyperkalemia are due either to abnormal shifts of potassium from the intracellular compartment to the extracellular compartment (e.g. rhabdomyolysis, tumor lysis) or dysfunction of renal potassium excretion (29).

Regulation of renal potassium excretion occurs over several hours. Therefore, changes in extracellular potassium concentration are initially buffered by movement of potassium into or out of skeletal muscle over seconds to minutes. Skeletal muscles play an important role mainly because skeletal muscle cells contain the largest single pool of potassium in the body. Moreover, due to a large number of NKA and K^+ channels, the skeletal muscle cells possess a huge capacity for potassium exchange (32, 33). The most important factors regulating the movement of potassium across the cell membrane under normal conditions are insulin and catecholamines. In

patients with chronic kidney disease, loss of nephron mass is counterbalanced by an adaptive increase in the secretory rate of potassium in remaining nephrons. In this way, potassium balance is well maintained until the glomerular filtration rate (GFR) falls below 15–20 mL/min (33). An increase in plasma-potassium to above 5.5 mmol/L is uncommon until over 90% of the renal function is lost and GFR is < 20 mL/min (34).

Potassium plays a crucial role in maintaining cell function. NKA leads to a K^+ gradient across the cell membrane [intracellular potassium concentration ($[K^+]_i$)> extracellular potassium concentration ($[K^+]_e$)] which is partially responsible for maintaining the potential difference across the membrane. This membrane potential is essential to the function of cells, especially in excitable tissues, such as nerve and muscle (33). Cellular uptake of K^+ is promoted by alkalemia, insulin, β-adrenergic stimulation, aldosterone, and xanthines such as caffeine by activating cell-membrane NKA (28).

Increasing potassium intake has many beneficial effects in humans, including reduction in blood pressure and the risk of fatal ventricular arrhythmias in patients with ischemic heart disease, heart failure, and left ventricular hypertrophy (34).

The difference between serum- and plasma-potassium

Potassium is usually measured using an ion-selective electrode, which converts the effective concentration of the ion dissolved in a solution into an electric potential measured by a voltmeter. Both plasma and serum can be used to measure potassium (35). Pseudohyperkalemia occurs when the *in vitro* serum-/plasma-potassium concentration is falsely elevated while the *in vivo* plasma-potassium concentration is not (36). Pseudohyperkalemia should be suspected when there is no apparent cause for the elevation in the serum-potassium in an asymptomatic patient who has no clinical or ECG manifestations of hyperkalemia.

In one study investigating pseudohyperkalemia in 182 previously healthy patients who had experienced trauma, several blood samples were obtained simultaneously from a radial artery catheter. Serum-potassium, plasma-potassium, and platelet counts were measured. They carried out 1105 of these parallel measurements in the 182 patients. The mean difference between serum- and plasma-potassium was 0.36 ± 0.18 mmol/L, resulting from platelet release during the clotting process when preparing serum from blood. A platelet count of 1000 x 10^9/L is associated with a

measurement error of nearly one mmol per liter of potassium. Thus, the use of plasma-potassium for determinations of potassium is superior to the use of serum (37).

Hypokalemia

Definition and epidemiology

Hypokalemia is defined as plasma-potassium <3.6 mmol/L. The severity of hypokalemia can be defined as mild (3.0-3.5 mmol/L), moderate (2.5-3.0 mmol/L), and severe (<2.5 mmol/L).

Over 20% of hospitalized patients are found to have hypokalemia (38). For hypokalemic patients, in-hospital mortality is 20.4%, or 10-fold that of the entire hospitalized population. In addition, 24% of the hypokalemic patients receive inadequate treatment of hypokalemia (39). The incidence of ventricular fibrillation is fivefold higher in patients with low serum-potassium than in patients with high serum-potassium. Moreover, no episodes of ventricular fibrillation were observed in patients with a serum-potassium of greater than 4.6 mmol/L (32).

Etiology

There are three main causes of hypokalemia: inadequate intake, excessive loss (gastrointestinal or renal), and transcellular fluxes of potassium (from the extracellular compartment into cells). However, medications are the most common causes of hypokalemia. Thus, it is always important to review the patient's medications (38). Hypomagnesemia, induced either by dietary restriction or by excessive loss (gastrointestinal or renal), causes renal potassium wasting. Hypomagnesemia often coexists with hypokalemia as a result of drug treatment (e.g., diuretics and amphotericin B) or conditions (e.g., hyperaldosteronism and diarrhea) that cause loss of both ions. Thus, it may be difficult to evaluate whether the hypokalemia is caused by the hypomagnesemia or is an independent effect (38). In patients with cardiovascular disease, hypokalemia is often caused by an increased loss of potassium through the kidneys due to nonpotassium-sparing diuretic therapy (32). Dietary potassium restriction alone is rarely a cause of hypokalemia, as renal potassium excretion can decrease to <15 mmol per day. Thus, even if potassium intake were zero, it would take 2–3 weeks for the serum-potassium concentration to

decrease to ~3 mmol/L (28). In the initial phase of hypokalemia, potassium is firstly lost from skeletal muscles, maintaining circulating potassium. Later, potassium is lost from blood and muscles, and finally, from other compartments, resulting in potassium concentrations that are too low to support life (32).

Diagnosis

A focused history includes evaluation for adequate nutrition, possible gastrointestinal losses, review of current medications, and if the patient has any cardiovascular comorbidities. It is essential to detect any renal loss of potassium that could be the cause of hypokalemia by taking a spot urine sample. The diagnosis is confirmed by measuring plasma-potassium. Other laboratory tests include serum analyses of glucose, magnesium, and creatinine, urine (spot) potassium and creatinine, and acid-base-status. An electrocardiogram (ECG) should be taken in order to detect any ECG changes or arrhythmias.

Symptoms

Symptoms of hypokalemia depend on the speed of the decrease in circulating potassium and the severity of hypokalemia. Patients with mild hypokalemia (plasma-potassium 3.0-3.5 mmol/L) often have no symptoms. However, in the presence of heart disease, hypokalemia carries an increased risk of cardiac arrhythmias. With more severe hypokalemia, nonspecific symptoms such as generalized weakness, tiredness, and constipation are more common. When plasma-potassium decreases to less than 2.5 mmol/L, ascending paralysis can develop with eventual impairment of respiratory function (38).

Treatment

Optimum treatment of hypokalemia requires identification and treatment of the underlying cause. Potassium can be given orally in liquid or tablet form or intravenously, usually as KCl. Standard intravenous administration is 40 mmol per liter saline. Continuous cardiac monitoring is indicated if the rate exceeds 10 mmol per hour. Concomitant hypomagnesemia should be treated concurrently.

Hypomagnesemia

Definition and epidemiology

Hypomagnesemia is defined as serum-magnesium <0.71 mmol/L. The severity of hypomagnesemia can be defined as mild (0.66-0.70 mmol/L), moderate (0.50-0.65 mmol/L), and severe (<0.50 mmol/L).

Hypomagnesemia is common with a prevalence of 12-20% in hospitalized patients and up to 65% in intensive care patients (11, 22, 40).

Etiology

The causes of hypomagnesemia can be inadequate magnesium intake, gastrointestinal or renal loss, or transcellular influx of magnesium (from the extracellular compartment into cells) (41). Gastrointestinal or renal losses are the two major causes of hypomagnesemia (40).

Diagnosis

Diagnosis is confirmed by measuring serum-magnesium. Low serum-magnesium indicates magnesium depletion. However, serum-magnesium may be normal in the presence of magnesium depletion (41). Hypomagnesemia is usually asymptomatic until serum-magnesium is <0.50 mmol/L.

Symptoms

Clinical manifestations to hypomagnesemia can be muscular weakness, nausea, vomiting, seizures, muscle cramps, nystagmus and cardiac arrhythmias (both supraventricular and ventricular) (41, 42). In addition, hypomagnesemia can lead to refractory hypokalemia and hypocalcemia (43). Up to 60% of patients with hypokalemia are hypomagnesemic, and the hypokalemia is refractory to potassium supplementation until magnesium is adequately repleted (41, 44). Magnesium deficiency leads to a decrease in intracellular magnesium. This releases the magnesium-mediated inhibition of the renal outer medullary potassium channel (ROMK) and increases potassium secretion (45).

Treatment

Patients with severe hypomagnesemia or symptomatic hypomagnesemia are treated with intravenous magnesium sulfate ($MgSO_4$) in addition to magnesium supplements per orally if possible. Patients with mild to moderate hypomagnesemia receive oral magnesium supplements. Magnesium infusion should be given slowly, preferentially

with a duration between 12 and 24 hours. This is because serum-magnesium is the major regulator of magnesium reabsorption in the loop of Henle (the major site of active magnesium transport), and an abrupt elevation in the serum-magnesium will partially remove the stimulus to magnesium reabsorption, and up to 50% of the infused magnesium will be excreted in the urine. In addition, magnesium uptake by the cells is slow and repletion requires sustained correction of the hypomagnesemia (40). It is essential to discontinue or avoid medications that can lead to hypomagnesemia. Potassium sparing diuretics may be helpful in patients who have renal magnesium wasting. Dietary intake of food containing high levels of magnesium (e.g. grains, green vegetables, beans, nuts, and seafood) should be encouraged (41, 46).

Cardiac electrophysiology

To understand the consequences of EIs for cardiac pathophysiology and particularly arrhythmia, it is important that we first review normal cardiac electrophysiology.

The cardiac action potential

The concentrations of ions inside the cell are different from the concentrations outside the cell. This difference of ion concentrations across the cell membrane is called the membrane potential. During diastole, which is the resting phase between heartbeats, the cell membrane maintains a stable negative potential, i.e. the resting membrane potential (RMP). During cardiac contraction, known as systole, the cellular membrane is depolarized, and when this increase in membrane potential reaches a threshold, an AP is triggered. The AP is characterized by a rapid depolarizing phase, followed by a plateau of positive potential before the membrane potential gradually returns to the resting level (repolarization). The five different phases of the AP (phase 0-4) are illustrated in Figure 3 (47). However, it should be noted that the cardiac AP differs significantly between cell types and across different regions of the heart, which ensures synchronized and efficient contraction.

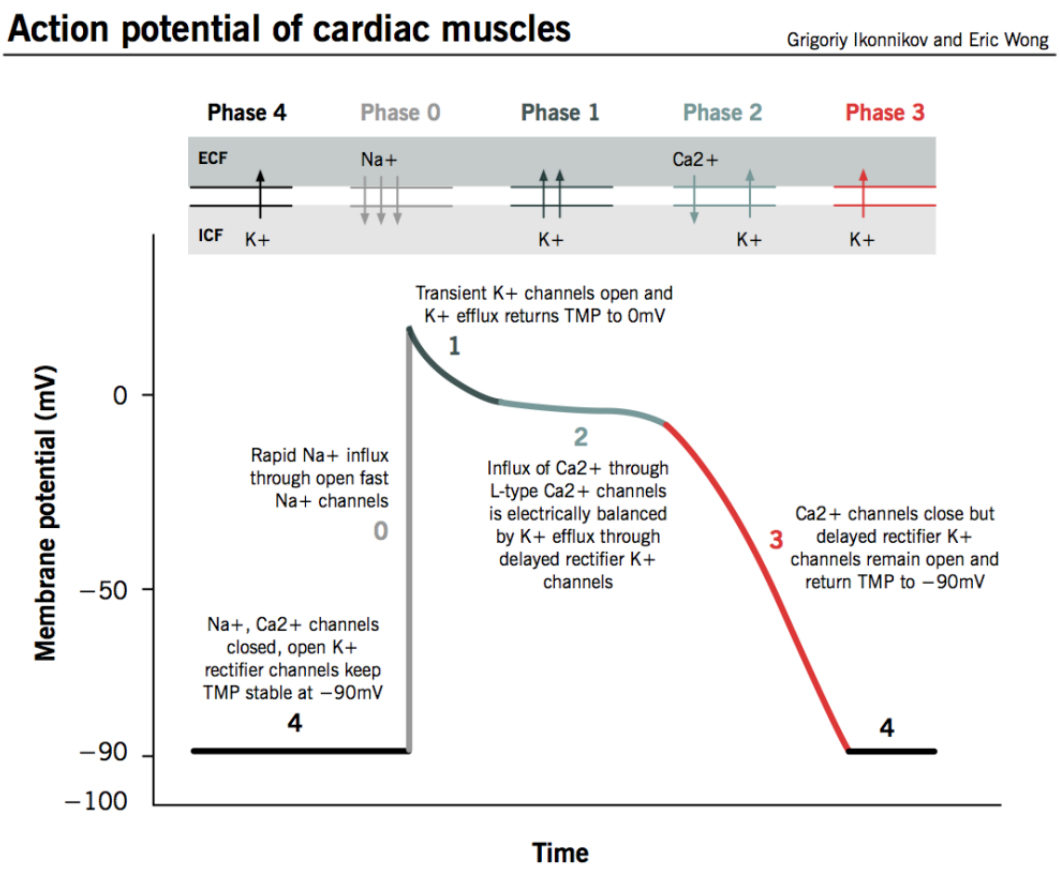

Figure 3. Used with permission from Grigoriy Ikonnikov, Eric Wong and Sultan Chaudhry. Action potential of cardiac muscles. McMaster Pathophysiology Review, www.pathophys.org. TMP: Transmembrane potential.

Phase 4 (resting, diastole): At RMP, i.e. during diastole, the membrane potential is predominantly determined by K^+. Indeed, in this phase, the membrane is permeable to K^+, and since the $[K^+]_i$ is higher than the $[K^+]_e$, K^+ diffuses out of the cell via the inward rectifier potassium channels (I_{K1}). I_{K1} thereby maintains the RMP.

Phase 0 (rapid depolarization): The cell is depolarized (increase in the membrane potential), and an AP is initiated. Normally, the AP is independent of the amplitude of the stimulus, since an "all or none" response is generated when the threshold potential is exceeded. The cardiomyocytes in the atria, ventricle, and His-Purkinje fibers are depolarized due to the opening of the voltage-activated Na^+ channels that lead to the influx of Na^+.

Phase 1 (early rapid repolarization): The membrane potential rapidly and transiently returns to near 0 mV (repolarizes) due to inactivation of I_{Na} channels and the activation of a transient outward K^+ current (I_{to}).

Phase 2 (the plateau phase): This phase may last several hundred milliseconds, the membrane potential changes slowly, and it is mainly calcium-mediated. L-type Ca^{2+} channels (LTCC) provide entry of Ca^{2+} into the cytosol, which is nearly in balance with outward K^+ current carried by rapid (I_{Kr}) and slow (I_{Ks}) delayed rectifier K^+ channels in atrial and ventricular myocytes, and also ultrarapid delayed rectifier K^+ channels (I_{Kur}) in atrial myocytes.

Phase 3 (rapid repolarizing phase): This final phase is predominantly driven by K^+ channels and mainly involves current carried by I_{K1}. The efflux of K^+ increases as the influx of Ca^{2+} and Na^+ decreases, which brings the membrane potential towards the RMP.

After initiation of an AP, the cardiomyocytes are unable to initiate another AP for about 200 ms. This period is called the refractory period and it allows the ventricles to complete their contractions and empty the blood before the next contraction. There are two refractory periods. During the *absolute refractory period* (phase 0 – 3), the cell cannot initiate another AP (depolarization is not possible) no matter how great the stimulus, because the cell is not fully repolarized and sodium channels remain inactivated. During the subsequent *relative refractory period* (phase 3), cardiomyocytes must be stimulated by a larger than normal stimulus to depolarize and initiate an AP.

Potassium channels in the heart

As briefly described above, cardiomyocytes contain a variety of K^+ channels, which function to control RMP and AP configuration.

Voltage-gated K^+ (K_V) channels are transmembrane channels specific for K^+ that open and close in response to changes in transmembrane voltage to allow passage of K^+ ions across the cell membrane (48). There are many types of K^+ channels in mammalian cardiomyocytes, and their expression varies greatly throughout the heart. This variety allows for precise and distinctive control of RMP, action potential duration (APD), and the refractory period throughout the heart. They affect APD by regulating the rate of repolarization (49). Ultrarapid delayed rectifier currents (I_{Kur}) play a role at the start of the rapid phase 1 of the AP and are the main delayed rectifier currents for the atria. This leads to a shorter APD seen in the atria compared to the ventricles (50). By studying Figure 4, we can see that ventricular myocytes have a higher density of I_{K1} which leads to a more hyperpolarized RMP compared with atrial

myocytes. The plateau phase of the AP is longer in ventricular myocytes since they have a lower density of I_{to} and lack I_{Kur}, in addition to larger Ca^{2+} current. Consequently, the prolonged plateau phase allows the I_{Kr} to recover from inactivation and therefore to a faster repolarization (49). The regiospecific localization of I_{Kur} in the atria has made it an attractive target for the development of atria specific therapy, particularly atrial fibrillation as inhibition of I_{Kur} would prolong the APD in the atria but not in the ventricles. This would prolong the refractory period and give a slower pulse conduction in the atria, leading to conversion of AF to sinus rhythm. The absence of I_{Kur} in the human ventricles reduces the risk of serious ventricular arrhythmias that can be induced by other anti-arrhythmic drugs targeting channels with broader expression (51).

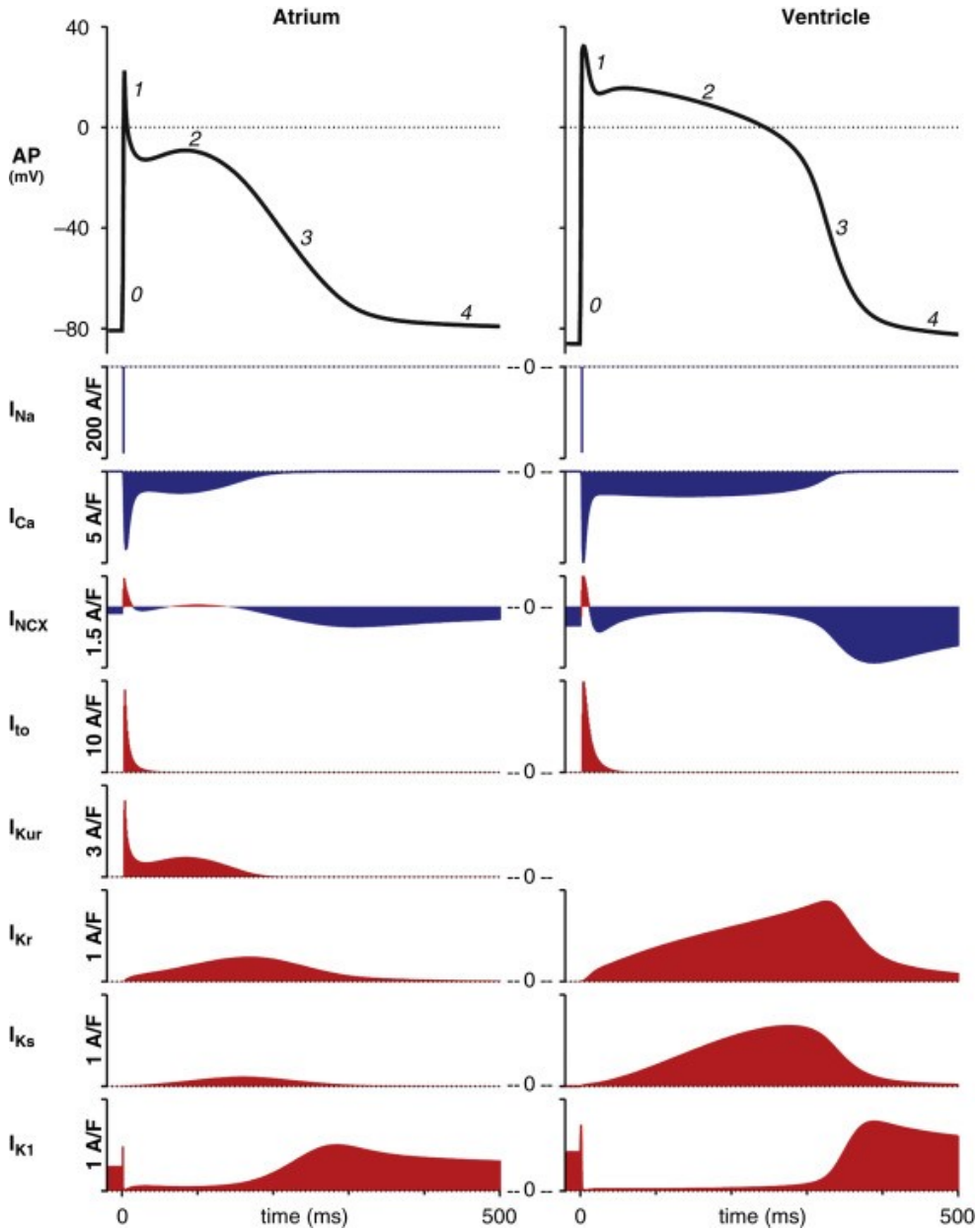

Figure 4. Transmembrane currents underlying the action potential (AP) in atrial and ventricular cardiomyocytes. Representative cardiac AP waveforms are shown in the top panel from atrial (left) and ventricular (right) myocytes. The five phases of the AP are labeled: 0, upstroke of the AP characterized by a rapid depolarization of the membrane; 1, initial repolarization; 2, plateau phase; 3, late repolarization phase; and 4, the resting (diastolic) phase. The rate of change of the membrane potential is proportional to the sum of the underlying transmembrane ion currents (lower panels). Inward currents (blue) depolarize the membrane, while outward currents (red) contribute to repolarization. Compared to an atrial AP, the ventricular AP typically has a longer duration, higher plateau potential (phase 2), and a more negative resting membrane potential (phase 4). The presence of I_{Kur} in atrial myocytes contributes to the lower plateau phase in the atrial AP. Greater I_{K1} in ventricular cells provides a faster phase 3 repolarization and a more negative resting membrane potential (phase 4). Figure and figure legend are reused with permission from Elsevier (52).

Excitation-contraction coupling

Cardiac excitation-contraction coupling (ECC) is the process linking electrical excitation of the myocyte during the AP to contraction of the heart. ECC is initiated by Ca^{2+} influx during phase 2 (the plateau phase) of the AP. This Ca^{2+} influx and resulting increase in $[Ca^{2+}]_i$ trigger the opening of sarcoendoplasmic reticulum (SR) calcium release channels called ryanodine receptors (RyR) (Figure 5). This process, termed calcium-induced calcium release, results in release of a much larger amount of Ca^{2+} from the SR. Following this process, Ca^{2+} binds to the myofilament protein troponin C, to activate the contractile machinery. For relaxation to occur, Ca^{2+} must be removed from the cytoplasm ($[Ca^{2+}]_i$ must decline) allowing Ca^{2+} to dissociate from troponin. This requires Ca^{2+} transport out of the cytosol by four pathways, including the sarcoendoplasmic reticulum Ca^{2+}-ATPase (SERCA), sarcolemmal Na^+/Ca^{2+} exchanger (NCX), sarcolemmal Ca^{2+}-ATPase, and the mitochondrial Ca^{2+} uniporter. The latter two mechanisms are referred to as “slow systems” (53, 54). In rats, the activity of SERCA accounts for 92%, NCX for 7%, and the slow systems for 1% of the efflux of Ca^{2+}. At steady-state, the amount of Ca^{2+} efflux during relaxation must balance the amount of Ca^{2+} influx during contraction to prevent net gain or loss of Ca^{2+} (53). Under normal conditions the NCX works mainly in the Ca^{2+} efflux mode, extruding one Ca^{2+} ion in exchange for three Na^+ ions. However, the amount of Ca^{2+} influx by NCX can be increased if the $[Na^+]_i$ is elevated by blocking NKA, if SR Ca^{2+} release and/or I_{Ca} by LTCC is inhibited, or if APD is prolonged (53).

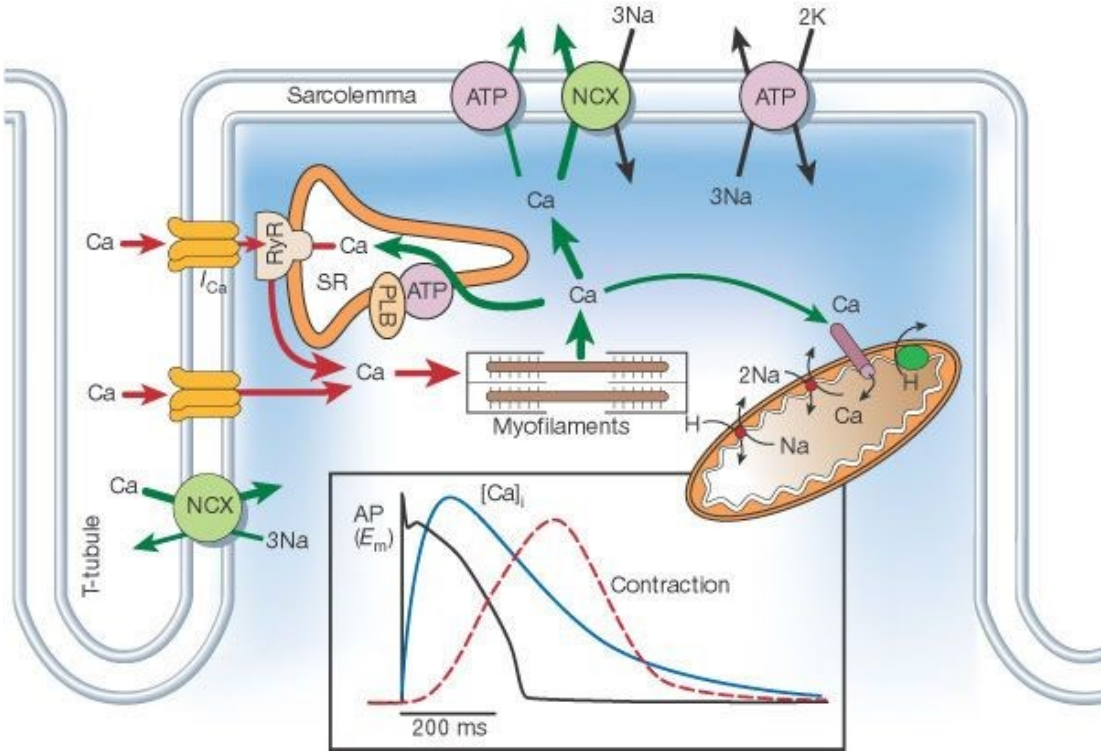

Figure 5. Ca^{2+} transport in ventricular myocytes. Inset shows the time course of an action potential, Ca^{2+} transient and contraction measured in a rabbit ventricular myocyte at 37 °C. NCX, Na^{+}/Ca^{2+} exchange; ATP, ATPase; PLB, phospholamban; SR, sarcoplasmic reticulum. Figure and figure legend are reused with permission from Nature Publishing Group (53).

Cardiac t-tubules – morphology and function

As mentioned above, ECC is initiated by Ca^{2+} influx during the plateau phase of the AP. This Ca^{2+} influx results in opening of the RyR which eventually results in contraction of the cardiomyocytes. The efficiency of this process is dependent on t-tubules; they spread the AP into the cell and enable close contact between the sarcolemma and the SR. Transverse (t-) tubules are invaginations of the surface sarcolemma occurring at the junction of each sarcomere (z-line), which have both transverse and longitudinal elements (54). They make up 21-64% of the total sarcolemma membrane area (55) and 0.8-3.6% of the cardiomyocytes volume (56). T-tubules have been found in cardiomyocytes from all mammalian species studied. Ventricular myocytes have the most developed t-tubule system, whereas atrial myocytes have more scarce and irregular t-tubules (57). In rat atria, about 30% of the cells are tubulated and about 10% of the atrial cells exhibit a well-organized t-tubule density equivalent to ventricular cells (58).

ECC depends on the close association between the SR network and t-tubule membranes. The junctional SR makes close contact with the t-tubule membrane so that RyRs on the SR are very closely apposed to LTCC on the t-tubule, thus forming the cardiac dyad that is fundamental to the processes initiating the systolic Ca^{2+} transient. The close contact between the RyRs and LTCC ensures a synchronous rise of the $[Ca^{2+}]_i$ during contraction (systole) and is a requirement for rapid and powerful contraction (54, 56). About 80% of the LTCC are at t-tubules (57). In atrial cardiomyocytes that lack t-tubules, $[Ca^{2+}]_i$ rises first at the edge of the cell and then propagates into the cell interior (55).

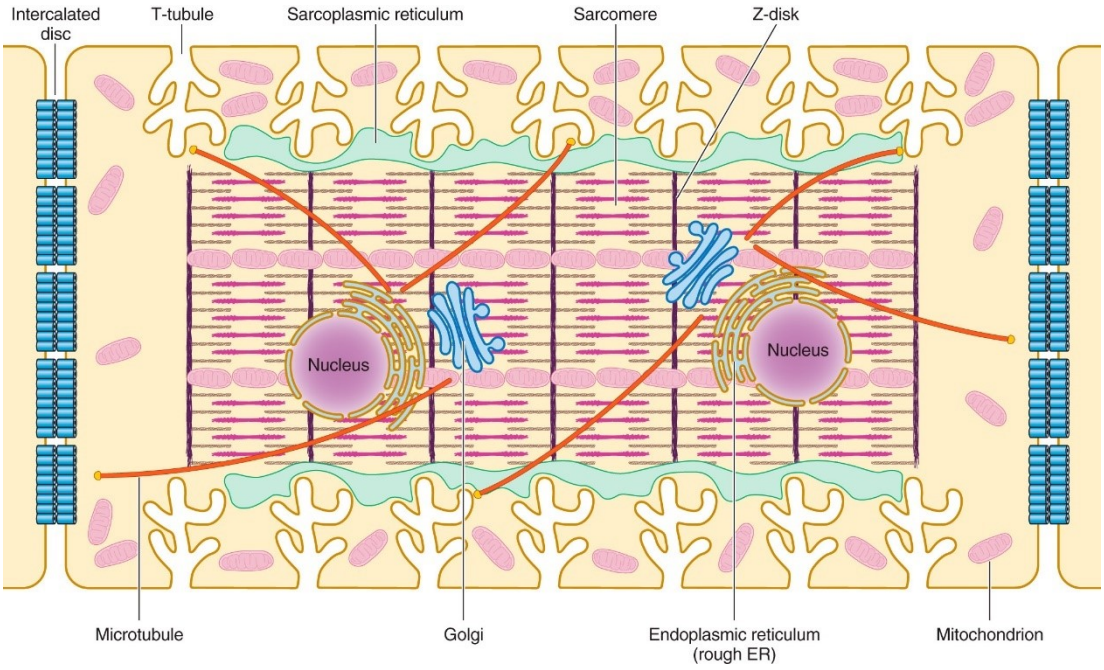

Figure 6. Schematic illustration of the internal structures of an adult ventricular cardiomyocyte. T-tubules, which are enriched with voltage-gated LTCC, are positioned closely near the SR, the primary internal calcium store. Sarcomeres form myofibrils, which are responsible for cardiomyocyte contraction upon calcium release. The Golgi apparatus and microtubules serve as the "loading dock" and "highways," respectively, to deliver ion channels to specific subdomains on the plasma membrane. Mitochondria provide the energy needed for the contraction of cardiomyocytes. Intercalated discs located at the longitudinal sides of each ventricular cardiomyocyte mediate the cell-to-cell propagation of action potentials. Figure and figure legend are reused with permission (57).

NKA-NCX crosstalk and local sodium

T-tubules are critical sites for not only Ca^{2+} homeostasis but also Na^+ regulation. NKA and NCX are essential for these processes.

The heart has several NKA isoforms. NKA-α_1 is the dominant isoform, whereas NKA-α_2 and NKA-α_3 are present in smaller amounts, and their expression differs significantly between species (59). All three isoforms may function differently, depending on their specific localization in the cell membrane (59). The α_1-isoform is the dominant isoform at the sarcolemma with 85-95% of the total NKA, whereas it constitutes over 55% of the NKA in the t-tubules and is prominent in both transverse and longitudinal t-tubules. The α_2-isoform density is 4-6 times higher in t-tubules compared with the sarcolemma and is almost entirely localized to the transverse elements (60, 61).

NCX activity is found predominantly in the t-tubules of ventricular cardiomyocytes, and over 60% of the Na^+ influx during the cardiac cycle enters the cell by this pump (62). During ECC, NCX removes Ca^{2+} following calcium-induced calcium release, by pumping in three Na^+ ions in exchange for one extruded Ca^{2+} ion. To maintain $[Na]_i$ at steady state, Na^+ must be pumped out via the NKA. Thus, the location and interplay between NKA and NCX are important with regards to $[Na]_i$ and $[Ca^{2+}]_i$ regulation, and consequently for the ECC (55). Indeed, it has been proposed that NKA-α_2 isoform and NCX share a common subsarcolemmal space and that within this restricted diffusion space or microdomain $[Na^+]_i$ may be different from bulk $[Na]_i$ (63). While the existence of such a microdomain remains controversial, it has been claimed that the subsarcolemmal $[Na]_i$ may be several fold higher than the $[Na]_i$ in the bulk cytosol, due to slow Na^+ diffusion from the NKA compartment. Furthermore, it has been shown that if the NKA is blocked (for example by ouabain, a potent and specific inhibitor), the $[Na]_i$ may be higher in sites where the NKA is blocked compared with $[Na]_i$ in the bulk cytosol. As a result, the high $[Na]_i$ induces reverse mode NCX (extruding three Na^+ in exchange for one Ca^{2+}), leading to an increase in $[Ca^{2+}]_i$. Thus, interaction between Na^+ fluxes and Ca^{2+} handling in the subsarcolemmal space may be important in determining cardiac contractility and promoting arrhythmia (63-65).

Early and delayed afterdepolarizations

As discussed in the above section, Ca^{2+} handling is essential with regards to arrhythmogenesis. Arrhythmias can be divided in bradyarrhythmias (heart rate < 50 beats/minute) and tachyarrhythmias (heart rate > 100 beats/minute). An imbalance in the cardiac Ca^{2+} handling can induce early or delayed afterdepolarizations (EADs,

DADs), which trigger tachyarrhythmias such as atrial fibrillation and ventricular tachycardia.

EADs are abnormal depolarizations that start during phase 2 (plateau) or phase 3 (repolarization) of the AP. An increase in Ca^{2+} inward current and/or a decrease in K^{+} outward current will prolong the APD. This prolongation of the APD promotes the reactivation of I_{Ca} which can lead to EADs (Figure 7A) (66, 67). Also, spontaneous SR Ca^{2+} release due to high SR Ca^{2+} levels promote an inward I_{NCX} as the released Ca^{2+} is extruded in exchange for three Na^{+}. As a result, this inward current depolarizes the membrane potential, and if sufficiently, it can trigger an EAD (Figure 7B). In atrial myocytes or in pathological conditions in ventricular myocytes (heart failure or ischemic heart disease) where the AP is shortened, EADs can be induced by non-equilibrium I_{Na} reactivation which is due to triggered (not spontaneous) SR Ca^{2+} release inward I_{NCX} (Figure 7D) (68, 69).

DADs (Figure 7C) begin during phase 4 of the AP after repolarization is completed but before another AP. They are caused by spontaneous SR Ca^{2+} release as described for EADs above. A DAD in a single cell cannot cause an arrhythmia because neighboring cells would provide a current sink, dissipating the depolarizing current. However, if DADs occur in a cluster of neighboring cells, the impulse can escape and propagate through the heart, resulting in arrhythmia (66).

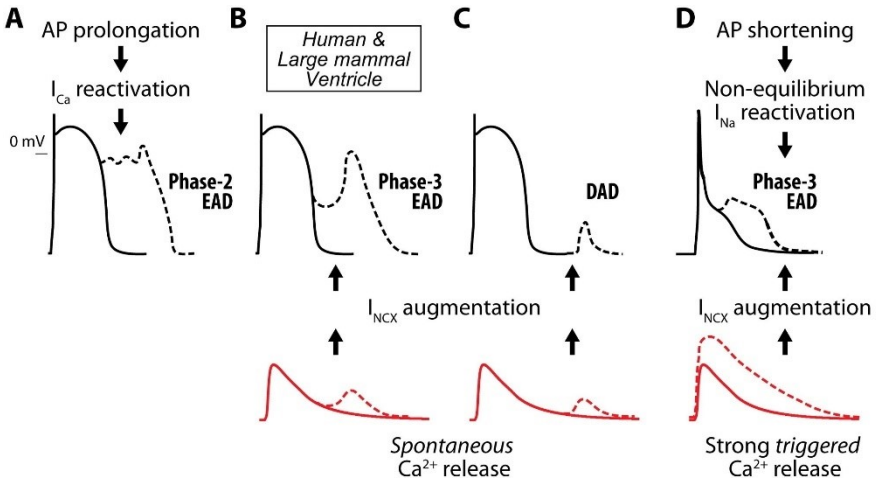

Figure 7. Mechanisms of afterdepolarization formation in cardiomyocytes. In ventricular myocytes from large mammals, phase-2 EADs are associated with I_{Ca} recovery from inactivation and reactivation during prolonged APs (A). Spontaneous SR Ca^{2+} release, which increases Ca^{2+} extrusion via NCX (inward current), can lead to phase-3 EADs (B) or DADs (C) when occurring during or after membrane potential repolarization, respectively. In murine ventricle, phase-3 EADs are favored by potentiated (triggered) Ca^{2+} transient and AP shortening (D). The former causes I_{NCX} augmentation and AP plateau prolongation (at negative membrane potential), during which non-equilibrium I_{Na} reactivation (permitted by rapid I_{Na} recovery during fast repolarization) can occur. This, which is shown to be relevant in the human atrium, could be a universal mechanism underlying EAD formation in both atria (especially pulmonary veins) and ventricles (70) of large mammals. Indeed, phase-3 EADs mediate re-initiation of atrial (and ventricular) fibrillation. Modified and reused with permission (67).

Electrophysiological effects of hypokalemia

Potassium is the most critical determinant of the RMP. The electrophysiological effects of potassium depend on its extracellular concentration (mild vs. severe), its direction (hypokalemia vs. hyperkalemia), and its rate of change (slow vs. fast) (3).

We can explore the mechanisms by which hypokalemia induces arrhythmia by applying *ex vivo* animal models of hypokalemia. In these models, cardiac tissue or isolated cardiomyocytes can be perfused with solutions that contain different K^+ concentrations and thereby studying the electrophysiological effects of hypokalemia (71).

Hyperpolarization and slowed conduction

In hypokalemia, the magnitude of the potassium gradient across the sarcolemma is increased, leading to hyperpolarization (more negative) of the RMP. Consequently, this leads to a decrease in membrane excitability in the beginning of the AP as a result of the increased difference between RMP and threshold potential. Also, the

hyperpolarization and increased threshold for excitation slow conduction in the cardiomyocytes (3, 71).

Prolonged repolarization

The activity of the potassium channels I_{K1}, I_{to}, and I_{Kr} as well as other potassium channels depends on the $[K^+]_e$. The outward current of I_{K1} is regulated by cytoplasmic Mg^{2+} and polyamines that prevent the passage of K^+ ions. Extracellular K^+ entering the channel removes these blocking cations and thereby restores the outward K^+ current. Consequently, hypokalemia increases the blocking of this potassium channel and thus decreases the outward current (72). Furthermore, hypokalemia leads to faster inactivation of I_{Kr} and downregulates its expression within hours. Hypokalemia also slows the reactivation I_{to} and reduces its density in cardiomyocytes (71, 72).

As a result of the suppressing effect of hypokalemia on the potassium currents I_{K1}, $I_{to,}$ and I_{Kr} (phase 1 to 3 of the AP), K^+ efflux is reduced, which in turn increases the APD and prolongs repolarization. Consequently, this increases the tendency to EADs due to reactivation of inward Na^+ and Ca^{2+} currents (3, 71).

Increased relative refractory period (phase 3 in AP)

Hypokalemia increases the relative refractory period (phase 3 in AP) and decreases the difference between RMP and the threshold potential during the terminal phase of the AP, thereby making the cardiomyocytes more excitable (3).

Inhibition of Na^+/K^+-ATPase and intracellular Ca^{2+} overload

In rat ventricular cardiomyocytes, a reduction in $[K^+]_o$ leads to a biphasic response in Ca^{2+} transient amplitude. Initially, the reduction in $[K^+]_o$ leads to hyperpolarization of the RMP and augmentation of the NCX-mediated Ca^{2+} efflux (forward mode), which temporarily reduces SR Ca^{2+} content and release. This results in a reduction of the Ca^{2+} transient amplitude. However, the reduction in $[K^+]_o$ also inhibits the NKA, reducing the NCX-mediated Ca^{2+} efflux, which subsequently leads to a progressive cellular Ca^{2+} overload and increased Ca^{2+} transient amplitude. Taken together, the reduction in $[K^+]_o$ gives a biphasic Ca^{2+} transient and subsequently Ca^{2+} overload and increased tendency for afterdepolarizations (73).

Activation of Ca^{2+}/calmodulin-dependent protein kinase II

As described above, hypokalemia inhibits the outward potassium currents and NKA. In isolated rabbit and rat hearts, moderate hypokalemia (2.7 mmol/L) is a potent

inducer of EAD-mediated ventricular arrhythmias. The inhibition of NKA leads to Na^+ and Ca^{2+} overload, which results in Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) activation. CaMKII activation initiates a positive feedback cascade, further exacerbating Na^+ and Ca^{2+} accumulation by activating the voltage-gated Na^+ channels and LTCC and increasing the influx of Na^+ and Ca^{2+}, which increasingly prolongs repolarization and promotes EADs (74).

Potassium and atrial fibrillation

Hypokalemia is associated with a higher risk of AF, which is independent of age, sex, serum-magnesium, and other possible confounders (75). It can induce AF through its above-mentioned electrophysiological effects in addition to causing sinoatrial node dysfunction and increased pulmonary vein arrhythmogenesis (27). Potassium intake reduces both blood pressure and the risk of lethal ventricular arrhythmias in patients with ischemic heart disease, heart failure, and left ventricular hypertrophy (34). Therefore, it could be possible that increasing plasma-potassium could be beneficial in AF patients. Interestingly, there are several case reports of patients with AF converting to SR during hyperkalemia (76-81).

Atrial fibrillation and flutter

Definition

AF is characterized by rapid and chaotic atrial activation, characterized by the lack of distinct and organized P waves and irregular ventricular activation (QRS complexes) on surface ECG (82). From a clinical standpoint, AF can be categorized into four types: 1) paroxysmal AF (PAF), which refers to self-terminating episodes that terminate within 7 days (in most cases within 48 hours); 2) persistent AF, which refers to AF that lasts more than 7 days and up to 1 year, or requires termination by cardioversion; 3) long-standing persistent AF, which refers to continuous AF lasting for 1 year or more when it is decided to choose a rhythm control strategy; and 4) permanent AF, when attempts to achieve sinus rhythm have been unsuccessful or continuous AF is accepted by the patient and physician and rhythm control interventions are not pursued (83).

Epidemiology

AF is the most common arrhythmia with an overall prevalence of 3% in adults aged 20 years or older (83). The prevalence increases from <0.5-1.0% at 50 years of age, and up to 15% at 80 years of age. It is also associated with reduced quality of life and increased morbidity and mortality (84). Furthermore, AF is associated with a 3-5-fold increased risk of stroke, accounting for about 20% of all strokes (84), and 10-40% of AF patients are hospitalized each year (83).

Etiology and risk factors

AF can result from an interplay between triggers (starting an electric stimulus such as EADs and DADs as described above) and a substrate (vulnerable tissue that allows AF to be induced). It is believed that triggers, usually arising from the pulmonary veins, are more critical than a vulnerable tissue in inducing PAF. As AF progresses from paroxysmal to permanent, the vulnerable tissue becomes more important (82). However, AF is a diverse condition and in addition to an isolated electrophysiological disorder, it can be a manifestation or consequence of other cardiac and noncardiac pathologies (84). The identification of these pathologies and their prevention and treatment are vital to prevent AF and its disease burden. Knowledge of these factors and their management is hence important for optimal management of AF patients (83).

Age and sex are two of the most potent predictors of AF. Other risk factors are genetic predisposition, hypertension, valvular heart disease, coronary artery disease, heart failure, cardiomyopathy, obesity, alcohol consumption, smoking, diabetes mellitus, chronic kidney disease, obstructive sleep apnea, thyroid dysfunction, vigorous exercise, inactivity, and inflammation (84).

Diagnosis

A 12-lead ECG is recommended to establish the diagnosis of AF, to determine the ventricular rate in AF, and to screen for other abnormalities. Initial blood tests should evaluate thyroid and kidney function as well as serum electrolytes, glucose, and full blood count. Transthoracic echocardiography is recommended in all AF patients to guide treatment decisions by identifying the increased ventricular and atrial size, myocardial hypertrophy, valvular disease, and ventricular dysfunction (83).

Symptoms

Patients with AF may experience a variety of symptoms including fatigue, palpitations, dyspnea, chest tightness, sleeping difficulties, and psychosocial distress (83). The most common symptoms are palpitations (defined as the increased perception of the heartbeat), chest pain, and a reduction in exercise capacity. There is substantial intra- and inter-individual diversity in the type and severity of symptoms. Within an individual, symptoms may fluctuate widely over time. Approximately 15-30% of patients with AF are asymptomatic (85).

Treatment

The aim with the current therapy for AF is to treat the symptoms and reduce the risk of tachycardia-induced cardiomyopathy and stroke (82). Oral anticoagulation can effectively prevent most ischemic strokes in AF patients (60-70% risk reduction) and can prolong life (83). It is recommended that patients with a CHA_2DS_2-VASc risk score of 2 or more in men, and 3 or more in women should receive treatment with oral anticoagulation (preferably a non-vitamin K oral anticoagulant) (83). Rate control in AF patients is often enough to improve AF-related symptoms. It can be achieved by atrioventricular blocking medications such as β-blockers, digoxin, L-type calcium channel blockers, or a combination of these (83). The optimal heart rate target in AF patients is unclear, but a resting heart rate of <110 beats per minute is recommended unless symptoms call for stricter rate control (83). Rhythm control therapy is indicated in patients who remain symptomatic on adequate rate control therapy. All trials that have compared rhythm control and rate control alone have resulted in neutral outcomes (83). Catheter ablation of AF is in general second-line treatment after failure or intolerance to antiarrhythmic drug therapy. It is successful in restoring and maintaining sinus rhythm in approximately 70% of selected patients with paroxysmal AF but only in approximately 50% of patients with long-standing AF (83).

Atrial flutter

Atrial flutter (AFl) is a related condition to AF, caused by an atrial macro-reentrant electric circuit leading to rapid and regular atrial activation [approximately 300 (240-400) circuits per minute], characterized by distinct and organized P waves (flutter waves) and typically regular but sometimes irregular ventricular activation (QRS complexes) on surface ECG depending on the degree of atrioventricular node

conduction block. AFl has lower prevalence, with an incidence <1/10 that of AF (83). While also associated with elevated stroke risk, morbidity, and mortality, the pathophysiology and outcomes of AFl have been considerably less investigated compared with AF (86).

Recent-onset atrial fibrillation or atrial flutter

Recent-onset atrial fibrillation (ROAF) or AFl, defined as an abrupt onset of symptoms within 48 hours from admission, are highly prevalent in EDs (87). The optimal management strategy for these conditions is unclear, and current European and American guidelines provide little direction (83). This lack of consensus is shown by different treatment modalities in clinical practice (87). Very often, the chosen strategy is either a “wait and see” approach or conversion to sinus rhythm by applying anti-arrhythmic medication or direct-current cardioversion (88).

Among patients with ROAF, about 50% convert spontaneously to sinus rhythm (89, 90). Patients with ROAF can be treated by a number of anti-arrhythmic medications, which can increase the likelihood of conversion to sinus rhythm to about 70% within 12 hours (91). However, most current anti-arrhythmic medications are not atrial specific, and they all have a significant risk of side-effects, including atrial and ventricular proarrhythmia, heart failure exacerbation, lung-, hepatic-, and thyroid toxicity, and death (82). Furthermore, anti-arrhythmic drugs have several contraindications, including bradycardia, known sinoatrial node dysfunction, AV conduction disturbances, and prolonged QT interval, heart failure, coronary artery disease, and structural heart disease (82). Another treatment option in ROAF patients is direct-current cardioversion, which has a success rate of more than 90% in these patients (92). However, this approach requires deep sedation and a six-hour postprandial period to ensure gastric emptying. In addition, it is associated with additional costs, resources, and risk. Furthermore, direct-current cardioversion also has potential complications, including skin burns, pacemaker dysfunction or damage, and ventricular fibrillation (if shocks are not QRS-synchronized) (92).

AIMS AND QUESTIONS

The overall aim of this thesis was to investigate EIs with special focus on hypokalemia and its association with arrhythmia. The specific aims of the separate studies were:

Article I (epidemiological study)
Electrolyte imbalances in an unselected population in an emergency department: a retrospective cohort study

There are studies describing the occurrence of EIs in the ED. However, few studies report EIs in an unselected population. Furthermore, the frequency of readmission among patients with other EIs than hyponatremia is unknown. In addition, LOS and mortality are little investigated in an unselected population with dyscalcemia, dysmagnesemia, and dysphosphatemia. This led us to the following questions:

1. *What is the frequency and severity of EIs in an unselected population in the ED?*
2. *Is there an association between EIs and patients age and sex?*
3. *Do patients with EIs have longer LOS and more readmissions than patients with normal electrolyte levels?*
4. *Do patients with EIs have higher mortality than patients with normal electrolyte levels?*

Article II (clinical study)
Potassium infusion increases the likelihood of conversion of recent-onset atrial fibrillation – a single-blinded, randomized clinical trial

Hypokalemia is prevalent in the ED, and it is associated with AF, which is the most common arrhythmia. It was interesting to read case reports describing patients with AF converting to sinus rhythm when they had hyperkalemia. This notion led us to the following question:

Does potassium infusion have the potential to convert recent-onset AF or AFl to sinus rhythm?

Article III (basic science study)

Hypokalemia promotes arrhythmia by distinct mechanisms in atrial and ventricular myocytes

Hypokalemia is associated with increased in-hospital mortality which is likely due to elevated incidence of ventricular fibrillation. Hypokalemic patients also have a higher risk of AF. Understanding the pro-arrhythmic role of hypokalemia has important implications for preventive treatment in many patients. It is unclear if and how hypokalemia promotes triggered activity in atrial cardiomyocytes and whether there are similarities with ventricular cardiomyocytes. Thus, in article III we aimed to investigate the arrhythmogenic effects of hypokalemia in atrial compared with ventricular cardiomyocytes:

1. *Does hypokalemia promote triggered activity in cardiomyocytes? If so, by what mechanism?*
2. *Are these mechanisms distinct in ventricular and atrial cardiomyocytes?*
3. *Do NKA and NCX localization and activity contribute to arrhythmogenesis in cardiomyocytes?*

METHODOLOGICAL CONSIDERATIONS

The epidemiological study – article I

Study design and participants

This study was a retrospective cohort study where all patients ≥ 18 years referred for any reason to the ED at Diakonhjemmet Hospital, a local hospital in Oslo, Norway, were included. During the study period from January 1, 2010, to December 31, 2015, 62 991 patient contacts were registered, involving 31 966 patients with laboratory blood analyses. One limitation to our study design is that due to involvement of many different physicians and nurses in patients care, the outcomes (LOS, readmissions, and mortality) would probably be less consistent compared with a prospective study design. Another limitation is that we did not have information about causes of death, and the diagnoses were based on ICD-10 coding which the attending physician found relevant. Furthermore, our study describes associations with the outcomes (LOS, readmissions, and mortality) and not causality. It is possible that the observed associations between EIs and the outcomes were due to other confounding factors such as medical history, vital status in the ED, and reason for referral to the ED. However, it is not possible to measure and correct for every possible factor that could affected the outcomes, even though we corrected for several comorbidities. Lastly, our results are from a local hospital and they could vary between different centers, regions, or countries, which limits the generalizability.

Biochemical analyses

In all medical patients, serum levels of sodium, potassium, calcium, albumin, and glucose were routinely measured. In surgical patients, serum levels of calcium, albumin and glucose were not routinely measured. Serum-magnesium and -phosphate and plasma-free calcium were measured when indicated and requested by the attending physician. Serum-sodium was corrected for serum-glucose (glucose-corrected serum-sodium=serum-sodium + [(serum-glucose – 5,6)/5,6] x 2,4), to account for the diluting effect of hyperglycemia (93). A correction formula was also used to calculate serum-albumin-corrected calcium (mmol/L) [= measured serum-calcium + 0.020 × (41.3 – serum-albumin) where 41.3 g/L was the albumin median] (94). One limitation of our study is the timing of blood samples at arrival of the patients to the ED. Ideally, the blood samples should be collected at arrival of the

patients and before any intervention. Although most blood samples were taken shortly after patients' arrival to the ED, we cannot exclude that in some patients the blood samples were collected after initiation of treatment. Data were extracted from the hospital's Department of Medical Biochemistry database.

Ethical considerations

The study was evaluated by the regional committee for medical and health research ethics (Regional Committee for Medical and Health Research Ethics South East) and defined as a quality study. It was also approved by the institutional review board (The Research Committee, Diakonhjemmet Hospital).

Statistics

Since many patients had more than one visit during the study period, the results were analyzed and presented for each ED visit. We presented our results as median with interquartile range (IQR) for continuous data and as percentages for categorical data. Univariable and multivariable logistic regression analyses were performed to study associations between EI and readmissions, in-hospital mortality, and 30-day mortality. Cox proportional hazard models were performed to analyze the association between EI and 1-year mortality. The multivariable analyses were adjusted for age, sex, and the comorbid conditions (hypertension, heart failure, chronic obstructive pulmonary disease, cancer, kidney failure, diabetes mellitus, atrial fibrillation, pneumonia, sepsis, dehydration, and hip fracture). We chose to adjust for conditions that could be related to the EIs. A two-sided *P*-value less than 0.05 was considered significant. Statistical analyses were performed using Stata/SE (version 14.2; Stata Corporation, College Station, TX, USA).

The clinical study –article II

Study design

This study was a single center, placebo-controlled, parallel-group study, which was conducted with equal randomization (1:1), blinded for patients but not for the healthcare providers. Our study was not double-blinded since plasma-potassium was monitored during the infusion with KCl, and an increase in plasma-potassium could therefore not be blinded for the healthcare providers.

Patients were assigned to either the KCl group or placebo group following blocked randomization by shuffling opaque concealed envelopes. The study was conducted at

Diakonhjemmet Hospital from April 2013 to November 2017. We informed the doctors and nurses regularly with maximum 3 months intervals and had information posters at the ED and the cardiac unit. In addition, study staff members were always available if there were any questions regarding patient inclusion and conduct of the study. Nevertheless, it took more time than we had anticipated to include the number of patients we needed according to the power analysis. Not all patients who met the inclusion and exclusion criteria were included, partly because doctors and nurses were very busy, especially in the ED, where the patients were supposed to be included. Furthermore, there was a turnover of doctors and nurses during the study period. It is possible that the inclusion would have been easier and more patients would have been included if we had a study nurse employed. Another hospital in Oslo was also included as a participating center but because of a very slow inclusion rate it was decided to exclude this hospital from including patients.

The KCl group was treated with 60 mmol KCl added to 1000 mL of 50 mg/mL glucose in an infusion bag, which was then inverted ten times to ensure that the solution was well-mixed. It is possible that this step was not done optimally which could be a reason to why some patients got pain at the infusion site. It would have been preferable if the infusion bag had been premixed. KCl was administered through a peripheral venous catheter in a large vein, preferably the antecubital vein. Unfortunately, in some patients the venous catheter was placed in a smaller vein on the back of the hand, which also could be another reason to why some of the patients got pain from the infusion site.

Participants

The patients included were referred to the ED from general practitioners, municipal emergency services, nursing homes, community health services, or directly by ambulance ordered from a dispatch center.

The inclusion criteria were age ≥18 years, recent-onset AF or AFl (onset of symptoms within 48 hours from admission) and plasma-potassium ≤4.0 mmol/L. The exclusion criteria were kidney failure with estimated GFR <30 mL/min, current usage of antiarrhythmic medication (flecainide, amiodarone, dronedarone, sotalol, dofetilide, or propafenone), infection, metabolic acidosis with pH <7.2, pregnancy, or lactation. Patients participating in other clinical trials or who had previously been included in our trial were also excluded.

Biochemical analyses

Serum levels of potassium, magnesium, and creatinine and plasma-potassium were measured at inclusion. Serum-potassium, -magnesium, and -creatinine were further analyzed every 8 hours after the start of infusion and when the infusion was stopped, whereas plasma-potassium were measured 4 hours after start of infusion and thereafter every 8 hours, i.e. in between the serum analyses, and when the infusion was halted. Thus, serum- or plasma-potassium was monitored every 4 hours. At the follow up about 3 months after discharge, serum- and plasma-potassium were measured. All plasma-potassium measurements were analyzed in a blood gas analyzer.

Echocardiography

Transthoracic echocardiography was performed a short time after conversion to sinus rhythm and before discharge. The patients had AF or AFl less than 48 hours before conversion to sinus rhythm, and it is possible that tachyarrhythmia affected our echocardiographic results. However, echocardiographic analyses would have been more challenging if the recordings had been done before the start of the infusion when the patients had AF or AFl. All patients were examined with an ultrasound scanner (GE Vivid E9) at the Cardiac Unit following the same procedure according to international recommendations.

All recordings were analyzed with the same software (GE EchoPAC). The recordings included standard parasternal (short- and long-axis) and apical (four-chamber, two-chamber, and long-axis) views. The following parameters were recorded: left atrial area, left ventricle end-diastolic diameter, left ventricle mass, left ventricular ejection fraction, ratio between mitral valve early and late diastolic inflow (E/A), ratio between early mitral inflow velocity and mitral annular early diastolic velocity (E/e'), and systolic pulmonary artery pressure.

Electrocardiography

At admission, a 12-lead ECG was recorded at rest and all included patients had continuous ECG-monitoring by telemetry until the end of the study period (maximum 24 hours). At the follow-up consultation about 3 months after discharge, a 12-lead ECG was recorded at rest to see if the patients had sinus rhythm or AF/AFl. If sinus rhythm, they were equipped with a hand-held "thumb ECG" recorder (Zenicor-EKG®; Zenicor Medical Systems AB, Stockholm, Sweden) for 3 days. Patients were

instructed how to use the recorder and we performed a test recording to ensure that the patient understood the instructions and the recorder was functioning as expected. Patients were then asked to perform 30 second rhythm recordings, once in the morning, once in the evening, and whenever they experienced palpitations or arrhythmia symptoms. The recorded signal corresponded to lead I in a standard 12-lead ECG. AF was defined as irregular ventricular rhythm without visible or regular P waves for 30 seconds, whereas AFl was defined by regular P waves at a rate of 250-350/minute. At times it was difficult to recognize the P waves due to poor quality of the recordings. However, it was easy to see if the rhythm was regular or irregular (RR-variability) during the 30-second recording. When any abnormality was detected, the patient was contacted and asked about symptoms and a follow-up consultation was arranged if necessary.

Zenicor-EKG® has shown to have a sensitivity of 96% and a specificity of 92% for detecting AF compared with 12-lead ECG (when rhythm registration was recorded for 30 days) (95). The device has also been shown to be superior to 24-hour ECG monitoring (96). Doliwa et al. studied patients with a verified diagnosis of paroxysmal AF and symptomatic episodes of palpitation at least once every three months. They used Zenicor-EKG® recordings over a 30 days period, and registrations were collected twice daily and in the event of arrhythmia symptoms for 30 days. AF was observed in 18 of 24 (82%) patients, and in 17 of 18 patients, the AF episodes were identified within 15 days of monitoring (96). Another study applying Zenicor to detect relevant arrhythmias in patients with palpitations or dizziness/presyncope identified AF in 9 of 95 (9.5%) patients during a 26 day period (97). In our study, the detection rate for AF was 8.6%. This value would probably have been higher if the monitoring time had been for 30 days. However, this was not one of our primary or secondary endpoints and might have been demanding from a patient perspective, and increased the risk for poor technical quality.

Ethical considerations

The study was approved by the Regional Committee for Medical and Health Research ethics, The Norwegian Medicines Agency, and the hospital's research committee, and was registered at Clinicaltirals.gov (NCT01818583). All study patients provided their informed written consent.

Statistics

We assumed that 50% of the patients would convert spontaneously to sinus rhythm and postulated that potassium infusion would increase the rate of conversion to 75%. With a power of 80% to detect this increase, at least 58 patients were predicted to be required in both the KCl and placebo groups to detect a significant treatment effect. Continuous variables were presented with median and IQR, and categorical variables were presented as count and percentage. Statistical differences between any two groups were examined with a 2-sample Wilcoxon rank-sum (Mann-Whitney) test applied for continuous variables and Pearson's Chi-squared test for categorical variables. Differences in time to conversion between groups were also assessed with Kaplan-Meier plots, a log-rank test, and Cox proportional hazard regressions.

The primary analysis was *intention-to-treat* and involved all randomized patients included. All patients who were randomized into either group were considered to be in the *intention-to-treat* sample (n=113). From these, 5 patients were excluded from the placebo group and 11 patients were excluded from the KCl group. All other patients for whom the protocol was followed (n=97) were included in a prespecified *per-protocol* analysis with the same endpoints as for the *intention-to-treat* analysis. Statistical analyses were performed using Stata/SE version 14.2 (Stata Corporation). A P-value ≤0.05 was considered statistically significant.

The basic science study – article III

Animal model

We used adult rats (~10 weeks old and ~300 g) as an animal model in our experiments. Although there are significant differences between rats and humans, the use of rodent animal models has provided a very useful insight into human cardiac physiology and disease. Small rodents are easier to handle and accommodate, and they have lower maintenance cost than larger animals. Cardiac excitation, contraction, and relaxation of small rodents and humans share many similarities and both groups express proteins with similar functions and roles (98). However, there are important differences between rodents and humans regarding cardiovascular physiology. The ventricular APD in small rodents is much shorter than humans, as the mouse AP has a rapid repolarization and lacks a prominent plateau phase unlike human cardiomyocytes (99). During relaxation (diastole), the fraction of Ca^{2+}

transported by SERCA is 92% and by NCX 7% in rats, while in humans this fraction is 77% and 23%, respectively (100, 101). These differences require careful interpretation and can limit translation of rodent studies to humans.

Isolation of cardiomyocytes

We isolated cardiomyocytes from the left and right atria separately as well as from the left ventricle using a Langendorff setup. On days with experiments, we isolated cardiomyocytes and used the cells within 10 hours. Isolated cardiomyocytes have several advantages such as the ability to select cells from different parts of the heart, and they are appropriate for experiments aiming to visualize cellular structure and the precise localization of intracellular molecules. Isolation of high-quality cardiomyocytes is a crucial factor for successful experiments, and there are several factors that can affect the procedure. Important factors that can affect the result of the cardiomyocyte isolation include the use of decontaminated equipment, correct solutions, careful mounting of the heart, good perfusion, prevention of air bubbles entering the aorta, correct temperature (36-37 ˚C), and pH, correct calculation of collagenase concentration in the perfusate, and duration of the perfusion. These factors ultimately affect the number and quality of the cells (102). We applied 200 U/mL collagenase type II (Worthington Biochemical, Lakewood, NJ, USA) for digestion during isolation. Since the collagenase batches do not have standardized enzyme activity or mixture of associated proteases, we tested each new batch on hearts from adult rats.

Field-stimulation of isolated cardiomyocytes

We triggered Ca^{2+} transients in the cardiomyocytes by field stimulation at 1 Hz through two platinum electrodes with biphasic electrical pulses. The frequency of 1 Hz is much slower than the physiological resting heart rate in rats which is between 250-493 beats per minute (98). The contraction of the cardiomyocytes was recorded by a video-edge detection system. The electrical pulses are thought to create a brief electrical field that triggers the activation of LTCC since the channel activity is voltage -dependent. This activation of LTCC is independent of Na^{+} current as opposed to initiation of AP *in vivo* (103). We executed the protocol for one cardiomyocyte per plate. We applied laminin-coated glass coverslips for adhesion of the cardiomyocytes. Laminin is a glycoprotein and a component of the basal lamina. By applying this technique, the contractile properties of the cardiomyocytes could be affected, since

the cells meet little resistance in the cell bath during contractions. This is different from the *in vivo* situation were the cardiomyocytes are connected to each other and their contractions are affected by the preload and afterload of the heart. Thus, our results cannot directly be transferred to the real-life situation.

The cardiomyocytes were stained with fluo-4 acetoxymethyl (AM) ester. This fluorescent indicator (compound that absorbs light photons at a certain wavelength and emits photons at a longer wavelength) is cell permeable and the cardiomyocytes can therefore get loaded by incubating them with this indicator. Once in the cytosol, cellular esterases remove the lipophilic groups (AM esters), making the fluo-4 membrane impermeant. The fluorescent indicator will therefore get trapped and accumulate inside the cell, making it less likely to leak outside the cell. However, a disadvantage of AM esters is that they can accumulate inside intracellular compartments making the fluorescent dye less sensitive to $[Ca^{2+}]_i$ (104). This would be a problem especially if the dye would leak into the mitochondria, which constitutes about 35% of the cell volume (105). Leakage of dye into intracellular compartment could influence the registered Ca^{2+} transients and lead to artefacts. To avoid this, we loaded the cardiomyocytes at room temperature, which is thought to slow the loading speed and potentially allow more time for de-esterification (104). The loading time in our experiments was 10 minutes. When the cardiomyocytes are excited by light of a specific wavelength, fluo-4 shows a rapid increase in fluorescence intensity when binding to Ca^{2+}.

T-tubule imaging in isolated cardiomyocytes

We visualized the t-tubules applying the fluorescent indicator di-8-amino-naphthyl-ethenyl-pyridinium (di-8-ANNEPS) (Invitrogen, Paisley, UK), which is a membrane binding molecule. It is less susceptible to internalization than other similar dyes, permitting extended observation and is less likely to label the membranes of intracellular compartments such as mitochondria or SR. The cell suspension was loaded with di-8-ANNEPS for 20 minutes and imaged using a confocal microscope (LSM 710, Zeiss, Jena, Germany, pinhole=0.9 μm) with a 60× magnification objective. In another set of experiments, the t-tubule labeling was paired with Ca^{2+} imaging and/or electrophysiological measurements. T-tubules were stained with di-8-ANEPPS, as described above, or with CellMask Orange (1:1000 dilution; Thermo Fisher Scientific, Waitham, MA, USA; C10045) for 10 minutes. Confocal images were

obtained with an LSM 510 confocal microscope (Zeiss), with a 40× magnification objective and pinhole set to 1 Airy unit (80 μm). In order to limit cell damage, the laser power and scanning time were reduced to a minimum.

Detubulation

In order to investigate whether untubulated ventricular cardiomyocytes would display similar results as untubulated atrial cells, we detubulated the ventricular cells. To achieve this, we used a technique described by Kawai et al (106). In this procedure we first apply formamide which is cell permeable. When the cell suspension with the ventricular cardiomyocytes is exposed to formamide, the osmolality of the solution is increased (from about 286 to about 1780 mosmol/kg H_2O). At first, this increase in osmolality leads to efflux of water (water moves out of the cells and into the surrounding solution), which makes the cell volume to decrease. After a while, the cell volume normalizes in the presence of formamide. After reapplication of the control solution/removal of formamide, the intracellular concentration of formamide is initially high, meaning that water will enter the cell. This causes a rapid cell swelling (the osmotic shock) that breaks the t-tubules from the cell membrane. Previous experiments have shown that over 80% of ventricular cardiomyocytes are detubulated after formamide treatment. Over time, formamide will leave the cell down its concentration gradient, water will follow, and cell volume and shape will normalize (106, 107).

Patch clamp experiments

We applied whole-cell patch clamp experiments including both current and voltage protocols in order to study the effects of low $[K^+]_e$ on the activity of NKA and NCX in ventricular cardiomyocytes, as well as in tubulated and untubulated atrial cardiomyocytes.

The patch clamp technique is a powerful technique which allows us to measure either the transmembrane voltage or the currents flowing across membranes using a glass micropipette. The glass micropipette (outer diameter 1-3 μm) is positioned to form a tight electrical seal with the cell membrane via strong chemical interactions between the microelectrode glass and the cell membrane. The connection between the micropipette and the cell membrane is called a gigaohm-seal because of the high electric resistance. The tip of the microelectrode is filled with a solution according to the planned recordings. After forming a seal between the fluid-filled microelectrode

and the cell membrane, suction is applied in the microelectrode glass and consequently a rupture in the membrane is created, which allows the microelectrode solution to have direct access to the inside of the cell (whole cell patch clamp). By voltage clamp, we control the membrane potential while simultaneously recording the current flowing across the membrane. Alternatively, current-clamp mode can be employed, where we control the current while simultaneously recording the electrical voltage across the cell membrane. In order to accomplish successful patch clamp experiments, it is vital that the isolated cardiomyocytes are viable and of good quality to enable seal formation. In addition, electrical noise should be minimized by effectively grounding all instruments (108).

Measurement of intracellular Na^+-concentration

The sodium-sensitive fluorescent indicator SBFI (sodium-binding benzofurzan isophthalate) is used to measure $[Na^+]_i$ in dual wavelength excitation ratio mode, where the ratio of fluorescence intensities is used to determine the $[Na^+]_i$. The cardiomyocytes are excited at 340 nm and 380 nm, while the emission is measured at 500 nm. However, binding of SBFI to intracellular proteins or membranes cause a shift of excitation and emission spectra. Baartscheer et al. showed that SBFI applied in dual emission mode provided a more sensitive and specific method to measure small changes in $[Na^+]_i$ in single cardiomyocytes down to $[Na^+]_i$ of about 1 mmol/L (109). We assessed the $[Na^+]_i$ in myocytes loaded with SBFI AM as described in (110).

Immunochemistry

We applied immunolabeling of intact atrial and ventricular tissue (immunohistochemistry) and isolated ventricular and atrial cardiomyocytes (immunocytochemistry) to look for the expression of α_1 and α_2 NKA isoforms as well as the expression of NCX in both the surface membrane and t-tubules.

The principle of immunohistochemistry and immunocytochemistry is to apply specific antibodies to determine the cellular location of proteins of interest in tissue sections (111) or location of proteins in isolated cells, respectively. In order to locate a protein or a pump of interest, we apply a specific primary antibody which binds to the protein or the pump (the antigen) at the epitope (site on the protein or pump at which the binding occurs). We then apply a specific secondary antibody coupled with a fluorochrome which binds to the primary antibody, which can then be visualized by fluorescence microscopy. Several fluorochromes can be coupled with the secondary

antibody and several secondary antibodies can bind to the primary antibody. Furthermore, several primary antibodies can bind to one cell or sub-cellular protein. In this way, a substantial amplification of fluorescence signaling permits detection of modest presence of antigen.

In our experiments, we applied a primary antibody against the α_1 and α_2 NKA isoforms as well as NCX. The specificity of the immunolabeling is crucial and depends on the specificity of the antibodies. We reduced the unwanted unspecific binding by incubating the cells with goat serum to reduce the number of regions of the pumps available for binding.

Mathematical modelling

We applied a mathematical model of the rat ventricular cardiomyocyte to study the t-tubular NKA-NCX crosstalk in driving Ca^{2+} overload during hypokalemia (112). By performing cellular experiments, we can study transmembrane ion channels, pumps, transporters, and intracellular processes like the occurrence of EADs and DADs. However, it is difficult to study the interactions between several of these factors simultaneously. Mathematical modelling makes this possible. The quality of data we obtain from mathematical modelling depends very much on the parameters from our cellular experiments. Thus, the uncertainties and limitations associated with our cellular experiments will affect the results from the mathematical modelling. One of the strengths of mathematical modelling is that it helps us to make different hypotheses and then to test our hypotheses by alterations of many different factors and to direct experiments to confirm these speculations.

Western blot and quantitative real-time polymerase chain reaction

Western blotting is often used to separate and detect specific proteins in a sample of tissue homogenate. In this procedure, a mixture of proteins is separated based on molecular weight, and thus by type, through gel electrophoresis. After separating the protein mixture, it is transferred to a membrane (blotting). The data produced by western blotting is semi-quantitative, since it provides a relative comparison of proteins levels, but not an absolute measure of quantity (113).

We applied western blotting to explore the expression of KCNA5/K_v1.5 in rat left atrial and left ventricular tissue. We used anti-K_v1.5 as the primary antibody and an anti-rabbit horseradish peroxidase-conjugated secondary antibody.

Polymerase chain reaction (PCR) is a technique by which a DNA or RNA sequence can be copied by applying oligonucleotide primers and DNA polymerase. The basic goal of real-time PCR is to precisely distinguish and measure (monitors the progress of amplification in real time) specific nucleic acid sequences in a sample even if there is only a very small quantity (114). The principle of quantitative PCR consists of three steps: conversion of RNA to complementary DNA (cDNA) by reverse transcriptase, the amplification of cDNA by PCR, and the detection and quantification of amplified products referred as amplicons (115).

We extracted mRNA from defrosted homogenates from rat right atrial and left ventricles and then applied quantitative PCR with TaqMan assays (Applied Biosystems, Foster City, USA) to quantify the expression of KCNA5.

Ethical considerations

This study was approved by the Norwegian Animal Research Authority (FDU application number 7786) under the Norwegian Animal Welfare Act, and conformed with Directive 2010/63/EU of the European Parliament.

When we apply an animal model, it is our responsibility and very important to *replace* animal models when possible, to *reduce* the number of animals used, and to *refine* the experiments in order to minimize the pain and distress of the animals. At our institute there is continuous optimization of surgical procedures, protocols, and animal handling. Furthermore, we do our best to minimize the number of animals used by planning and coordinating our experiments.

Statistics

All data were tested for normality of distribution using a Shapiro-Wilk test. Normally distributed data were compared with Student's *t*-test or ANOVA, as appropriate. Non-normal distributions were examined with a Wilcoxon signed rank test or Kruskal-Wallis Analysis on Ranks. Two-factor comparisons were performed with two-way ANOVA. Differences in proportions were determined by z-test. P values < 0.05 were considered statistically significant. All data were analyzed by Sigmaplot software (Systat Software, Chicago, USA) and are presented as mean ± SE.

SUMMARY OF RESULTS

Article I: Electrolyte imbalances in an unselected population in an emergency department: a retrospective cohort study

In this study, we investigated EIs in the ED. In total, 62 991 ED-visits involving 31 966 patients were registered between 2010 and 2015. The median age of all patients was 69 years (IQR, 51-82 years), most of them were older (65.5% above 60 years), and they were mostly (70.8%) referred for medical and not surgical reasons. EIs were mostly mild, and the most common EI was hyponatremia [24.6% (glucose-corrected)]. Among the patients who were admitted (70,6%), the median LOS was 3 days (IQR 1-5 days), with 21.3% admitted for 5 days or longer. Patients with increasing severity of EI had longer LOS than patients with normal electrolyte measurements. Among admitted patients, there were 20.5% readmissions within 30 days from discharge. Hyponatremic patients accounted for 23.6% of these readmissions. Hypocalcemia and hypomagnesemia were also associated with readmissions. Dysnatremia, dyskalemia, hypercalcemia, hypermagnesemia, and hyperphosphatemia were associated with increased in-hospital mortality, and all EIs except hypophosphatemia were associated with increased 30-day and 1-year mortality compared with patients not having the specific EI.

Article II: Potassium infusion increases the likelihood of conversion of recent-onset atrial fibrillation – a single-blinded, randomized clinical trial

In this study, we studied the effect of potassium infusion in conversion of recent-onset AF or AFl to sinus rhythm. We included a total of 113 patients between 2013 and 2017, of whom 53 were allocated to the placebo group and 60 to the KCl group. Over 90% of the patients had AF and baseline characteristics were mainly similar between the groups. KCl infusion had no significant effect regarding time to or frequency of conversion to sinus rhythm compared with the placebo group. Ten patients had to prematurely stop the KCl infusion because of pain at the infusion site. After excluding these patients, the *per-protocol* analysis showed that significantly more patients converted to sinus rhythm in the KCl group with the fastest infusion rate (15 mmol/h) compared with the placebo group (82 % vs 52%, P=0.018).

Furthermore, KCl-infused patients who achieved an above-median hourly increase in plasma-potassium (>0.047 mmol/h) exhibited a significantly higher conversion rate, compared with both the placebo group and the group with below-median change in plasma-potassium. However, there was no difference in time to conversion compared with placebo. The group which received KCl at the highest infusion rate, exhibited a significantly greater increase in plasma-potassium than patients who received 12 mmol/h or 9,4 mmol/h. In addition, patients that converted to sinus rhythm had a significantly higher change in both serum- and plasma-potassium per hour than those that did not convert to sinus rhythm.

Article III: Hypokalemia promotes arrhythmia by distinct mechanisms in atrial and ventricular myocytes

In this study, we investigated the pro-arrhythmic mechanisms of hypokalemia on cardiomyocytes. We performed experiments in isolated rat cardiomyocytes when extracellular potassium was reduced from 5.0 to 2.7 mmol/L. These experiments were supported by mathematical modeling studies. We observed that ventricular cardiomyocytes and a subpopulation of atrial cardiomyocytes exhibited a biphasic change in Ca^{2+} transient amplitude, with subsequently increased incidence of spontaneous Ca^{2+} waves. Conversely, some atrial cardiomyocytes showed a steady-state reduction in Ca^{2+} transient amplitude (monophasic change) without an increased incidence of spontaneous Ca^{2+} waves.

All ventricular and a group of atrial cardiomyocytes exhibited t-tubules. These cells showed a biphasic response to hypokalemia, whereas the untubulated atrial cells showed a monophasic response. Furthermore, after de-tubulating the ventricular cardiomyocytes the monophasic response was reproduced. Thus, our data indicated that the presence of t-tubules promoted the biphasic response.

We showed that the NKA-α_1 isoform was presented in the sarcolemma and the t-tubules of the ventricular and tubulated atrial cells as well as in the sarcolemma of the untubulated atrial cells. However, while the expression of NKA-α_2 isoform was robustly present in the ventricular cells, it was very low in both tubulated and untubulated atrial cells. Furthermore, blockade of NKA-α_2 isoform inhibited the biphasic response in ventricular cells, but not in untubulated atrial cells. This finding suggests that the presence of the NKA-α_2 isoform in t-tubules was not a requirement

for a biphasic response to hypokalemia. Similarly, measurement of NKA activity did not identify differences between tubulated and untubulated cells which could account for distinct responses to hypokalemia.

Next, we examined the localization of NCX and showed similar dense NCX localization in the sarcolemma and t-tubules in both ventricular and atrial cells. In addition, during hypokalemia there was a reduction in NCX-mediated Ca^{2+} efflux with subsequent elevation of SR Ca^{2+} content. This finding suggests that although no change in global cytosolic Na^{+} levels was detected, NKA inhibition during hypokalemia leads to Na^{+} accumulation which was sensed by t-tubular NCX, probably by crosstalk between NKA and NCX at the same localization. Conversely, in untubulated atrial cells NCX-mediated Ca^{2+} efflux was not slowed during hypokalemia, and thus any local elevation of Na^{+} was not sensed by NCX. Consequently, Ca^{2+} efflux lead to reduced SR Ca^{2+} content. These findings were also verified by employing a mathematical model.

In ventricular and tubulated atrial cells hypokalemia hyperpolarized the resting RMP and increased the occurrence of both DAD and EADs. The EADs occurred during the plateau phase of the prolonged AP and were Ca^{2+}dependent and associated with LTCC reopening. In contrast, untubulated atrial cells showed no significant increase in DADs. They had significantly shorter AP due to larger outward of K^{+} current which we observed resulted from the presence of I_{Kur}. However, EADs were frequent and, unlike the tubulated cells, were not Ca^{2+} dependent but occurred during the phase-3 of the AP as the membrane potential rapidly repolarized. These EADs were triggered by non-equilibrium I_{Na}. These findings suggest that the presence of I_{Kur} and a short AP in untubulated hypokalemic cells increased the Na^{+} channel availability. This led to EADs in phase 3 induced by non-equilibrium reactivation of the fast I_{Na}. These findings were also verified in a mathematical model of the human atrial cardiomyocyte.

DISCUSSION OF THE RESULTS

In this thesis, the aim was to investigate EIs with special focus on hypokalemia, how they may induce arrhythmia, and whether potassium infusion could hold therapeutic promise for patients with atrial fibrillation and atrial flutter, two common arrhythmias.

Article I describes the first study of a non-selected, adult ED patient population describing the prevalence of all clinically important EIs according to type, severity, and associations with the outcomes LOS, readmission, and mortality. Nearly half of the patients presented with at least one type of EI, and EI was associated with longer LOS, increased readmissions, and mortality. To our knowledge, this study was the first to report serum-sodium and glucose-corrected serum-sodium, in addition to comparing albumin-corrected with free calcium in an unselected population admitted to the ED.

Article II reports the first study to investigate the effects of KCl infusion in patients with recent-onset AF or AFl and plasma-potassium ≤4 mmol/L. Our results indicate that potassium infusion in this setting may increase the likelihood of conversion of ROAF to sinus rhythm.

In article III we showed that hypokalemia can induce triggered activity in ventricular and atrial cardiomyocytes by distinct mechanisms. This was the first study describing the triggered activity in hypokalemic atrial cardiomyocytes. Furthermore, the distribution of NKA and NCX in atrial cardiomyocytes in addition to the absence of I_{Kur} in tubulated atrial cells are other novel findings.

Prevalence of electrolyte imbalances and their association with age

The reported prevalence of EI in previous studies among ED patients was generally in accordance with our results from article I. The wide intervals of prevalence, such as those previously reported in hyponatremic patients (2.3-44%), are most likely due to different definitions of EIs, the time of the measurement (e.g., at admission, during hospitalization), and the study population (i.e., young vs. older patients). In our study, serum-magnesium and serum-phosphate were measured only when decided

by the attending physician. Thus, the prevalence of dysmagnesemia and dysphosphatemia was unknown.

We reported both serum-sodium and glucose-corrected serum-sodium since hyperglycemia has a diluting effect on serum-sodium, and thus the actual value of serum-sodium in hyperglycemic patients is glucose-corrected sodium (93). Furthermore, the superiority of glucose-corrected serum-sodium over measured serum-sodium to predict mortality has been demonstrated, and it is therefore suggested that glucose-corrected serum-sodium should be considered in studies analyzing sodium in serum (116).

Most studies report calcium levels either as plasma-calcium or albumin-corrected calcium. However, several studies have shown that the measurement of plasma-free calcium is superior to plasma-calcium and albumin-corrected calcium (117-122). Therefore, we reported both albumin-corrected calcium and free calcium. We could find only one study investigating the frequency of plasma-free calcium among ED patients, and our results were in accordance with this study (123). Furthermore, we found that there was a discrepancy between these two calcium-measurements, with albumin-corrected calcium classifying greater proportions of patients as hypercalcemic than free-calcium, which classified a greater proportion of patients as hypocalcemic. In other words, knowing that plasma-free calcium is superior, albumin-corrected calcium overestimated the actual calcium value.

As demonstrated in previous studies, the majority of our patients with EIs were over 60 years old, indicating that older patients have a higher risk for developing EI (4, 5, 124). With aging the renal function decreases, and there are neurohumoral changes that increase the risk for EIs (1). Furthermore, aging is associated with increasing prevalence of morbidities (malignant, cardiovascular, and pulmonary diseases), and greater usage of several drugs that can contribute to EIs (1, 4, 124).

Hospital length of stay and readmission among patients with electrolyte imbalance

Dysnatremia is known to be associated with longer LOS (17, 125). Accordingly, our results (article I) showed that median LOS for patients with dysnatremia was increased 3-6 days depending on the severity of the dysnatremia compared with normonatremic patients who had a median LOS of 2 days.

We demonstrated a similar increase in median LOS in patients with hypokalemia depending on its severity. However, the median LOS for hyperkalemia was 4 days independent of the severity. This could be due to relatively few patients with moderate and severe hyperkalemia and that most of these patients probably had severe renal failure and were transferred to a nearby university hospital for dialysis. Additionally, high mortality in these patients reduced the LOS. We found only a single retrospective study of dyskalemia among ED patients. In line with our findings, this study demonstrated longer LOS among patients with dyskalemia but without defining the severity of dyskalemia. Furthermore, the mean LOS for normokalemic patients was substantially longer than at our hospital (12).

In patients with hypercalcemia measured either as free or albumin-corrected calcium, median LOS was increased compared with normocalcemic patients. This finding was in line with a previous study where serum-calcium was measured and corrected for albumin (16).

Our patients who had serum-magnesium and serum-phosphate measurements were older than the other patients and could have malnutrition, weight loss, arrhythmias, renal failure, and other EIs. Patients with moderate hyperphosphatemia had longer LOS in comparison with normophosphatemic patients. This difference could reflect renal failure, which is a common cause of hyperphosphatemia, and also was shown in a retrospective study of ED patients at admission (25).

Besides some studies describing an association between hyponatremia and readmission (17-19, 26), the frequency of readmissions among patients with other EIs is unknown. We defined readmission as a new admission within 30 days post-discharge. Our multivariate analysis adjusted for age, sex, and comorbid conditions, showed that only hyponatremia, hypocalcemia, and hypomagnesemia were significantly associated with increased probability for readmission. Hypernatremia was not associated with increased likelihood for readmission, probably because of the high in-hospital mortality among these patients. Similarly, we did not observe an increased odds ratio for readmission with increasing severity of EIs, most likely due to higher mortality in patients with the most severe EIs.

Electrolyte imbalances and mortality

We found that dysnatremia, dyskalemia, hypercalcemia, hypermagnesemia, and hyperphosphatemia were associated with increased in-hospital mortality, whereas all EIs except hypophosphatemia were associated with increased 30-day and 1-year

mortality. Except for dysphosphatemia, we observed that the greater the severity of the EIs, the higher the risk of mortality. These results are in accordance with previous studies (9, 11, 12, 22, 25, 123, 126-128).
Elderly patients are more susceptible to severe hypernatremia which increases the rate of mortality. This increased vulnerability reflects an increase in the proportion of body fat, and a decrease in total body water as there is a decrease in the perception of thirst and reduced capacity of the kidneys to concentrate urine (126). Hyperkalemia has the potential for causing fatal arrhythmias (129), and hypercalcemia, which is a common finding among cancer patients, indicates poor prognosis (130). Approximately 50% of hypercalcemic cancer patients die within 30 days of diagnosis (131). Chronic kidney disease is prevalent, especially in older patients with other co-morbidities, and is the most common cause of hyperphosphatemia and hypermagnesemia (132). Moreover, it has been shown that hyperphosphatemia is associated with increased all-cause mortality in the general population without apparent kidney disease (133). Hyperphosphatemia can lead to adverse cardiovascular outcomes and mortality, particularly in patients with chronic kidney disease, by promoting endothelial dysfunction, vascular stiffness, and vascular calcification (133). Hypermagnesemia, on the other hand, may cause impairment of both cardiac systolic contractility and diastolic relaxation in addition to serious arrhythmia (134).

From the abovementioned EIs we chose to study the association between potassium and arrhythmia. More specifically, if potassium infusion could promote conversion of recent-onset AF and AFl to sinus rhythm (article II) and how hypokalemia may promote arrhythmia in cardiomyocytes (article III).

Potassium infusion and conversion to sinus rhythm

Our results (article II) from the placebo group were in accordance with previous studies showing that about 50% of patients with ROAF convert spontaneously to sinus rhythm within 48 hours (89, 135). Moreover, it has been shown that antiarrhythmic drugs increase the probability of conversion of ROAF to sinus rhythm to approximately 70% within 12 hours (91). In line with this, almost 70% of our AF patients who received KCl infusion with a rate of 15 mmol/h converted to sinus rhythm within a median infusion time of 5.3 hours.

However, the gold standard of prospective clinical trials is *intention-to-treat* analysis, i.e., that all randomized patients are included in the final statistical analyses regardless of whether they received intended treatment or not. If a medical treatment is truly effective, an intention-to-treat analysis will provide an unbiased estimate of the efficacy of the treatment at the level of adherence in the study. After *intention-to-treat* analysis, we found no significant effect of KCl infusion with regards to time or frequency of conversion to sinus rhythm compared with the placebo group. This either indicates that KCl has no effect in promoting conversion of ROAF to sinus rhythm or that the study included too few patients or there were too many dropouts to have the statistical power to detect an effect. For instance, there may have only been a positive effect of KCl within in a subgroup or subgroups of patients. However, an argument against *intention-to-treat* analysis is that heterogeneity might be introduced if noncompliant patients, dropouts, and compliant subjects are mixed together in the final analysis. Furthermore, it could be argued that if patients who actually did not receive the investigated treatment are included as patients who received treatment then it indicates very little about the efficacy of the treatment. *Per-protocol* analysis may therefore give important additional information since such an analysis is a comparison of treatment groups that includes only those patients who completed the treatment originally allocated. Our *per-protocol* analysis showed as mentioned above that the subgroup of patients receiving KCl with a rate of 15 mmol/h had a significantly and substantially higher conversion rate in patients with ROAF than the placebo group (82 vs. 52%), indicating that a positive effect of KCl was dependent on the rate of infusion, i.e. a dose-dependent effect. Further support of this hypothesis was the observation that an above-median hourly increase in plasma-potassium exhibited a significantly higher conversion rate (91.7%). Moreover, patients who converted to sinus rhythm had a significantly higher change in both serum- and plasma-potassium per hour than those who did not convert to sinus rhythm. The final support of the hypothesis that the positive effect of KCl infusion is dependent on an hourly increase in plasma-potassium is that the significantly increased likelihood of conversion to sinus rhythm was independent of the total amount of KCl infused.

Another interesting finding was that KCl significantly promoted conversion to sinus rhythm in patients without a history of AF compared with the placebo group, also in the *intention-to-treat* analysis. This indicates that the fewer the episodes of AF, the

higher the success rate for conversion to sinus rhythm, probably because these patients have less atrial remodeling.

Finally, our results indicate that the lower the plasma-potassium at admission, the higher the success rate of KCl infusion for conversion to sinus rhythm. In fact, all patients with plasma-potassium <3.8 mmol/L at baseline converted to sinus rhythm.

Taken together, our results may indicate that among patients with ROAF, a sufficiently high increase in plasma-potassium may increase the likelihood of conversion to sinus rhythm, independent of the amount of KCl, and that KCl has a more profound effect in AF naïve patients and in those with the lowest plasma potassium levels. Thus, KCl may be as effective as other anti-arrhythmic drugs, without their risk or contraindications and without the burden of additional resources related to direct-current cardioversion therapy.

With regards to AFl, we could not make any conclusions since we had only nine patients of whom two converted to sinus rhythm and both were in the placebo group. It is possible that our results would be strengthened if we had not included patients with AFl, since these patients have another arrhythmogenic mechanism than AF.

Plasma-potassium versus serum-potassium

Plasma or serum can be used to assess circulating potassium concentration using an ion-selective electrode (35). Normally, serum-potassium as reported by Nijsten et al. is 0.36 ± 0.18 mmol/L higher than plasma-potassium due to potassium release from platelets during the clotting process when preparing serum from blood (37). Thus, potassium measured in serum can lead to pseudohyperkalemia, i.e. falsely high potassium concentration characterized by marked elevation of serum-potassium without clinical evidence of hyperkalemia (37, 136, 137). Therefore, in our study (article II), we chose to measure plasma-potassium at inclusion. At the follow-up consultation, we measured serum- and plasma-potassium simultaneously and found that the median difference was 0.3 mmol/L in accordance with the previous study by Nijsten et al (37). However, in contrast to our patients who were asymptomatic and without arrhythmia, the measurement of potassium in the previous study was in 182 healthy patients who had experienced trauma.

Potassium infusion and the risk of hyperkalemia

The kidneys are mainly responsible for maintaining total body potassium constant by increasing the potassium excretion as the circulating potassium levels increase. The healthy kidney has a robust capacity to excrete potassium, and under normal conditions most persons can ingest enormous quantities of potassium without development of clinically significant hyperkalemia (31). Thus, most hyperkalemia cases are due either to abnormal shifts of potassium from the intracellular compartment to the extracellular compartment (e.g., rhabdomyolysis, tumor lysis) or dysfunction of renal potassium excretion (29). In patients with chronic kidney disease, hyperkalemia does not develop until the GFR falls below 15–20 mL/min (33). An increase in plasma potassium to above 5.5 mmol/L is uncommon until over 90% of the renal function is lost and GFR is <20 mL/min (34).

It could be argued that administrating KCl in normokalemic patients could lead to severe hyperkalemia and lethal arrhythmia, especially when there was no comparable previous study. However, in our study, no patients had estimated GFR <30 mL/min since this was an exclusion criterion, and no patients developed clinically significant hyperkalemia despite an infusion rate of 15 mmol/h and administration of up to 366 mmol KCl. This finding verifies the above-mentioned pathophysiological considerations, and indicates that potassium infusion could be safe when the estimated GFR is above 30 mL/min.

Potassium chloride is known to be vesicant. To avoid this side-effect, our staff were instructed to position the catheter properly in the antecubital vein and to make sure that the infusion bag was well-mixed by inverting it ten times after adding KCl. Despite this, we were surprised that 12 of our patients reported pain at the infusion site, which was alleviated when the infusion was stopped. However, it is possible that the infusion bag was not well mixed, and in a few patients the peripheral vein catheter was placed on the back of the hand, not following the study instructions. Therefore, we recommend using a pre-mixed potassium infusion.

Magnesium infusion and conversion to sinus rhythm

Our trial is the first to investigate a potentially beneficial effect of potassium infusion to convert AF and AFl to sinus rhythm. However, similar trials have been done with magnesium infusion. It is, however, still not clear if magnesium infusion can promote the conversion of AF to sinus rhythm, as the findings of two previous meta-analyses

were contradictory (138, 139). The contradictory results in these meta-analyses could be due to different causes of AF and different sample size. Only a few studies with small sample size were found and included. The trials included investigated different doses of intravenous magnesium and compared magnesium with different antiarrhythmic drugs. Furthermore, with regards to adequate randomization and blinding, most of the included studies were found to have medium quality. In addition, meta-analysis can overestimate the clinical efficacy of magnesium since negative studies are usually underreported. Taken together, we need more clinical trials looking into the effect of magnesium on conversion of AF.

It is known that magnesium depletion reduces intracellular potassium concentration due to renal potassium wasting (38). For this reason, patients in the KCl group who at the time of inclusion had serum-magnesium levels ≤0.8 mmol/L, also received magnesium infusion. However, few patients had serum-magnesium ≤0.8 mmol/L and therefore received magnesium infusion. Furthermore, the median serum-magnesium level at baseline was >0.80 mmol/L in all groups, and with regards to conversion to sinus rhythm, there was no significant difference between those patients who received magnesium and those who received only KCl. This suggests that magnesium infusion in a subset of patients did not affect the study results significantly.

Glucose infusion and decrease in plasma-potassium

Administration of glucose induces insulin secretion, which can promote cellular uptake of potassium by activating cell-membrane NKA (28). In keeping with this, we observed a small reduction in plasma-potassium in our patients in the placebo group receiving infusion with 50 mg/mL glucose. However, the placebo group's reduction of plasma-potassium was minimal, with unchanged median values and only a small change in IQR from 3.6-4.0 mmol/L to 3.6-3.8 mmol/L. Thus, this small reduction probably had no significant effect on our results.

Heart rate-reducing medications

At admission, our patients with tachycardia received heart rate-reducing medications (beta-blocker, calcium antagonist, or digoxin), which have little converting properties and are not regarded as antiarrhythmic drugs (83). Seventy percent of the patients in the KCl group and 91% in the placebo group received heart rate-reducing medications, mostly the beta-blocker metoprolol. Moreover, a higher proportion of

patients in the placebo group who converted to sinus rhythm received metoprolol compared with the KCl group. Thus, we believe that metoprolol did not contribute to an increased conversion rate in the KCl group.

Post-discharge follow-up

At the follow-up about three months post-discharge, numerically, more patients in the placebo group had documented recurrence of AF [10 (22,3%) vs. 5 (9,5%)], but this difference was not significant. Furthermore, there was no significant difference in serum- or plasma-potassium between the groups. However, our study did not have enough power to detect such differences.

An interesting question is whether continuous oral potassium supplementation will be preventive with regards to recurrence of AF in normokalemic patients with normal kidney function. Unlike intravenous infusion of high-concentrated KCl, oral potassium supplementation will probably not increase circulating potassium as healthy kidneys will increase the excretion of potassium. Theoretically, it would be possible that potassium sparing diuretics could prevent AF by keeping the plasma-potassium in the upper normal range. However, this remains to be shown and hopefully future studies will give an answer.

Electrophysiological effects of potassium

The extracellular concentration, whether it is too high (hyperkalemia) or too low (hypokalemia) and its rate of change, has electrophysiological effects (3). As the plasma-potassium increases, the magnitude of the potassium gradient across the sarcolemma *decreases*, leading to *depolarization* of the RMP. Consequently, the RMP gets closer to the threshold potential which enhances the excitability of the cell. However, with further increase in plasma-potassium the excitability is reduced, probably due to excessive depolarization ("inactivation") and thereby reduced conduction and triggered activity (140). This applies to all cardiac muscle, but atrial myocytes are more sensitive than ventricular myocytes to an increase in plasma-potassium, whereas specialized fibers such as in the sinoatrial node, internodal tracts, and the bundle of His are practically resistant. Thus, the reduced excitability and conduction occur at lower plasma-potassium in atrial myocytes than the other types of cardiac cells (141, 142).

Considering these electrophysiological effects of potassium, our observation of conversion of ROAF to sinus rhythm upon increasing plasma-potassium could be

explained by a transient conduction acceleration which could terminate a re-entrant circuit in the atria. Alternatively, reduced conduction and triggered activity at higher plasma-potassium levels in atrial myocytes could promote conversion to sinus rhythm. Thus, an increase in plasma-potassium may either eliminate a re-entrant circuit or suppress an ectopic focus in the atria, letting the hyperkalemia-resistant sinoatrial node to reestablish a sinus rhythm (77, 143).

In article III, we studied the arrhythmogenic effects of potassium in rat cardiomyocytes. Indeed, we found distinct arrhythmogenic mechanisms of hypokalemia in atrial and ventricular cardiomyocytes. These findings, which are discussed and elaborated upon below, indicate that increasing circulating plasma levels may favor conversion to sinus rhythm via a direct cellular action.

Hypokalemia's effect on Ca^{2+} transients and waves in ventricular and atrial cardiomyocytes

We simulated hypokalemic conditions in isolated rat cardiomyocytes by lowering the $[K^+]_o$ from 5.0 mmol/L to 2.7 mmol/L. At first, hypokalemia results in hyperpolarization of the RMP, which in turn stimulates the NCX to extrude Ca^{2+} and thereby a reduction in SR Ca^{2+} content. Consequently, this reduces the Ca^{2+} transient amplitude in both atrial and ventricular cardiomyocytes (73, 144-146). At the same time, hypokalemia also inhibits NKA, which results in the accumulation of Na^+ intracellularly, consequently stimulating the reverse NCX, leading to an increase in SR Ca^{2+} content and release. This results in the subsequent increase in Ca^{2+} transient amplitude and the display of the biphasic response, which is in accordance with previous findings in ventricular rat cardiomyocytes (73). However, in our study (article III), in addition to displaying the same findings in tubulated atrial cells as in ventricular cells, we also showed that t-tubules are required for the biphasic response, as it was absent in untubulated atrial and de-tubulated ventricular cells. Furthermore, we showed that the increase in Ca^{2+} transient amplitude was associated with an increased incidence of Ca^{2+} waves and DADs following the cessation of the cell stimulation. The increase in the incidence of Ca^{2+} waves and DADs was displayed in biphasic (tubulated) atrial and ventricular cells, but not in the monophasic (untubulated) atrial cells. These are novel findings and propose that the presence of t-tubules increases the frequency of Ca^{2+} waves and DADs, and thus is pro-arrhythmic

in hypokalemic exposed cardiomyocytes, with differences in two subpopulations of atrial cardiomyocytes.

The localization of NKA and NCX and their cooperation in t-tubules

As we observed that hypokalemia increased the frequency of Ca^{2+} waves in tubulated cardiomyocytes, we looked further into this by investigating the localization and activity of NKA and NCX.

It is known from our experiments and previous work in ventricular cardiomyocytes that the NKA-α_1 isoform is abundant in both the t-tubules and sarcolemma, whereas NKA-α_2 is most prominently localized in the t-tubules (60, 61). However, the localization of NKA was unknown in atrial cardiomyocytes. In these cells, we observed that the NKA-α_1 was present both in the sarcolemma and t-tubules, when they were present, whereas NKA-α_2 was sparsely expressed in the sarcolemma and not expressed in the t-tubules.

So far, we observed a biphasic response to hypokalemia in ventricular and tubulated atrial cells and that NKA-α_2 was present in the t-tubules of the ventricular but not the atrial cardiomyocytes. This indicates that hypokalemia could induce a biphasic response without the presence of the NKA-α_2. We also strengthened this finding by showing that the blockade of this isoform with low-dose ouabain inhibited the biphasic response in ventricular but not tubulated atrial cells. Furthermore, we observed that the NKA current was similarly reduced during hypokalemia in all cell types and was insufficient to elevate global $[Na^+]_i$.

Since the biphasic response could be induced without the presence and activity of NKA-α_2, could it be explained by the presence and activity of NCX? We and others have observed that NCX is densely localized in the sarcolemma and the t-tubules in ventricular cells (147, 148). However, as with NKA, the localization of NCX had not previously been described in atrial cells. We found that NCX was localized both in the sarcolemma and the t-tubules when they were present in these cells. With regards to NCX activity, we found that it was slowed during hypokalemia in tubulated cardiomyocytes but not in the untubulated atrial cells. This indicated that NCX did not sense the local accumulation of Na^+ in untubulated atrial cells and thus Ca^{2+} removal by NCX continued to increase due to the hyperpolarized RMP. Consequently, this increase in NCX activity led to reduced SR Ca^{2+} content, a steady-state reduction in Ca^{2+} transient amplitude and Ca^{2+} waves.

The above-mentioned findings suggested a co-localization and cooperation between t-tubular NKA and NCX, which were also supported by our mathematical modeling and shown to depend on a shared local Na^+ domain (60, 63, 149). It is believed that in this subsarcolemmal domain surrounding NKA and NCX, the concentration of Na^+ is higher compared with the bulk cytosolic concentration of Na^+ (55, 60, 64, 65, 149). This $[Na^+]_i$ gradient is believed to be regulated by the higher abundance of the NKA-α_2 isoform in the ventricular t-tubules, which was in accordance with our findings (61). However, we observed this NKA-NCX cooperation in tubulated atrial cells where the NKA-α_2 isoform was not present. Therefore, the NKA and NCX cooperate within the t-tubules of atrial and ventricular cells and are not dependent only on the NKA-α_2 isoform.

Hypokalemia's different mechanisms of promoting EADs in tubulated and untubulated cardiomyocytes

As mentioned above, when tubulated cardiomyocytes are exposed to hypokalemia, the close interaction between NKA and NCX leads to Ca^{2+} overload. This Ca^{2+} overload increases the propensity of spontaneous Ca^{2+} release, leading to EADs and DADs. However, in ventricular cells, EADs are additionally promoted by a prolonged plateau phase of the AP during hypokalemia, which allows recover of LTCCs from inactivation (71). This prolongation partly results from activation of CaMKII following Ca^{2+} overload, which stimulates voltage-gated Na^+ channels and LTCC, thereby increasing the influx of Na^+ and Ca^{2+} (74). AP prolongation additionally results from inhibition of potassium currents during hypokalemia, as indicated by our steady-state K^+ current measurements. Thus, hypokalemia promotes EADs in ventricular cells by multiple mechanisms.

Conversely, we observed that the EADs in untubulated atrial cells were not calcium-dependent, but were displayed during phase 3 of the AP due to I_{Na}. Considering the important role of I_{Na} in triggering phase-3 EADs, we observed that these events were rapidly blocked by tetrodotoxin treatment which is a I_{Na} blocker. To induce phase-3 EADs, the APD has to be shortened so that the Na^+ channels can reactivate (67, 68). Also, it has been shown that the short APD in mouse ventricular cells (68) or human atrial cells (69, 150) is similarly susceptible to EADs triggered by non-equilibrium I_{Na}.

In line with this, we observed a significantly shorter APD in untubulated atrial cells than in ventricular and tubulated atrial cells. Furthermore, the shorter APD in

untubulated atrial cells was linked to a larger outward K^+ current mainly due to the presence of I_{Kur}, and when inhibited by 4-AP, which selectively blocks I_{Kur} (151, 152), the APD was prolonged. These observations were supported by our mathematical model of the human atrial cardiomyocyte. The application of a model of human atrial cardiomyocytes was a limitation in our study, since it was compared with experiments in rat atrial cardiomyocytes. However, no mathematical model of the rat atrial cardiomyocyte is currently available.

It is known that I_{Kur} is displayed in atrial but not ventricular cardiomyocytes, despite the expression of the channel's RNA (KCNA5) and protein ($K_v1.5$) in both atria and ventricles (153). Accordingly, we found that KCNA5 and $K_v1.5$ were expressed both in atria and ventricles. However, we observed that I_{Kur} was only displayed in untubulated atrial cells. It is not known why this potassium channel is expressed in ventricular cells without displaying the current. It may be that it has another function in ventricular than in atrial cells.

In summary, hypokalemia inhibits the outward potassium currents in ventricular and atrial cardiomyocytes, which prolongs the plateau phase and thereby reactivates I_{Ca}, ultimately triggering phase 2-EADs. In contrast to this, in untubulated atrial cells the APD is abbreviated due to the exclusive presence of I_{Kur}, resulting in phase 3-EADs triggered by reactivation of I_{Na}.

CONCLUSIONS

The main conclusions from this thesis are that EIs are common in patients visiting the ED, and that EIs are associated with increased LOS, readmissions, and mortality. Furthermore, in patients with ROAF and plasma-potassium in the lower normal range, KCl infusion may increase the likelihood of conversion to sinus rhythm if the dose is high enough. Lastly, hypokalemia increases the susceptibility to arrhythmia by distinct mechanisms in atrial and ventricular cardiomyocytes.

Below are answers to the questions mentioned under the chapter "Aims and questions"

Article I

Electrolyte imbalances in an unselected population in an emergency department: a retrospective cohort study

1. Electrolyte imbalances are common in the ED. They are mostly mild, and the most common EI is hyponatremia with the prevalence of 24.6%.

2. Most patients with EIs are older (> 60 years of age) and females (53,5%).

3. Patients with increasing severity of EI have longer LOS compared with patients with normal electrolyte measurements. However, increasing severity of EI is not associated with readmissions, probably because of high mortality among patients with severe EIs. Hyponatremia, hypocalcemia, and hypomagnesemia are associated with increased risk of readmission.

4. Dysnatremia, dyskalemia, hypercalcemia, hypermagnesemia, and hyperphosphatemia are associated with increased in-hospital mortality, whereas all EIs except hypophosphatemia are associated with increased 30-day and 1-year mortality.

Article II

Potassium infusion increases the likelihood of conversion of recent-onset atrial fibrillation – a single-blinded, randomized clinical trial

High dose potassium infusion may increase the likelihood of conversion to sinus rhythm in patients with ROAF and plasma potassium in the lower normal range. Potassium infusion likely has higher efficacy the lower the plasma potassium is and in patients that have experienced fewer AF episodes.

Article III

Hypokalemia promotes arrhythmia by distinct mechanisms in atrial and ventricular myocytes

1. Hypokalemia does promote triggered activity in cardiomyocytes. It leads to hyperpolarization of the membrane potential and reduced NKA activity in both ventricular and atrial cardiomyocytes. Subsequently, this leads to Ca^{2+} overload which increases the incidence of Ca^{2+} waves and DADs in ventricular and tubulated atrial cardiomyocytes. These effects are due to a functional co-localization of NKA and NCX within the t-tubules. In addition, in ventricular and tubulated atrial cardiomyocytes hypokalemia promotes reactivation of the L-type I_{Ca} and thus increased incidence of EADs during the plateau phase of the prolonged APD. Conversely, in untubulated atrial cells EADs occur without the occurrence of Ca^{2+} overload. In these cells, hypokalemia leads to abbreviation of the APD and reactivation of I_{Na}, which promotes EADs in phase 3 of the AP. The brief APs in untubulated atrial cardiomyocytes are maintained by I_{Kur}, which is exclusively present in untubulated atrial cardiomyocytes.

2. Hypokalemia in tubulated atrial cardiomyocytes results in Ca^{2+} overload (biphasic response) in the same way as it does in ventricular cardiomyocytes. However, it does not lead to Ca^{2+} overload in untubulated cardiomyocytes.

3. Hypokalemia in atrial cardiomyocytes increases the susceptibility to phase-3 EADs by non-equilibrium reactivation of I_{Na}. This is due to the presence of the I_{kur} which is absent in ventricular and tubulated atrial cells.

4. The NKA-α_1 isoform is present in the sarcolemma and the t-tubules of both ventricular and tubulated atrial cells as well as in the sarcolemma of the untubulated atrial cells. However, while the expression of NKA-α_2 isoform is robustly present in the ventricular cells, it is very low in tubulated and untubulated atrial cells. NCX shows similar dense NCX localization in the sarcolemma and t-tubules of both ventricular and atrial cells.

CONSEQUENCES OF THIS THESIS FOR CLINICAL PRACTICE AND FUTURE RESEARCH

EIs are common and they are associated with increased LOS, readmissions and motality. Moreover, patients with EIs may have nonspesific symptoms that can reduce their quality of life. It is therefore crucial that health personnel are effectively trained in the diagnosis and management of EIs. Although designed as a before-after study without a control group, we have recently shown that diagnostic and treatment of patients with EI may reduce readmisions and improve symptoms and quality of life (154). Thus, there is a need for large, randomized controlled trials to investigate whether optimal diagnosis and treatment of EIs can reduce the above-mentioned outcomes as well as reduce the consumption of health care resources.

Our results from article II indicate that among patients with ROAF and plasma-potassium in the lower normal range, a sufficiently high increase in plasma potassium may increase the likelihood of conversion to sinus rhythm and that this likelihood may be even higher the lower the plasma-potassium is and with fewer AF episodes experienced. Thus, KCl may be as effective as other antiarrhythmic drugs without their risk or contraindications and without the burden of additional resources related to direct-current cardioversion therapy. Our findings should be verified in a larger study with premixed KCl infusion bags and an infusion rate of 15 mmol/h. It is essential that the infusion bag is well mixed, preferably by a pharmacist, and that it is infused in a larger vein like the cubital vein to minimize the risk for pain at the infusion site.

If our findings were to be verified in a larger study, KCl would have clinical consequence as it would be a cheaper, less risky drug with almost no serious side-effects and no contraindications. Hypothetically, potassium tablets could be used as a “pill in the pocket” to treat ROAF if they are designed to rapidly release and allow potassium absorption in the gut. Additionally, it would be interesting to see if AF patients who kept their plasma-potassium in the upper reference range by taking for example a potassium sparing diuretic would have fewer episodes with AF.

Our findings in article II are supported by our experimental and modeling results from article III showing that hypokalemia increases the propensity to EADs and DADs and therefore is proarhythmic in cardiomyocytes.

Antiarrhythmic drugs are important for the treatment of arrhythmia. As our understanding of cardiac arrhythmic mechanisms and the role of the ion channels in normal AP generation increases, we can develop cardiac ion channel activators or blockers directed at modulating the cardiac AP or its refractory period. More specifically, these drugs could increase the success rate of the treatment and at the same time minimize the potential risk for cardiac and extracardiac toxicity. There has been interest in studying I_{Kur} blockade for the treatment of AF. The aim is to prolong the AP and refractory period and thereby reducing the risk of reentry (155). Our results from article III suggest that blockade of I_{Kur}, which is atrial specific, may be especially effective in hypokalemic patients with AF where the APD is abbreviated. At the same time, AF like other arrhythmias is heterogeneous and the efficacy of targeting ion channels varies according to the cause and extent of the arrhythmia. In addition, various physiological and pathological conditions can lead to remodeling of K^+ channel expression, which can alter the AP and increase the risk of sudden cardiac death (50). Thus, to develop specific novel antiarrhythmic medications to treat patients with AF as well as other arrhythmias, we need much more research to understand the mechanisms leading to AF.

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ARTICLES

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RESEARCH ARTICLE

Electrolyte imbalances in an unselected population in an emergency department: A retrospective cohort study

Kiarash Tazmini[1¤]*, Ståle H. Nymo[1], William E. Louch[2,3], Anette H. Ranhoff[1,4], Erik Øie[1,3]

1 Department of Internal Medicine, Diakonhjemmet Hospital, Oslo, Norway, **2** Institute of Experimental Medical Research, Oslo University Hospital, Ullevål and University of Oslo, Oslo, Norway, **3** Center for Heart Failure Research, University of Oslo, Oslo, Norway, **4** Department of Clinical Science, University of Bergen, Bergen, Norway

¤ Current address: Department of Endocrinology, Morbid Obesity and Preventive Medicine, Faculty of Medicine, Oslo University Hospital, Oslo, Norway.
* kiakol3@gmail.com

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Abstract

Background

Although electrolyte imbalances (EIs) are common in the emergency department (ED), few studies have examined the occurrence of such conditions in an unselected population.

Objectives

To investigate the frequency of EI among adult patients who present to the ED, with regards to type and severity, and the association with age and sex of the patient, hospital length of stay (LOS), readmission, and mortality.

Methods

A retrospective cohort study. All patients ≥18 years referred for any reason to the ED between January 1, 2010, and December 31, 2015, who had measured blood electrolytes were included. In total, 62 991 visits involving 31 966 patients were registered.

Results

EIs were mostly mild, and the most common EI was hyponatremia (glucose-corrected) (24.6%). Patients with increasing severity of EI had longer LOS compared with patients with normal electrolyte measurements. Among all admitted patients, there were 12928 (20.5%) readmissions within 30 days from discharge during the study period. Hyponatremia (glucose-corrected) was associated with readmission, with an adjusted odds ratio (OR) of 1.25 (95% CI, 1.18–1.32). Hypomagnesemia and hypocalcemia (albumin-corrected) were also associated with readmission, with ORs of 1.25 (95% CI, 1.07–1.45) and 1.22 (95% CI, 1.02–1.46), respectively. Dysnatremia, dyskalemia, hypercalcemia, hypermagnesemia, and hyperphosphatemia were associated with increased in-hospital mortality, whereas all EIs except hypophosphatemia were associated with increased 30-day and 1-year mortality.

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Conclusions

EIs were common and increasing severity of EIs was associated with longer LOS and increased in-hospital, 30-days and 1-year mortality. EI monitoring is crucial for newly admitted patients, and up-to-date training in EI diagnosis and treatment is essential for ED physicians.

Introduction

Electrolyte imbalance (EI) is common in hospitalized patients as well as in the general population and is associated with increased morbidity and mortality [1–9]. Clinically important EIs include dysnatremia, dyskalemia, dyscalcemia, dysmagnesemia, and dysphosphatemia. The prevalence of hyponatremia in the emergency department (ED) is reported to range from 2.3–44%, while prevalence of hypernatremia is 1.1–4.4%, hypokalemia 10.2–39%, hyperkalemia 0.8–13%, and albumin-corrected hypercalcemia 0.7–7.5% [1, 2, 6, 10–13].

EIs have previously been investigated in several different cohorts. However, most previous studies have investigated one or two specific electrolytes in a selected group of patients with a single disease (e.g., heart or kidney disease) [14, 15], or in patients in a particular risk group (e.g., intensive care patients or patients using diuretics) [16, 17]. Previous studies have shown an association between EI and increased hospital length of stay (LOS) [8, 9, 13, 18–26] and a correlation between hyponatremia and rate of readmission [18–20, 27]. Although EI is frequently found in clinical practice, there are few studies which have examined frequency and outcomes in an unselected group of adult patients admitted to the ED. Apart from some studies of hyponatremia and readmission [18–20, 27], the frequency of readmissions among patients with other EIs is unknown. Data are particularly sparse regarding outcomes of dyscalcemia, dysmagnesemia, and dysphosphatemia.

We aimed to investigate the prevalence and severity of sodium and potassium imbalances among all adult patients visiting the ED, as well as imbalances of albumin-corrected calcium, free calcium, magnesium and phosphate levels in patients where these electrolytes were measured. We also examined possible associations between EIs and patient age and sex and with the clinically important outcomes LOS, readmission, in-hospital mortality and mortality 30 days and one year after discharge.

Methods

Study population, design and data source

All patients 18 years or older referred for any reason to the ED at Diakonhjemmet Hospital between January 1, 2010, and December 31, 2015, were included in this retrospective cohort study. During the study period, 62 991 patient contacts were registered, involving 31 966 unique patients with laboratory blood analyses (S1 Fig). Diakonhjemmet Hospital is a local urban hospital in Oslo, Norway, which serves approximately 135 000 inhabitants. Patients are referred to the ED from general practitioners, municipal emergency services, nursing homes, community health services or directly by ambulance ordered from a dispatch center. For every visit to the ED, age, sex, patient category (medical or surgical), serum-electrolyte values and serum-albumin and glucose levels were registered.

Serum-sodium levels were corrected for serum-glucose by lowering the sodium concentration by 2.4 mmol/L for every 5.5 mmol/L increase in glucose (glucose-corrected serum-

Table 1. The reference range [*], and definition of electrolyte imbalances by degree of severity. All values are in conventional units with SI units in parentheses.

Electrolyte	Ref. range	Hypo-			Hyper-		
		Mild	Moderate	Severe	Mild	Moderate	Severe
S-sodium mmol/L	137–145	130–136	125–129	<125	146–154	155–165	>165
S-potassium mmol/L	3.6–5.0	3.0–3.5	2.5–2.9	<2.5	5.1–5.9	6.0–6.9	≥7.0
S-Albumin-corrected- calcium mmol/L	2.15–2.51	1.90–2.14	1.65–1.89	<1.65	2.52–2.75	2.76–3.00	3.01–3.50
P-free-calcium mmol/L	1.14–1.28	1.00–1.13	0.80–0.99	<0.80	1.29–1.50	1.51–1.70	1.70–2.00
S-magnesium mmol/L	0.71–0.94	0.66–0.70	0.50–0.65	<0.5o	0.95–2.0	2.10–5.00	>5.00
S-phosphate mmol/L	Female 0.85–1.50 Male 18–49 yrs 0.75–1.65 Male > 49 yrs 0.75–1.35	Female 0.65–0.84 Male 0.65–0.74	0.30–0.64	<0.30	Female 1.66–1.74 Male 18–49 yrs 1.66–1.74 Male > 49 yrs 1.36–1.74	1.75–2.00	>2.00

[*] According to our hospitals laboratory which applies SI units.

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sodium = serum-sodium + [(serum-glucose– 5,6)/5,6] x 2,4), to account for the diluting effect of hyperglycemia [28]. The reference range for glucose according to our hospital's laboratory was 4.0–6.0 mmol/L. A correction formula was also used to calculate albumin-corrected calcium levels (mmol/L) [= measured serum-calcium level + 0.020 × (41.3 –serum-albumin) where 41.3 g/L is the albumin median] [29].

At Diakonhjemmet Hospital, measurement of serum levels of sodium, potassium, calcium, albumin and glucose are performed routinely in all medical patients in the ED (serum levels of calcium, albumin and glucose are not measured in surgical patients), whereas measurement of serum-magnesium, -phosphate and plasma-free calcium are carried out when indicated. We registered EIs for every ED visit.

We also recorded LOS, readmission within 30 days post-discharge, and in-hospital, 30-day, and 1-year mortality. Primary and secondary diagnoses as classified by the International Classification of Diseases, 10th revision (ICD-10), were registered. Data were extracted from the hospital's Department of Medical Biochemistry database and the patient administrative system. After obtaining the data, the electrolyte values were categorized into groups based on the severity of imbalance (mild, moderate and severe) according to Table 1.

The study was evaluated by the regional committee for medical and health research ethics (Regional Committee for Medical and Health Research Ethics South East) and defined as a quality study. It was also approved by the institutional review board (The Research Committee, Diakonhjemmet Hospital). Since all data were fully anonymized before we accessed them, the regional committee for medical research ethics and the institutional review board waived the requirement for informed consent.

Statistical analyses

The results are analyzed and presented in relation to each ED visit, not patient, since many patients had more than one visit during the study period. The results are presented as median with interquartile range (IQR) for continuous data, and as percentages for categorical data. Univariable and multivariable logistic regression analyses were performed to study associations between EI and readmissions, in-hospital mortality, and 30-day mortality. Cox proportional hazard models were performed to analyze the association between EI and 1-year

mortality. The multivariable analyses were adjusted for age, sex and the comorbid conditions (hypertension, heart failure, chronic obstructive pulmonary disease, cancer, kidney failure, diabetes mellitus, atrial fibrillation, pneumonia, sepsis, dehydration, and hip fracture). A two-sided *P*-value less than 0.05 was considered significant. Statistical analyses were performed using Stata/SE (version 14.2; Stata Corporation, College Station, TX).

Results

Baseline characteristics

The median age of all patients was 69 years (IQR 51–82 years). 33 705 patients (53.5%) were female, 44 381 (70.8%) were referred for medical reasons, and 18 325 (29.2%) were referred for surgery. Most of the patients were older (S2 Fig); 12 005 (19.1%) were 40–59 years, 21 970 (35.0%) were 60–79 years, and 19 146 (30.5%) were 80 years and older.

Electrolyte imbalances were mostly mild, and the most common EI was hyponatremia (glucose-corrected) (24.6%) (Table 2).

Hyponatremia, hypokalemia, hypercalcemia, dysmagnesemia, and hypophosphatemia were more frequent among female patients (S1 Table).

Among all visits (62 991), all electrolytes were measured in 7438 (11,8%), and among these visits, there were 2783 (37.4%) who had no EI, 2546 (34.2%) had one EI, 1290 (17.3%) had two EIs, and 819 (11%) had three or more EIs. Furthermore, among all visits who had measured sodium, potassium, and albumin-corrected calcium (45 500), 19207 (42,2%) had at least one of these EIs. The median serum-glucose levels for all ED-visits was 6.2 (5.5–7.4) mmol/L, and

Table 2. Electrolyte imbalance frequency by degree of severity for all ED visits 2010–2015.

	Proportion (%)	Mild n (%)	Moderate n (%)	Severe n (%)
Sodium				
Hyponatremia	15 030/62 929 (23.9)	13 186 (87.7)	1350 (9.0)	494 (3.3)
Hypernatremia	1076/62 929 (1.7)	989 (91.9)	69 (6.4)	18 (1.7)
Glucose corrected sodium				
Hyponatremia	11 692/47 467 (24.6)	10 224 (87.4)	1051 (9.0)	417 (3.6)
Hypernatremia	1894/47 467 (4.0)	1781 (94.0)	81 (4.3)	32 (1.7)
Potassium				
Hypokalemia	5376 /62 730 (8.6)	4931 (91.7)	396 (7.4)	49 (0.9)
Hyperkalemia	2080/62 730 (3.3)	1834 (88.2)	197 (9.5)	49 (2.4)
Calcium (albumin-corrected)				
Hypocalcemia	713/45 675 (1.6)	657 (92.2)	40 (5.6)	16 (2.2)
Hypercalcemia	4981/45 675 (10.9)	4527 (90.9)	341 (6.9)	113 (2.3)
Calcium (free)				
Hypocalcemia	3559/14 835 (24.0)	3454 (97.1)	97 (2.7)	8 (0.20)
Hypercalcemia	555/14 835 (3.7)	501 (90.3)	41 (7.4)	13 (2.3)
Magnesium				
Hypomagnesemia	1226/8512 (14.4)	594 (48.5)	563 (46.0)	69 (5.6)
Hypermagnesemia	691/8512 (8.1)	691 (100)	0	0
Phosphate				
Hypophosphatemia	715/7621 (9.4)	533 (74.6)	174 (24.3)	8 (1.1)
Hyperphosphatemia	634/7621 (8.3)	357 (56.3)	116 (18.3)	161 (25.4)

Abbreviations: ED, Emergency Department.

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there were 26 303 (55.4%) visits with serum-glucose > 6.0 mmol/L. Among ED visits with hyperglycemia (s-glucose > 6.0 mmol/L), 10 905 (26.1%) had hyponatremia and 672 (1.6%) hypernatremia.

Hospital length of stay

18 541 (29.4%) patients were discharged from the ED and not admitted. The median LOS among admitted patients was 3 days (IQR 1–5 days). While 31 062 (49.3%) of the patients were admitted for 1–4 days, 13 388 (21.3%) were admitted for 5 days or longer. Patients with increasing severity of EI had longer LOS compared with patients with normal electrolyte measurements (Table 3).

Readmission

Among all admitted patients, there were 12 928 (20.5%) readmissions within 30 days from discharge during the study period. Patients with hyponatremia accounted for 3557 (23.6%) of these readmissions (Table 3). Among those who were not readmitted, 27 170 (54.3%) were females and the median age was 68 years (IQR, 49–82 years). By comparison, among those who were readmitted, 6 535 (50.6%) were females and the median age was 71 (IQR 57–82 years). The median LOS was 1 day (IQR 1–4 days) for both groups. EIs were not significantly different between patients who were not readmitted compared with those who were readmitted, whereas 30-days mortality was higher in patients who were readmitted (40%) compared with patients who were not readmitted (26.5%). Hyponatremia (glucose-corrected) was associated with readmission with an adjusted odds ratio (OR) of 1.25 (95% CI, 1.18–1.32). Similar associations were observed for hypomagnesemia and hypocalcemia (albumin-corrected calcium) with ORs of 1.25 (95% CI, 1.07–1.45) and 1.22 (95% CI, 1.02–1.46) (Table 4). Among all admissions with mild EIs the readmission rate was 10–20%, whereas it was 0.2–10% for patients with moderate to severe EIs (S2 Table). Increasing severity of EIs was not associated with an elevated OR for readmission (S3 Table).

In-hospital, 30-day and 1-year mortality. Dysnatremia, dyskalemia, hypercalcemia, hypermagnesemia, and hyperphosphatemia were associated with increased in-hospital mortality, and all EIs except hypophosphatemia were associated with increased 30-day and 1-year mortality compared with patients not exhibiting the specific EI (Table 4). After adjusting for other EIs and comorbidities, hypernatremia, hyperkalemia, hypercalcemia, and hyperphosphatemia were still associated with increased in-hospital mortality and 30-day mortality. Hypernatremia, hypercalcemia, and hyperphosphatemia were associated with increased 1-year mortality (Fig 1).

Discussion

This is the first study of a non-selected, adult ED patient population describing the prevalence of EI according to type, severity, and associations to outcomes such as LOS, readmission, and mortality. Nearly half of the patients exhibited at least one type of EI, but most were mild. Increasing severity of EI was associated with longer LOS, increased in-hospital, 30-day and 1-year mortality.

Prevalence of EIs

In our patient population, we observed a high prevalence of hyponatremia (24.6% of patients, glucose-corrected sodium), frequent hypokalemia and albumin-corrected hypercalcemia (8.6% and 10.9%, respectively), and lower prevalence of hypernatremia, hyperkalemia, and

Table 3. Median length of hospital stay by degree of severity of the electrolyte imbalance and number of readmissions within 30 days after discharge for all admissions 2010–2015.

	Normo- days (IQR)	Mild days (IQR)	Moderate days (IQR)	Severe days (IQR)	Readmitted n (%)
Sodium					
Normonatremia (n = 31 154)	2 (1–5)				9169 (19.6)
Hyponatremia (n = 12 125)		3 (2–6)	4 (2–8)	5 (3–8)	3557 (23.6)
Hypernatremia (n = 854)		3 (1–7)	5 (3–9)	6 (3–11)	187 (17.4)
Glucose corrected sodium					
Normonatremia (n = 23 490)	2 (1–5)				6319 (18.7)
Hyponatremia (n = 9479)		3 (2–6)	4 (2–7)	5 (3–8)	2693 (23.0)
Hypernatremia (n = 1521)		3 (2–6)	4 (3–8)	6 (4–10)	381 (20.1)
Potassium					
Normokalemia (n = 38 241)	2 (1–5)				11 352 (20.5)
Hypokalemia (n = 4351)		3 (2–6)	5 (2–9)	6 (2–11)	1023 (19.0)
Hyperkalemia (n = 1663)		4 (2–7)	4 (2–7)	4 (1–10)	500 (24.2)
Calcium (albumin-corrected)					
Normocalcemia (n = 28 746)	3 (1–5)				7781 (19.5)
Hypocalcemia (n = 546)		4 (2–7)	4 (2–7)	7.5 (2–11)	164 (23.0)
Hypercalcemia (n = 4063)		3 (1–6)	3 (1–7)	5 (3–11)	1111 (22.3)
Calcium (free)					
Normocalcemia (n = 8430)	3 (1–6)				2003 (18.7)
Hypocalcemia (n = 3056)		4 (2–7)	5 (2–7)	6.5 (2–10)	739 (20.8)
Hypercalcemia (n = 472)		4 (2–7)	5 (2–11)	9.5 (4–13.5)	116 (20.9)
Magnesium					
Normomagnesemia (n = 5778)	4 (2–7)				1193 (18.1)
Hypomagnesemia (n = 1135)		4 (2–7)	4 (2–7)	4 (2–7)	264 (21.6)
Hypermagnesemia (n = 622)		4 (2–8)	NA	NA	133 (19.3)
Phosphate					
Normophosphatemia (n = 5563)	4 (2–7)				1151 (18.4)
Hypophosphatemia (n = 658)		4 (2–8)	4 (2–7)	3 (2–11)	122 (17.1)
Hyperphosphatemia (n = 580)		4 (2–9)	6 (3–10.5)	4 (2–10)	125 (19.8)

Abbreviations: NA, not applicable, IQR, inter quartile range.

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albumin-corrected hypocalcemia (1.7%, 3.3%, and 1.6%, respectively). These findings are in line with previous prevalence studies in ED which have shown prevalence of hyponatremia to be 2.3–44%, hypernatremia 1.1–4.4%, hypokalemia 10.2–39%, hyperkalemia 0.8–13%, and albumin-corrected hypercalcemia 0.7–7.5% [1, 2, 6, 10–13]. The large variation in the reported prevalence of these EIs is likely related to differences in the threshold used to define the imbalances, the time of the measurement (e.g., at admission, during hospitalization), and the study population (i.e. young vs. older patients). Most of our patients with EI were older (>60 years of age), suggesting that aging is an important determinant for developing EI. Indeed, impairment of renal function and changes in neurohumoral homeostasis during aging are well established. In addition, medical conditions and polypharmacy which are more prevalent in older people likely contribute to EI in this population [30].

The prevalence of abnormal levels of magnesium and phosphate in our overall patient cohort is unknown since these two electrolytes were only measured when indicated by the attending physician. When measured, patients with magnesium and phosphate values in the

Table 4. Multivariate analysis for readmission, in-hospital, 30-day and 1-year mortality for all ED visits 2010–2015.

	Multivariable analysis readmission*		Multivariable analysis in-hospital mortality[a]		Multivariable analysis 30-days mortality[a]		Multivariable analysis 1-year mortality[a]	
	Odds ratio (95% CI)	p-value	Odds ratio (95% CI)	p-value	Odds ratio (95% CI)	p-value	Hazard ratio (95% CI)	p-value
Normonatremi (reference)								
Hyponatremia	1.19 (1.14–1.25)	<0.0001	1.58 (1.40–1.78)	<0.0001	1.25 (1.21–1.29)	<0.0001	1.46 (1.39–1.54)	<0.0001
Hypernatremia	0.82 (0.70–0.96)	0.016	4.66 (3.72–5.82)	<0.0001	1.62 (1.46–1.80)	<0.0001	1.79 (1.54–2.08)	<0.0001
Glucose corrected normonatremi (reference)								
Hyponatremia	1.25 (1.18–1.32)	<0.0001	1.53 (1.34–1.74)	<0.0001	1.24 (1.19–1.28)	<0.0001	1.45 (1.37–1.53)	<0.0001
Hypernatremia	1.01 (0.90–1.14)	0.802	3.69 (3.06–4.46)	<0.0001	1.40 (1.30–1.52)	<0.0001	1.59 (1.42–1.79)	<0.0001
Normokalemia (reference)								
Hypokalemia	0.93 (0.86–1.00)	0.042	1.73 (1.46–2.06)	<0.0001	1.20 (1.14–1.26)	<0.0001	1.25 (1.15–1.35)	<0.0001
Hyperkalemia	1.09 (0.98–1.21)	0.126	3.33 (2.81–3.95)	<0.0001	1.45 (1.35–1.55)	<0.0001	1.54 (1.39–1.70)	<0.0001
Normocalcemia (albumin-corrected calcium, reference)								
Hypocalcemia	1.22 (1.02–1.46)	0.032	1.38 (0.91–2.09)	0.126	1.42 (1.25–1.60)	<0.0001	1.52 (1.27–1.81)	<0.0001
Hypercalcemia	1.06 (0.99–1.14)	0.119	2.35 (2.05–2.70)	<0.0001	1.45 (1.38–1.51)	<0.0001	1.70 (1.59–1.82)	<0.0001
Normocalcemia (free-calcium)								
Hypocalcemia	1.11 (1.00–1.22)	0.037	1.10 (0.92–1.31)	0.281	1.09 (1.03–1.17)	0.005	1.14 (1.03–1.26)	<0.009
Hypercalcemia	1.00 (0.81–1.25)	0.947	2.10 (1.57–2.80)	<0.0001	1.43 (1.25–1.64)	<0.0001	1.61 (1.33–1.95)	<0.0001
Normomagnesemia (reference)								
Hypomagnesemia	1.25 (1.07–1.45)	0.004	1.02 (0.74–1.40)	0.919	1.12 (1.03–1.22)	0.010	1.29 (1.12–1.48)	<0.0001
Hypermagnesemia	1.10 (0.90–1.35)	0.353	2.45 (1.83–3.28)	<0.0001	1.13 (1.00–1.27)	0.046	1.27 (1.06–1.52)	0.009
Normophosphatemia (reference)								
Hypophosphatemia	0.95 (0.77–1.16)	0.601	0.82 (0.53–1.26)	0.368	0.94 (0.84–1.04)	0.226	0.76 (0.63–0.94)	0.009
Hyperphosphatemia	1.08 (0.87–1.34)	0.498	3.80 (2.86–5.06)	<0.0001	1.29 (1.13–1.48)	<0.0001	1.37 (1.13–1.67)	0.001

Abbreviations: ED, Emergency Department.

* Adjusted for age, sex and comorbid conditions (hypertension, heart failure, atrial fibrillation/atrial flutter, chronic pulmonary disease, cancer, kidney failure, dehydration, diabetes mellitus, pneumonia, sepsis and hip fracture).

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normal range had a median LOS of 4 days, compared with only 2 days in the unselected population of patients with sodium and potassium values in normal range. A probable explanation for this observation is that magnesium and phosphate levels are measured in patients with malnutrition, weight loss, arrhythmias, renal failure, and other EIs as well as in the oldest patients. Among patients presenting with dysmagnesemia and dysphosphatemia, 14.4% exhibited hypomagnesemia, 8.1% hypermagnesemia, 9.4% hypophosphatemia, and 8.3% hyperphosphatemia, and these patients accounted for 25.6% of ED visits.

Because of the diluting effect of hyperglycemia on sodium concentration, the true value of s-sodium in hyperglycemic patients is glucose-corrected sodium [28]. One previous study demonstrated the superiority of glucose-corrected serum-sodium to predict mortality over measured serum-sodium, and the authors suggested that glucose-corrected serum-sodium should be considered in studies analyzing serum-sodium [31]. To our knowledge our study is the first study to report serum-sodium and glucose-corrected serum-sodium in an unselected hospital population.

Since most of our patients had serum-calcium and serum-albumin levels analyzed, albumin-corrected calcium could be calculated. However, in as many as 23.6% of the ED visits free-calcium was analyzed in plasma. Of these, 24.0% of patients exhibited hypocalcemia and 3.7% hypercalcemia. In comparison with albumin-corrected calcium levels, hypercalcemia was

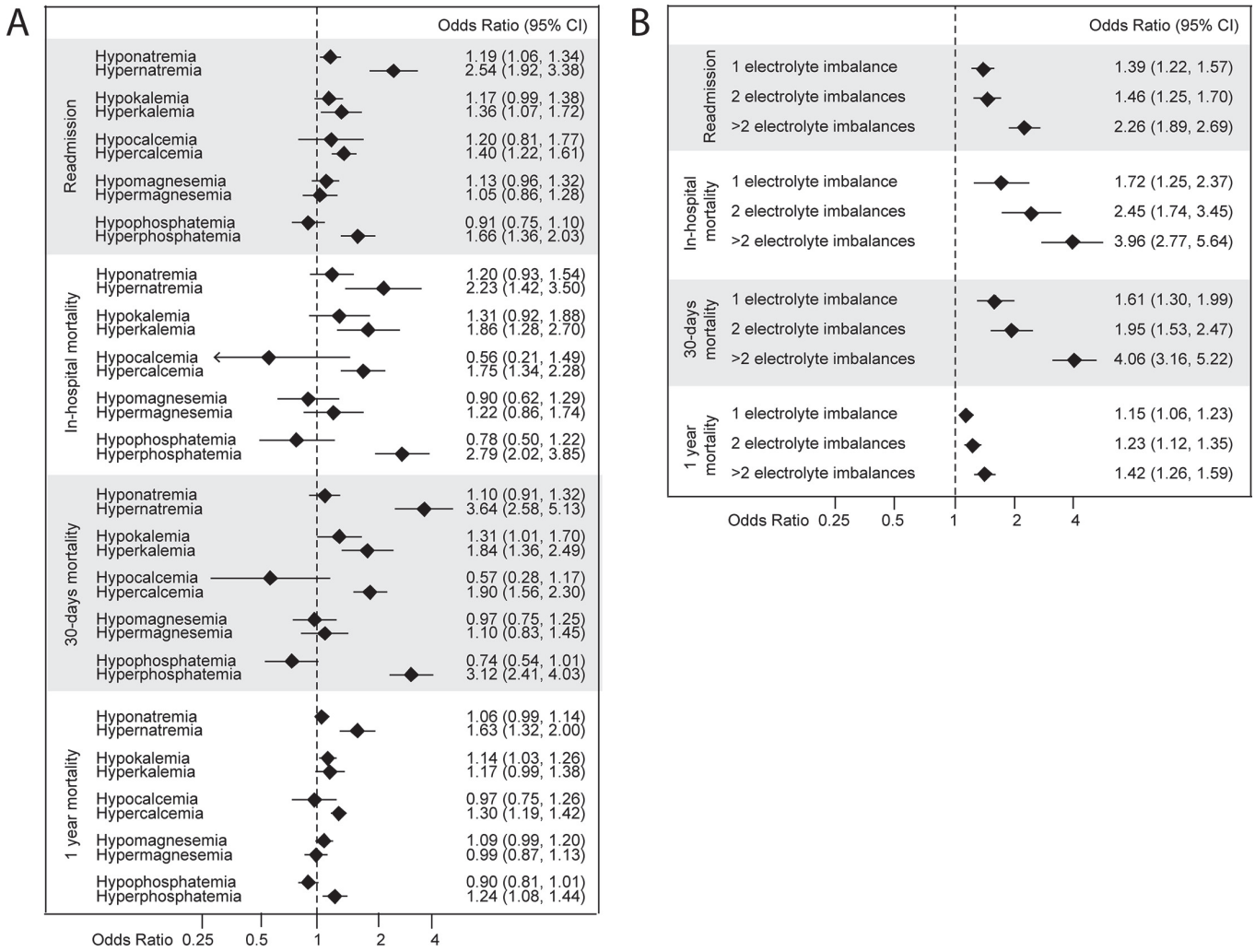

Fig 1. In-hospital, 30-days, 1-year mortality and readmission. A. Adjusted for all electrolyte imbalances, age, sex and comorbid conditions (hypertension, heart failure, atrial fibrillation/atrial flutter, chronic pulmonary disease, cancer, kidney failure, dehydration, diabetes mellitus, pneumonia, sepsis and hip fracture). **B.** Adjusted after number of electrolyte imbalances; 1, 2, or >2 electrolyte imbalances.

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less frequently observed when measured as free calcium in plasma. Conversely, hypocalcemia was more frequent when measured as plasma-free calcium. This discrepancy could be due to a selection of patients where arterial blood was drawn for blood gas analysis, in addition to absence of both hemolysis and venous stasis which can lead to an increase in calcium. Therefore, it seems like albumin-corrected calcium measurements overestimates calcium levels. Similar results have been previously reported in a study investigating blood gas analyses in patients visiting the ED (25.3% hypocalcemia and 3% had hypercalcemia) [32]. Numerous studies have identified specific clinical situations in which direct measurement of free-calcium has been shown to be superior to its calculation from total calcium and albumin, even with corrections for pH [33]. Thus, in our study, the results from free-calcium may be closer to the actual dyscalcemia than albumin-corrected calcium. To our knowledge, the present study is the first to compare albumin-corrected with free calcium in an unselected population admitted to the ED.

Hospital length of stay

It is well known that dysnatremia is associated with longer LOS. According to a recent meta-analysis investigating 46 studies, mean LOS was 3 days longer in hyponatremic patients[18]. In another recent study, LOS was 10% longer for patients with community-acquired hypernatremia [34]. In our study, the median LOS for patients with mild, moderate, and severe hyponatremia and hypernatremia was 3, 4, and 5 days and 3, 5, and 6 days, respectively, compared with 2 days for normonatremic patients. Our results demonstrate a similar increase in LOS for patients with hypokalemia as patients with dysnatremia, with median hospital stays of 3, 5, and 6 days for mild, moderate, and severe hypokalemia. For hyperkalemia, median LOS was 4, 4, and 4 days, respectively. Of note, the number of patients with severe dyskalemia was very low. Importantly, while the median LOS is perhaps shorter than expected in patients with severe hyperkalemia, it should be noted that most of these patients likely suffered from severe renal failure and thus were transferred to the nearby university hospital for dialysis. High mortality in this patient group also likely reduced LOS values. We are aware of only a single retrospective study of potassium levels in ED patients, which reported a mean LOS of 5.8 days for patients with hypokalemia and 6.6 days for patients with hyperkalemia. However, in this study, the mean LOS for normokalemic patients was substantially longer than at our hospital (4.8 days) [9].

In our study, the median LOS for patients with severe hypocalcemia was 6.5 days when measured as free calcium and 7.5 days when measured as albumin-corrected calcium, respectively. For patients with severe hypercalcemia, the median LOS was 9.5 days when measured as free calcium and 5 days when measured as albumin-corrected calcium, respectively. Thus, these patients had significantly longer hospital stays than normocalcemic patients, for whom median LOS was 3 days. An earlier study reported a mean LOS of 10 days in patients with hypercalcemia, mirroring our results. In this previous study, however, the mean LOS was much longer for all patients than in our study, and there was no significant correlation between serum-calcium level and the length of hospital stay [13].

Although the population of patients where magnesium and phosphate were measured was likely more morbid, median LOS was only slightly increased for those exhibiting dysmagnesemia and hypophosphatemia compared with normomagnesemia and normophosphatemia. Patients with moderate hyperphosphatemia, however, exhibited longer LOS in comparison with normophosphatemic patients (median LOS = 6 vs 4 days). This difference may at least partly reflect renal failure, which is a common cause of hyperphosphatemia. This increase in LOS was lower in patients with severe hyperphosphatemia compared with patients with moderate hyperphosphatemia, again possibly reflecting transfer of patients with severe hyperphosphatemia to the nearby university hospital for dialysis. Indeed, a previous retrospective study investigating ED patients found a substantial reduction of renal function in patients with hyperphosphatemia upon admission[26]. In parallel to our findings, a mean LOS of 6 days was previously reported in patients with hyperphosphatemia compared with 3 days for patients with normophosphatemia [26].

Readmission

In a multivariate analysis adjusted for age, sex and comorbid conditions, only hyponatremia, hypocalcemia, and hypomagnesemia were significantly associated with increased probability for readmission. Hypernatremia was not associated with increased probability for readmission due to the high in-hospital mortality for these patients. A similar lack of increased OR for readmission with increasing severity of EIs likely also reflects higher mortality in patients with the most severe EIs.

Mortality

In our study, hyponatremia and hypokalemia were associated with 58% and 73% increases in risk of in-hospital mortality, but such associations were not observed for hypocalcemia, hypomagnesemia, and hypophosphatemia. However, for all electrolytes increased levels were associated with a substantial increase in-hospital mortality (2.1–4.6-fold increase). For all EIs except dysphosphatemia, the risk of mortality increased with greater EI severity. Except for hypophosphatemia, all other EIs (decreased and increased electrolyte levels) were associated with increased 30-day and 1-year mortality values. However, this association was much more marked when electrolyte levels were *increased*, as 30-day and 1-year mortality values were elevated by 13–79%. These results are in line with previous studies [6, 8, 9, 23, 26, 32, 35–37]. The association between increased electrolyte levels and mortality may largely reflect very high mortality rates in hypernatremic patients. Indeed, a previous study reported a mortality rate of 61% in these patients, and 50% mortality even after correction of hypernatremia[35]. Hyperkalemia has the potential for causing fatal arrhythmias especially in patients with kidney disease, cardiovascular disease, and diabetes mellitus [38]. These conditions are common and require therapeutic interventions which can induce or worsen hyperkalemia [38]. Hypercalcemia is common in patients with cancer, occurring in 20–30% of cases [39], comprising 44% of all cases of hypercalcemia in the ED [13], and is indicative of very poor prognosis; approximately 50% of hypercalcemic cancer patients die within 30 days of diagnosis [40]. The most common cause of hyperphosphatemia and hypermagnesemia is chronic kidney disease, which is particularly prevalent in older patients with other co-morbidities including cardiovascular disease [41]. Moreover, it has been shown that hyperphosphatemia is associated with increased all-cause mortality in the general population without apparent kidney disease [42]. Hyperphosphatemia can lead to adverse cardiovascular outcomes and mortality, particularly in patients with chronic kidney disease, by promoting endothelial dysfunction, vascular stiffness, and vascular calcification [42]. Hypermagnesemia, on the other hand, may cause impairment of both cardiac systolic contraction and diastolic relaxation in addition to serious arrhythmia [43].

Limitations

Our study has limitations due to its retrospective design. We linked laboratory data to administrative data (age, sex, diagnoses, hospital LOS, readmission and mortality). The diagnoses were based on the ICD-10 coding which the responsible physician found relevant, and we did not have information concerning causes of death. We have adjusted for chronic diseases that are likely to have influenced outcomes, but there might be multiple other factors affecting LOS, readmissions and mortality which were not accounted for. Since the electronic patient journal systems in Norway are not searchable, we were unable to link the laboratory data to other potentially confounding variables, including past medical history, reason for visiting and vital status in the ED, and known history of chronic electrolyte imbalances and treatment. Although the routine at Diakonhjemmet Hospital is to take blood samples shortly after the patient has arrived in the ED, we cannot exclude that blood from some patients was collected after initiation of treatment. Furthermore, since the study was only performed in one hospital, the results could vary between centres, regions, or countries, which limits the generalizability. The results of our study regarding magnesium, free calcium, and phosphate levels appear to reflect only a subset of patients from whom these measurements were performed for clinical reasons. Thus, the true prevalence of these EIs could not be shown in this study.

Conclusion

Our results demonstrate that EIs are common in patients admitted to an ED at a local urban hospital, and that patients with EI have an increased risk of prolonged LOS, readmission, and mortality. Thus, EIs increase consumption of health care resources. EIs may be reflect serious underlying conditions or the EI in itself may contribute to the increased risk of prolonged LOS, readmission, and mortality. It is therefore crucial that health personnel are effectively trained in the diagnosis and management of EIs. We further suggest that future studies should investigate whether an increased focus on EI detection, follow-up, and treatment can decrease the risk of prolonged LOS, readmission, and mortality.

Supporting information

S1 Fig. Flowchart and overview of analyses; Electrolyte imbalance. * LOS, length of stay.
(PNG)

S2 Fig. Distribution of age categories for all visits to the emergency department 2010–2015.
(PNG)

S1 Table. Electrolyte imbalance categorized by sex for all ED visits 2010–2015. Abbreviations: ED, Emergency Department.
(DOCX)

S2 Table. Readmissions within 30 days after discharge by degree of severity of the electrolyte imbalance for all admissions 2010–2015. Abbreviations: NA, not applicable.
(DOCX)

S3 Table. Multivariate analysis for readmission, in-hospital, 30-day and 1-year mortality by degree of severity of the electrolyte imbalance for all ED visits 2010–2015. Abbreviations: ED, Emergency Department. NA, not applicable. * Adjusted for age, sex and comorbid conditions (hypertension, heart failure, atrial fibrillation/atrial flutter, chronic pulmonary disease, cancer, kidney failure, dehydration, diabetes mellitus, pneumonia, sepsis and hip fracture).
(DOCX)

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Author Contributions

Conceptualization: Kiarash Tazmini, Anette H. Ranhoff, Erik Øie.

Data curation: Kiarash Tazmini.

Formal analysis: Kiarash Tazmini, Ståle H. Nymo.

Project administration: Anette H. Ranhoff, Erik Øie.

Writing – original draft: Kiarash Tazmini.

Writing – review & editing: Kiarash Tazmini, Ståle H. Nymo, William E. Louch, Anette H. Ranhoff, Erik Øie.

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II

Potassium infusion increases the likelihood of conversion of recent-onset atrial fibrillation—A single-blinded, randomized clinical trial

Kiarash Tazmini, MD,[a,b,c,1] Mai S. Aa. Fraz, MD,[a] Ståle H. Nymo, MD, PhD,[a] Mathis K. Stokke, MD, PhD,[c,d] William E. Louch, PhD,[b,c] and Erik Øie, MD, PhD[a] *Oslo, Norway*

Background The optimal antiarrhythmic management of recent-onset atrial fibrillation (ROAF) or atrial flutter is controversial and there is a considerable variability in clinical treatment strategies. It is not known if potassium infusion has the potential to convert ROAF or atrial flutter to sinus rhythm (SR). Therefore, we aimed to investigate if patients with ROAF or atrial flutter and plasma-potassium levels ≤4.0 mmol/L have increased probability to convert to SR if the plasma-potassium level is increased towards the upper reference range (4.1-5.0 mmol/L).

Methods In a placebo-controlled, single-blinded trial, patients with ROAF or atrial flutter and plasma-potassium ≤4.0 mmol/L presenting between April 2013 and November 2017 were randomized to receive potassium chloride (KCl) infusion (n = 60) or placebo (n = 53). Patients in the KCl group received infusions at one of three different rates: 9.4 mmol/h (n = 11), 12 mmol/h (n = 19), or 15 mmol/h (n = 30).

Results There was no statistical difference in the number of conversions to SR between the KCl group and placebo [logrank test, P = .29; hazard ratio (HR) 1.20 (CI 0.72-1.98)]. However, KCl-infused patients who achieved an above-median hourly increase in plasma-potassium (>0.047 mmol/h) exhibited a significantly higher conversion rate compared with placebo [logrank P = .002; HR 2.40 (CI 1.36-4.21)] and KCl patients with below-median change in plasma-potassium [logrank P < .001; HR 4.41 (CI 2.07-9.40)]. Due to pain at the infusion site, the infusion was prematurely terminated in 10 patients (17%).

Conclusions Although increasing plasma-potassium levels did not significantly augment conversion of ROAF or atrial flutter to SR in patients with potassium levels in the lower-normal range, our results indicate that this treatment may be effective when a rapid increase in potassium concentration is tolerated and achieved. (Am Heart J 2020;221:114-24.)

Atrial fibrillation (AF) is the most common arrhythmia, with an estimated prevalence of approximately 3% in adults aged 20 years or older. Atrial flutter has lower prevalence, with an incidence <1/10 that of AF.[1] Recent-onset atrial fibrillation (ROAF) or atrial flutter, defined as an abrupt onset of symptoms within 48 hours from admission, are highly prevalent in emergency departments.[2] Current European and American guidelines describe different treatment options and strategies for management of ROAF.[1] This is reflected by a wide variability of treatment approaches employed in clinical practice.[2] Indeed, common strategies range from a "wait and see" approach to conversion to sinus rhythm (SR) by use of anti-arrhythmic medication or direct-current cardioversion.[3]

About 50% of patients with ROAF with duration less than 48 hours convert spontaneously to SR.[4, 5] Several antiarrhythmic drugs have been shown to increase the likelihood of conversion to SR to about 70% within 12 hours.[6] However, most current antiarrhythmic drugs are not atrial specific, and they all have significant risk of side-effects, including atrial and ventricular proarrhythmia, heart failure exacerbation, lung-, hepatic-, and thyroid toxicity, and death.[7] Furthermore, antiarrhythmic drugs have several contraindications including bradycardia, sinoatrial node dysfunction, AV conduction

From the [a]Department of Internal Medicine, Diakonhjemmet Hospital, Oslo, Norway, [b]Institute of Experimental Medical Research, Oslo University Hospital, Ullevål and University of Oslo, Oslo, Norway, [c]K.G. Jebsen Center for Cardiac Research and Center for Heart Failure Research, University of Oslo, Oslo, Norway, and [d]Clinic of Internal Medicine, Lovisenberg Diaconal Hospital, Oslo, Norway.
[1]Current workplace: Department of Endocrinology, Morbid Obesity and Preventive Medicine, Faculty of Medicine, Oslo University Hospital, Postbox 4950 Nydalen, 0424 Oslo.
Clinical Trial Registration – URL: https://clinicaltrials.gov. Unique identifier: NCT01818583
Submitted December 20, 2018; accepted December 21, 2019.
Reprint requests: Kiarash Tazmini, MD, Department of Internal Medicine, Diakonhjemmet Hospital, PO BOX 23, Vinderen, 0319, Oslo, Norway.
E-mail: kiakol3@gmail.com
0002-8703

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disturbances, prolonged QT interval, heart failure, coronary artery disease, and structural heart disease.[7] By comparison, direct-current cardioversion has been shown to have a success rate of more than 90% in ROAF patients.[8] However, this approach requires deep sedation and a 6 hours postprandial period to ensure gastric emptying, associated with additional costs, resources, and risk.

Clinical studies have shown that lower serum concentrations of potassium (<3.5 mmol/L) are associated with a higher risk of AF.[9] This association is independent of age, sex, serum-magnesium, antihypertensives, myocardial infarction, heart failure, and other potential confounders.[9] It has been suggested that hypokalemia may trigger AF through adverse effects on the sinoatrial node and increased pulmonary vein arrhythmogenesis.[10] Hypokalemia causes resting membrane hyperpolarization, Na^+-K^+ ATPase inhibition and suppression of K^+ channel conductances resulting in action potential duration prolongation, reduced repolarization reserve, early afterdepolarizations, delayed afterdepolarizations, and automaticity. These changes are believed to increase the risk of developing AF.[11] Thus, increasing potassium intake with subsequently increased or normalized plasma-potassium levels might be beneficial in AF patients, mirroring previous work showing that this approach reduces both blood pressure and the risk of lethal ventricular arrhythmias in patients with ischemic heart disease, heart failure, and left ventricular hypertrophy.[12] There are several case reports of patients with AF converting to SR during hyperkalemia.[13-18] However, to our knowledge there are no studies investigating whether potassium infusion has the potential to convert AF to SR. We presently hypothesized that patients with ROAF or atrial flutter with plasma-potassium levels ≤4.0 mmol/L have increased probability to convert to SR if the plasma-potassium level is increased towards the upper part of the reference range; between 4.1 and 5.0 mmol/L. Thus, our aim was to investigate whether potassium, a cheap treatment with few contraindications, could be an alternative to more expensive drugs with potentially serious side-effects.

Materials and methods

Study design

A single center, placebo-controlled, parallel-group study was conducted with equal randomization (1:1), blinded for patients but not for the healthcare providers, at Diakonhjemmet Hospital, Oslo, Norway, from April 2013 to November 2017. Diakonhjemmet Hospital is a local urban hospital in Oslo, Norway, serving approximately 135,000 inhabitants. The patients are referred to the emergency department from general practitioners, municipal emergency services, nursing homes, community health services, or directly by ambulance ordered from a dispatch center.

Participants

Eligible participants were patients ≥18 years of age visiting the emergency department with AF or atrial flutter with abrupt onset of symptoms within 48 hours from admission and plasma-potassium ≤4.0 mmol/L. Patients with or without a documented history of previous AF/atrial flutter were included. A 12-lead ECG was recorded (Mortara ELI 280, Mortara Instruments, Inc, Milwaukee, WI), at rest and interpreted by the treating physician. Exclusion criteria were kidney failure with estimated glomerular filtration rate (eGFR) <30 mL/min, current usage of antiarrhythmic medication (flecainide, amiodarone, dronedarone, sotalol, dofetilide or propafenone), infection, metabolic acidosis with pH <7.2, pregnancy, or lactation. Patients participating in other clinical trials or who had previously been included in our trial were also excluded. Duration of symptoms, age, gender, body mass index (BMI), co-morbidities and medications were registered shortly after patient inclusion, based on a review of medical records. After conversion to SR and before discharge, transthoracic echocardiography was performed, registering the following parameters: left ventricular (LV) ejection fraction, left atrial area, LV end-diastolic diameter (LVEDD), LV mass, mitral regurgitation, systolic pulmonary artery pressure (SPAP), and the LV diastolic parameters E/A-ratio (ratio between mitral valve early and late diastolic inflow) and E/e' (ratio between early mitral inflow velocity and mitral annular early diastolic velocity).

The study was approved by the Regional Committee for Medical and Health Research ethics, The Norwegian Medicines Agency, and the hospital's research committee, and was registered at Clinicaltirals.gov (NCT01818583). All study patients provided their informed written consent. KT was funded by The South-Eastern Norway Regional Health Authority (Helse Sør-Øst RHF); grant number: 2013011. Helse Sør-Øst RHF had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. The authors are solely responsible for the design and conduct of this study, all study analyses, the drafting and editing of the paper and its final contents.

Randomization, study treatment, and monitoring

Patients were assigned to either the potassium chloride (KCl) group or placebo group following blocked randomization by shuffling opaque concealed envelopes. The KCl group was treated with 60 mmol KCl added to 1000 mL of 50 mg/mL dextrose in an infusion bag, which was then inverted 10 times to ensure that the solution was well-mixed. KCl was administered through a peripheral venous catheter in a large vein, preferably the antecubital vein, at a rate of 166 mL/h (9.4 mmol KCl/h), 212 mL/h (12 mmol KCl/h), or 265 mL/h (15 mmol KCl/h). An interim analysis was performed to determine whether plasma-potassium increased as hypothesized. Since there was a smaller

increase than expected, only the highest infusion rate was continued. Patients in the KCl group exhibiting serum-magnesium levels ≤0.80 mmol/L also received magnesium sulfate infusion (0.5 mmol/kg added to 1000 mL of 9 mg/mL sodium chloride) over 24 hours at a rate of approximately 42 mL/h.

KCl infusion was stopped if the patients converted to SR, the plasma-potassium level reached ≥5.5 mmol/L, severe ventricular arrhythmia or symptomatic bradycardia occurred, eGFR declined to <30 mL/min, or more than weak pain was experienced at the infusion site. The placebo group received only 50 mg/mL dextrose at the same infusion rate as in the KCl treatment group.

One of the trial investigators (KT) performed a day to day monitoring of potential adverse clinical events. Patients who did not convert to SR during infusion of KCl or placebo were treated by direct-current cardioversion the day after the start of infusion. The minimum infusion time for patients who did not convert to SR during infusion was 12 hours and the maximum infusion time was 24 hours. Patients with tachycardia (heart rate >100 beats/min) could receive rate-reducing treatment with a beta-blocker, a calcium-antagonist, digoxin or a combination of agents (beta-blocker + digoxin or calcium antagonist + digoxin) after the treating physician's assessment since these drugs are considered to have little converting properties and not regarded as antiarrhythmic drugs.[1]

Serum levels of potassium, magnesium, and creatinine (venipuncture) and plasma-potassium (arterial blood gas) were measured at inclusion. Serum-potassium, magnesium, and creatinine were further analyzed every 8 hours after the start of infusion and when the infusion was stopped, whereas plasma-potassium were measured 4 hours after start of infusion and thereafter every 8 hours, i.e. in between the serum analyses, and when the infusion was halted. Thus, serum- or plasma-potassium was monitored every 4 hours.

Continuous ECG-monitoring by telemetry was performed on all patients.

At the follow-up consultation performed roughly 3 months after discharge, a 12-lead ECG was recorded at rest, and all patients with SR were equipped with a hand-held "thumb ECG" recorder (Zenicor-EKG®; Zenicor Medical Systems AB, Stockholm, Sweden) for 3 days. Patients were instructed to perform 30 second rhythm recordings once in the morning, once in the evening, and whenever they experienced palpitations or arrhythmia symptoms. The recorded signal corresponded to lead I in a standard 12-lead ECG. AF was defined as irregular ventricular rhythm without visible or regular P waves for 30 seconds, whereas atrial flutter was defined by regular P waves at a rate of 250-350/min.

End points

The co-primary endpoints were time to and frequency of conversion to SR. The secondary endpoints were serious adverse (e.g. ventricular arrhythmia, symptomatic bradycardia, death) and adverse events (e.g. pain at the infusion site) during infusion, detection of AF or atrial flutter, hospital visits due to AF or atrial flutter, episodes with tachycardia lasting for at least 1 hour, and serum- and plasma-potassium during follow up.

Statistics

We assumed that 50% of the patients would convert spontaneously to SR[4, 19] and postulated that potassium infusion would increase the rate of conversion to 75%. With a power of 80% to detect this increase, at least 58 patients were predicted to be required in both the KCl infusion and placebo groups to detect a significant treatment effect.

Continuous variables are presented with median and interquartile range (IQR), and categorical variables are presented as count and percentage. Statistical differences between any two groups were examined with Pearson's Chi-squared test for categorical variables and a 2-sample Wilcoxon rank-sum (Mann-Whitney) test applied for continuous variables. Differences in time to conversion between groups were also assessed with Kaplan-Meier plots, a log-rank test, and Cox proportional hazard regressions. The assumption of proportional hazard was tested using Schoenfeld residuals as well as visual inspection of a log-log plot.

The primary analysis was *intention-to-treat* and involved all randomized patients included. All patients who were randomized into either group were considered to be in the *intention-to-treat* sample (n = 113). From these, 5 patients were excluded from the placebo group and 11 patients were excluded from the KCl group (Figure 1). All other patients for whom the protocol was followed (n = 97) were included in a prespecified *per-protocol* analysis with the same endpoints as for the *intention-to-treat* analysis.

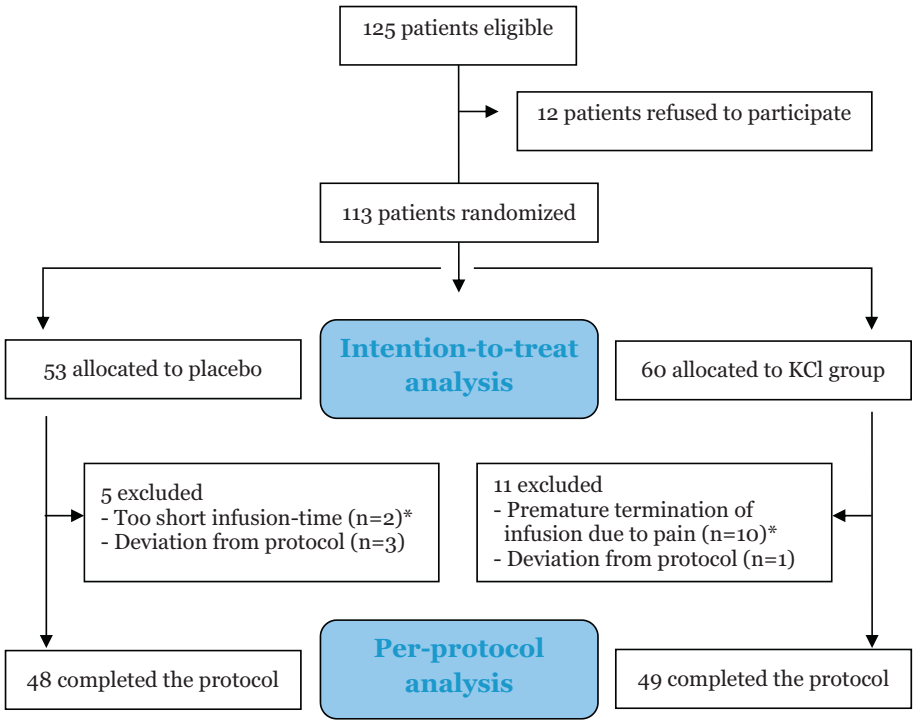
Statistical analyses were performed using Stata/SE version 14.2 (Stata Corporation, College Station, TX). A $P \leq .05$ was considered statistically significant.

Results

Study participants

Due to slower inclusion than expected, a total of 113 patients were randomized, of whom 53 were allocated to the placebo group and 60 to the KCl group (Figure 1). The vast majority of included patients presented exclusively with AF (>90%), while 9 patients (8%) had atrial flutter and 2 patients (1.8%) alternated between AF and atrial flutter. Eleven patients received KCl at an infusion rate of 166 mL/h (9.4 mmol KCl/h), 19 patients at a rate of 12 mmol KCl/h, and 30 patients at a rate of 15 mmol KCl/h. Forty-eight patients in the placebo group and 49 patients in the KCl group completed the protocol. Baseline characteristics (Table I) were similar between these groups, apart from a higher percentage of men and a higher median LVEDD in

Figure 1



Study enrollment and randomization.KCl, potassium chloride. *Patients who had received fluid corresponding to <10 mmol KCl in the placebo group and <10 (1.25-6) mmol KCl in the KCl group.

the KCl group. However, adjusting for these variables in Cox regression models did not change our result to any significant degree.

Outcomes

Time to and frequency of conversion to SR. There was no significant effect of KCl infusion on the co-primary endpoint of time to or frequency of conversion to SR compared with placebo when all KCl patients were grouped independent of infusion rate (Tables II and III).

A Kaplan-Meier plot showed only a trend towards increased number of patients who converted to SR, which was non-significant by logrank test (P = .29, Figure 2a), and Cox regression showed a hazard ratio (HR) of 1.20 (CI 0.72-1.98, P = .72) for KCl infusion compared with placebo. When analyzing the KCl group receiving the fastest infusion KCl rate of 15 mmol/h, there was still no significant difference in conversion rate nor in time to conversion in the *intention-to-treat* analysis compared with placebo. However, when comparison was done in patients without a history of AF, significantly more patients in the KCl group converted to SR compared with the placebo group (85.7 vs 52.4%; P = .019; Supplementary Table 1a) independently of infusion rate of KCl.

Twelve patients in the KCl group experienced pain at the infusion site (20%), and most of these were allocated to receiving KCl at the fastest infusion rate of 15 mmol/h. In 10 of these patients, the KCl infusion had to be prematurely terminated due to the pain (Table II). After excluding these patients, the *per-protocol* analysis showed that significantly more patients converted to SR in the KCl 15 mmol/h group compared with the placebo group (82% vs 53%. P = .018, Table III). Significant differences between the KCl 15 mmol/h and placebo groups were also observed when tested with a Kaplan Meier plot (logrank P = .02) and Cox regression (HR 2.26, CI 1.09-3.71, P = .024, Figure 2b). Again, when comparison was done in patients without a history of AF, significantly more patients in the KCl group converted to SR compared with the placebo group (85% vs 47.4%; P = .013; Supplementary Table 2a) independently of infusion rate of KCl.

Of note, KCl-infused patients who achieved an above-median hourly increase in plasma-potassium (>0.047 mmol/h) exhibited a significantly higher conversion rate, but no difference in time to conversion compared with placebo (Tables II and III and Supplementary Table 1a and 2a). Kaplan Meier plots also showed a significant difference between this above-median group compared

Table I. Baseline characteristics.

	Placebo (n = 53)	KCl (n = 60)
Age, y	63 (54-73)	67 (53-74)
Sex, % male	27 (50.9%)	44 (73.3%)*
BMI, kg/m^2	24.6 (22.5-27.2)	25.8 (22.9-28.1)
Duration of symptoms, h	2.75 (1.8-5.0)	4.2 (2.0-12.5)
Heart rate, bpm	128 (116-143)	131 (111-148)
Medical history		
Atrial fibrillation	32 (60.4%)	39 (65.0%)
Kidney function (eGFR) ≥60 mL/min	44 (83.0%)	53 (88.3%)
Hypertension	19 (35.9%)	19 (31.7%)
Stroke/TIA	5 (9.4%)	2 (3.3%)
Coronary heart disease	3 (5.7%)	7 (11.7%)
Previous ablation	3 (5.7%)	5 (8.3%)
Diabetes mellitus	2 (3.8%)	4 (6.7%)
Hypo-/hyperthyroidism	3 (5.7%)	3 (5%)
Medications		
ASA	8 (15.1%)	12 (20.0%)
Warfarin	5 (9.4%)	6 (10%)
NOAC	12 (22.6%)	10 (16.7%)
β-Blocker	14 (26.4%)	20 (33.3%)
Digoxin	1 (1.9%)	0
ACE-I or ARB	15 (28.9%)	15 (25%)
Diuretic (loop or thiazide)	9 (17.0%)	13 (21.7%)
Potassium-sparing diuretic	1 (1.9%)	1 (1.7%)
CCB (non-dihydropyridines)	6 (11.3%)	8 (13.3%)
CCB (dihydropyridines)	1 (1.9%)	2 (3.3%)
Statin	12 (22.6%)	13 (21.7%)
Levothyroxine	2 (3.8%)	2 (3.3%)
Echocardiographic characteristics		
LAA, cm^2, n = 107	21 (18-27)	22.5 (19-27)
LVEDD, mm, n = 108	47 (44-52)	50 (47-54)*
LV mass, g, n = 107	60.7 (45.8-83.3)	66.3 (52.8-81.7)
LVEF ≥50%, n = 107	51 (98.1%)	52 (94.6%)
Mitral regurgitation†, n = 108	4 (7.7%)	7 (12.5%)
E/A-ratio, n = 89	1.2 (0.9-1.6)	1.2 (1.1-1.7)
E/e', n = 95	7.9 (6.0-10.1)	7.7 (5.8-10.5)
SPAP, mmHg, n = 59	30.5 (26.5-36.0)	26.5 (25.0-30.0)
Pre-infusion		
S-potassium, mmol/L	4.1 (3.8-4.2)	4.1 (3.9-4.2)
S-potassium <3.5 mmol/L, n = 3	3.3 (3.3-3.3)	3.4 (3.3-3.4)
P-potassium, mmol/L	3.8 (3.6-4.0)	3.8 (3.6-3.9)
P-potassium <3.5 mmol/L, n = 11	3.3 (3.3-3.4)	3.4(3.2-3.4)
Magnesium, mmol/L, n = 111	0.81 (0.78-0.85)	0.83 (0.76-0.87)
Creatinine, μmol/L	79 (66-91)	80 (71-90)
NT-proBNP, ng/L, n = 97	262 (80-1023)	304 (127-1023)
Troponin T, ng/L, n = 111	11 (10-16)	11 (10-17)
CRP, mg/L	1 (1-2)	1 (1-2)
TSH, mIU/L, n = 100	2.2 (1.1-3.1)	2.3 (1.5-3.4)
Free T_4, pmol/L, n = 100	16 (15-18)	16 (15-17)

Values are median and interquartile range or number and percentage.
BMI: body mass index; TIA: transient ischemic attack; ASA: Acetylsalicylic acid; NOAC: Non-vitamin K antagonist oral anticoagulant; ACE-I: angiotensin converting enzyme inhibitor; ARB: Angiotensin receptor II blocker; CCB: calcium channel blocker; LAA: left atrial area; LVEDD: left ventricle end-diastolic diameter; LV: left ventricle; LVEF: left ventricle ejection fraction; E/A: ratio between mitral valve early and late diastolic inflow; E/e': ratio between early mitral inflow velocity and mitral annular early diastolic velocity; SPAP: Systolic pulmonary artery pressure.
† Moderate to severe. *p value <.05.

with both the placebo group (logrank $P = .002$) and KCl patients with below-median change in plasma-potassium (logrank $P < .001$). There was no difference between the below-median group and placebo (logrank $P = .09$, Figure 2c). Cox regression showed an HR of 2.40 (CI 1.36-4.21, $P = .002$) when comparing above median

Table II. Outcomes by *intention-to-treat* analysis.

Variables	All participants		265 mL/h	All KCl	
	Placebo (n = 53)	KCl (n = 60)	KCl (15 mmol/h) (n = 30)	ΔP-potassium below median p-potassium/h (n = 28)	ΔP-potassium above median p-potassium/h (n = 28)
Time to conversion, h	6.8 (1.6-15.5)	5.5 (2.3-12.1)	5.3 (1.8-10.9)	8.1 (3.0-13.2)	5.4 (2.2-11.7)
Number of patients who converted to SR	28 (52.8%)	34 (56.7%)	20 (66.7%)	10 (35.7%)	23** (82.1%)
Infusion time, h	13.0 (4.7-17.2)	9.6 (2.8-16.0)	6.0 (2.2-13.5)	13.8 (9.8-22.7)	5.6* (2.2-10.7)
Total potassium infused, mmol	0	120.1 (34.1-209.4)	90.0 (32.5-202.5)	169.5 (107.8-255)	82 (33.1-143.8)
Pain from the infusion	0	12** (20%)	9*** (30%)	2* (7.1%)	6*** (21.4%)
S-potassium, mmol/L†	4.0 (3.8-4.2)	4.7 (4.3-5.1)	4.6 (4.2-5.1)	4.7 (4.4-5.1)	4.6 (4.2-5.1)
P-potassium, mmol/L†	3.8 (3.6-3.8)	4.3 (4.1-4.6)	4.4 (4.0-4.7)	4.3 (4.2-4.6)	4.4 (4.0-4.7)

Values are median and interquartile range or number and percentage.
*P value <.05; **P value <.01; ***P value <.001
† After stopping the infusion (post-infusion).
All treatment groups were statistically compared with the placebo group.

change with placebo, and HR of 4.41 (CI 2.07-9.40, $P < .001$) compared with below median change.

The group which received KCl at a rate of 15 mmol/h exhibited a greater increase in plasma-potassium (0.07 [0.04-0.18] mmol/L/h) than patients who received 9.4 mmol/h (0.04 [0.03-0.05] mmol/L/h; $P = .015$) and 12 mmol/h (0.04 [0.03-0.06]; $P = .043$). Patients that converted to SR had a higher change in both serum- and plasma-potassium per hour than those that did not convert to sinus rhythm (0.08 [0.04-0.18] and 0.07 [0.03-0.18] mmol/L vs. 0.05 [0.02-0.06] and 0.04 [0.03-0.05] mmol/L, respectively, $P < .01$).

When analyzing whether lower baseline plasma-potassium levels yield higher probability for conversion to SR after KCl infusion, we found that in the KCl group receiving the highest infusion rate of 15 mmol/h, all patients with plasma-potassium at baseline <3.8 mmol/L (n = 13) converted to SR, whereas 70% with plasma-potassium between 3.8-4.0 mmol/L (n = 17) converted to SR.

Atrial flutter. For the small subgroup of patients with atrial flutter, only 2 of 9 patients (22%) converted to SR, and both of these were in the placebo group. However, these findings should be interpreted cautiously due to the small number of atrial flutter patients included in this trial.

Administration of $MgSO_4$. A total of 14 (23.3%) patients in the KCl group were also administered $MgSO_4$; 4 in the 9.4 mmol/h group, 1 in the 12 mmol/h group, and 9 in the 15 mmol/h group. There was no significant difference ($P = .26$) between this subset of patients and those receiving only KCl regarding conversion to SR during the infusion.

Heart rate-reducing medication. Seventy percent of the patients in the KCl group and 91% of the patients in the placebo group received heart rate-reducing medication. In the KCl group, 62% of the patients were treated with metoprolol, 5% with verapamil, and 3% with digoxin, respectively vs. 79%, 2%, and 9%, respectively, in the placebo group. Among the patients who converted to SR, 68% were treated with metoprolol, 6% with verapamil, and 6% with digoxin, respectively, in the KCl group vs. 82%, 0%, and 11%, respectively, in the placebo group.

Follow-up

At the follow-up consultation after discharge, most patients in both groups had SR at rest and during the 3 days with ambulatory ECG recording (Table IV). No patients had atrial flutter. Numerically there was a higher proportion of patients in the placebo group than in the KCl group who exhibited AF at rest or during ambulatory ECG registration. There was also a higher number of contacts with the hospital for AF among patients in the placebo group compared to the KCl group. However, these differences were not significant.

Discussion

This is the first study to investigate the effects of KCl infusion in patients with ROAF or atrial flutter. Our results did not demonstrate any significant effect of potassium infusion on the primary endpoint, time to and frequency of conversion to SR. However, the *per-protocol* analysis indicates that an increase in circulating potassium may increase the likelihood of conversion of ROAF to SR if the patient does not experience pain at the

Table III. Outcomes by *per-protocol* analysis.

Variables	All participants		265 ml/h	All KCl	
	Placebo (n = 48)	KCl (n = 49)	KCl (15 mmol/h) (n = 22)	ΔP-potassium below median p-potassium/h (n = 25)	ΔP-potassium above median p-potassium/h (n = 24)
Time to conversion, h	7 (2-15.6)	5.7 (2.3-12.4)	5.3 (2.2-12.1)	8.1 (3.0-13.2)	5.4 (2.2-11.7)
Number of patients who converted to SR	25 (52.1%)	32 (65.3%)	18* (81.8%)	10 (40.0%)	22** (91.7)
Infusion-time, h	13.4 (5.8-17.6)	12.1 (3.9-17.9)	6.8 (2.2-14.2)	14 (11.8-23.0)	5.7* (2.2-12.9)
Total potassium infused, mmol	0	141.0 (37.1-222.7)	102.6 (33.7-213.7)	180.0 (119.0-242.0)	80.5 (33.1-192.9)
Pain from the infusion	0	2 (4.1%)	2* (9.1%)	1 (4.0%)	1 (4.2%)
S-potassium, mmol/L†	4.0 (3.8-4.2)	4.7 (4.3-5.1)	4.6 (4.2-5.1)	4.7 (4.4-5.1)	4.6 (4.2-5.1)
P-potassium, mmol/L†	3.8 (3.6-3.8)	4.3 (4.1-4.6)	4.4 (4.0-4.7)	4.3 (4.2-4.6)	4.4 (4.0-4.7)

Values are median and interquartile range or number and percentage.
*P value <.05; **P value <.01; ***P value <.001
† After stopping the infusion (post-infusion).
All treatment groups were statistically compared with the placebo group.

infusion site but only if the hourly increase is sufficiently high.

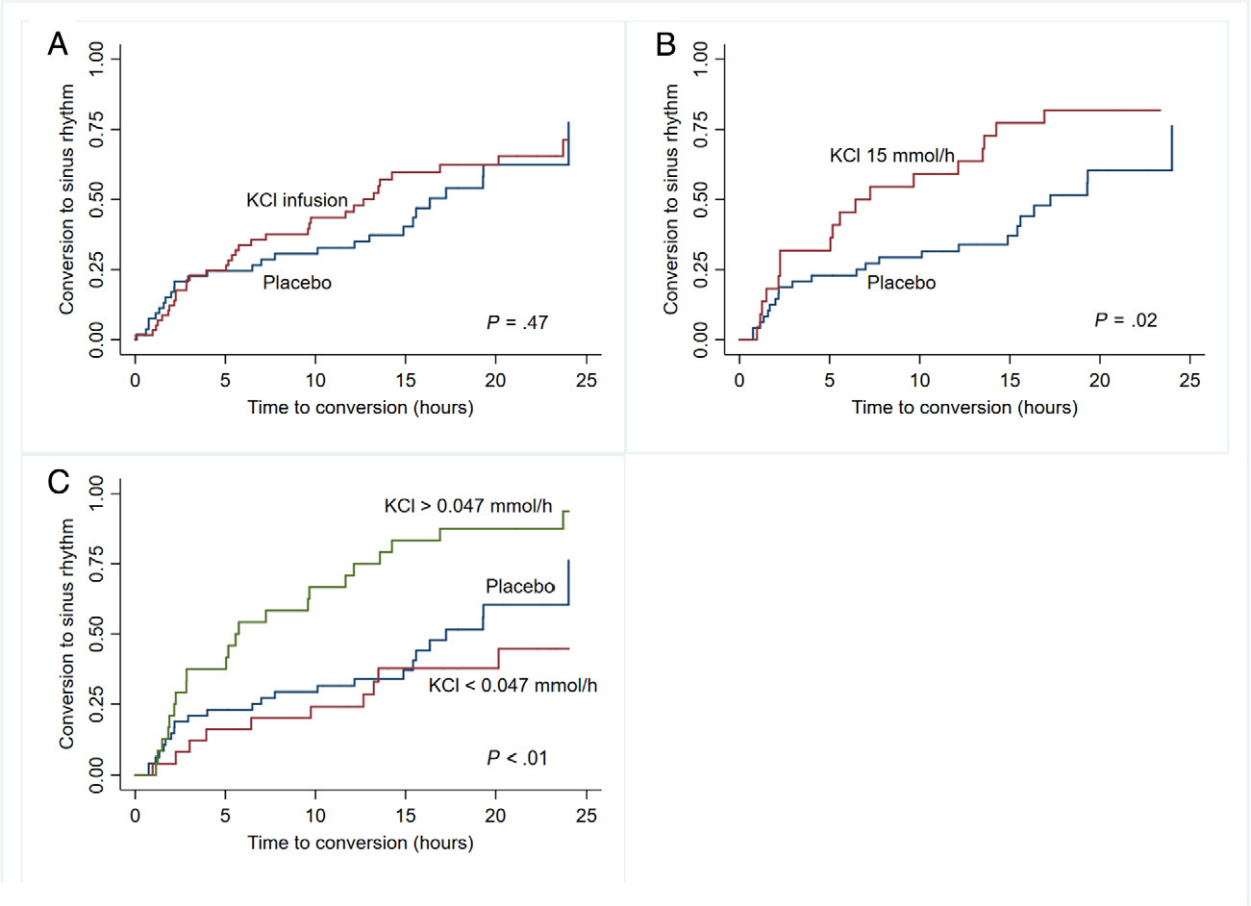
Previous work has shown that roughly 50% of patients with ROAF convert spontaneously to SR within 48 hours,[4, 19] well in accordance with the results in the placebo group in our study. However, few patients with atrial flutter converted to SR indicating that this arrhythmia may be less likely to spontaneously convert to SR and be less affected by potassium correction. Of note, the number of patients in our study who converted to SR after KCl infusion was the same as for the placebo-treated patients. However, in a *post-hoc* analysis, we found that an increase in circulating potassium in our patients with potassium levels in the lower normal range may promote conversion to SR if the speed of potassium increase is sufficiently high, independent of the total amount infused. In fact, the median total amount of potassium infused was lowest in the group of patients receiving this rapid infusion, and there was no significant difference in the final plasma-potassium level between this KCl group and the two other KCl groups. Furthermore, no patients developed clinically significant hyperkalemia despite infusion of up to 366 mmol KCl.

Antiarrhythmic drugs have previously been shown to increase the likelihood of conversion of ROAF to SR to approximately 70% within 12 hours.[6] Interestingly, the patients in our study receiving KCl infusion at a concentration of 15 mmol/h exhibited a similar conversion rate to SR (66.7%) within a median infusion time of just 5.3 hours. Unfortunately, in 17% of the patients receiving KCl infusion at the highest infusion speed, the infusion had to be stopped due to pain at the infusion site. Of the remaining fast-infusion patients that completed the study protocol, the vast majority (82%) converted to SR, and in the group of patients with an increase of plasma-potassium above median levels of increase, as many as 91.7% of these patients and all patients without a history of AF achieved conversion to SR. Furthermore, our results may suggest that KCl infusion is particularly effective in patients with the lowest plasma-potassium levels. Thus, in patients without potassium-induced infusion pain, rapidly applied KCl may be as effective as other antiarrhythmic drugs and does not carry the risk or burden of additional resources inherent with direct-current cardioversion therapy. We therefore suggest that patients with ROAF with plasma-potassium $\leq$4.0 mmol/L are candidates for receiving KCl infusion at 15 mmol/h for up to 12 hours. Indeed, of the patients in our study that experienced a rapid and substantial increase in circulating potassium, 75% converted to SR within this time. However, if a patient experience more than weak pain at the infusion site or if there is only a slight increase in plasma/serum-potassium, the KCl infusion should be terminated and another treatment strategy should be chosen.

None of our patients developed clinically significant hyperkalemia despite infusion of up to 366 mmol KCl. This can be explained by the fact that the healthy kidney has a robust capacity to excrete potassium. Under normal conditions, most persons can ingest very large quantities of potassium (400 mmol per day or more) without clinically significant hyperkalemia. In the kidneys, potassium ingestion stimulates potassium secretion and inhibits potassium reabsorption.[20]

It is known that KCl is vesicant and to avoid this, proper catheter position prior to and during infusion, in addition to a well-mixed infusion bag is important. Despite taking

Figure 2



Kaplan-Meier curve for time to conversion and conversion to sinus rhythm (*per-protocol* analysis). All patients who received KCl infusion (n = 49) versus placebo (n = 48) **(a)**. Patients in the 15 mmol/h group (n = 22) versus placebo (n = 48) **(b)**. All patients who received KCl with a median plasma potassium change per hour above (n = 24) and below (n = 25) median (0.047 mmol/h) versus placebo (n = 48) **(c)**.

these steps, we were surprised that 12 of our patients reported pain at the infusion site, which was alleviated when the infusion was stopped. According to our protocol, staff were instructed to invert the infusion bag 10 times after adding KCl to the dextrose, to be sure that the solution was well-mixed. In addition, the protocol dictated that the peripheral vein catheter was to be placed in the antecubital vein and not at a more distal location. In some of the patients who experienced side-effects, the infusion bag may not have been well mixed, and in a few patients the peripheral vein catheter was placed on back of the hand, not following the study instructions. We suggest that in future investigations the solution should be pre-mixed by a pharmacist.

It is uncertain whether magnesium infusion can promote conversion of AF to SR, as the findings of two previous meta-analysis studies were contradictory.[21, 22] Our patients in the KCl group who exhibited serum-magnesium levels ≤0.8 mmol/L also received magnesium infusion since magnesium depletion reduces intracellular potassium concentration due to renal potassium wasting.[23] However, few patients fit this criterion and received magnesium infusion, and indeed, median serum-magnesium level at the start of the protocol was >0.80 mmol/L in all groups. In addition, magnesium infusion was not associated with altered conversion to SR, suggesting that inclusion of magnesium infusion in a subset of patients did not markedly affect the study results.

It could be argued that the placebo infusion with 50 mg/mL dextrose may decrease plasma-potassium and in this way augment the likelihood that the active arm of KCl infusion would work. However, the reduction of plasma-potassium in the placebo group was very small, with unchanged median values and only a small change in

Table IV. Post-discharge follow-up.

	Placebo (n = 48)	KCl (n = 54)
Time of follow-up after discharge (months), n = 102	3.8 (3.3-4.8)	3.9 (3.3-4.5)
Heart rate, bpm	65 (57-75)	65 (57-76)
ECG at rest, n = 102*		
- Sinus rhythm	43 (89.6%)	49 (92.6%)
- Atrial fibrillation	4 (8.3%)	3 (5.5%)
- Atrial flutter	0	0
Ambulatory ECG, n = 93†		
- Sinus rhythm	37 (86.0%)	48 (96.0%)
- Atrial fibrillation	6 (14.0%)	2 (4.0%)
- Atrial flutter	0	0
Number of contacts with Diakonhjemmet Hospital for atrial fibrillation/atrial flutter after discharge, n = 102*		
- No contacts	36 (67.9.0%)	49 (81.7%)
- One or more times	17 (32.1%)	11 (18.3%)
Total number of ECG-documented atrial fibrillation/atrial flutter, n = 113	25 (47.2%)	15 (25.0%)
History of palpitations during ≥1 hour, n = 102*	17 (35.4%)	12 (22.2%)
Serum-potassium, mmol/L, n = 102*	4.4 (4.2-4.6)	4.3 (4.1-4.4)
Plasma-potassium, mmol/L, n = 101‡	4.1 (3.9-4.3)	4.0 (3.9-4.2)
Difference between serum- and plasma-potassium, mmol/L, n = 101‡	0.3 (0.2-0.3)	0.3 (0.2-0.4)

* Eleven patients did not attend the follow-up consultation.
† Eleven patients did not attend the follow-up consultation, and nine patients did not receive ambulatory ECG monitoring since they exhibited atrial fibrillation in an ECG performed at rest.
‡ Eleven patients did not attend the follow-up consultation. In one patient plasma-potassium was not measured.

IQR from 3.6-4.0 mmol/L to 3.6-3.8 mmol/L. Thus, we believe this very small reduction in plasma-potassium had little effect on our results.

Seventy percent of the patients in the KCl group and 91% of the patients in the placebo group received heart rate-reducing medication after admission. Of these, most received metoprolol while only a few patients received verapamil or digoxin. Among the patients who converted to SR, 68% were treated with metoprolol in the KCl group vs. 82% in the placebo group, indicating that metoprolol did not contribute to the increased conversion rate in the KCl group.

At the post-discharge follow-up, we did not find any significant difference in the number of patients with AF between the KCl and placebo groups. However, this study had a low statistically power to detect such a difference. Moreover, since patients who did not convert to SR after KCl or placebo infusion were treated by direct-current cardioversion the day after the start of infusion, and since plasma- and serum-potassium levels at post-discharge follow-up were similar in the KCl and placebo groups, a significant difference in the frequency of AF is not to be expected. Whether potassium supplementation may have long-term effects in preventing AF in patients with circulating potassium in the lower normal range is not known and should be investigated in future studies.

The electrophysiological effects of potassium depend not only on its extracellular concentration but also on the direction (hypokalemia vs. hyperkalemia) and rate of change.[24] Gradually increasing plasma-potassium induces a biphasic electrical sequence, with initial augmentation and then depression of excitability, conduction, and automaticity.[25] Atrial muscle is more sensitive than ventricular muscle to elevated potassium levels, whereas specialized fibers such as in the sinoatrial node, internodal tracts, and the bundle of His are practically resistant.[26, 27] The presently observed conversion of AF to SR upon increasing potassium levels could thus be due to a transient conduction acceleration which could terminate a re-entrant circuit in the atria.[14, 28] Alternatively, return to SR may result from reduced triggered activity of atrial cardiomyocytes. Previous work in isolated ventricular myocytes has shown that hypokalemic conditions result in inhibition of the Na^+-K^+ ATPase, and subsequent cellular calcium overload associated with generation of both early and delayed afterdepolarizations.[29, 30] If similar pro-arrhythmic alterations occur in atrial myocytes, elevating blood potassium levels may favor a return to SR via a direct cellular action.

We measured plasma-potassium levels for the inclusion of patients, since the use of plasma is superior to serum for accurate determination of potassium.[31] While either plasma or serum can be used to assess potassium using an ion-selective electrode,[32] as presently employed, platelet release from platelets during the clotting process results in serum-potassium levels

which are 0.36 ± 0.18 mmol/L higher in serum than those measured in plasma.[31] This difference is in accordance with our findings of serum- and plasma-potassium levels measured at baseline (Table IV).

Limitations

Our study has several limitations. Firstly, due to slower than expected inclusion rate, five fewer patients in the placebo group were recruited than planned based on the pre-study power analysis. Secondly, since our results may indicate that KCl should be given at a speed of 15 mmol/h, more significant results would have been expected if a greater number of patients had been assigned to this patient group. Thirdly, our beneficial findings are results of a *post-hoc* analysis and thus should be regarded as hypothesis-generating. Fourthly, although earlier studies have shown conflicting results regarding the ability of magnesium to convert ROAF to SR, we cannot rule out a confounding effect of magnesium in our study. However, few patients received magnesium infusion and the treatment was not associated with increased rate of conversion. Nevertheless, our study was not powered to address the effect of parallel infusion of magnesium and KCl. Fifthly, due to our exclusion criteria it is possible that our results do not apply to the whole ROAF population.

Conclusion

Overall, our data do not demonstrate any increase in frequency or rate of conversion of ROAF to SR after increasing plasma-potassium levels in patients with levels in the lower normal range. However, in a *post-hoc* analysis, we found that a rapid increase in circulating potassium may increase the likelihood of conversion of ROAF to SR. However, pain at the infusion site was relatively frequent and thus, not all patients with ROAF are able to complete such a treatment. Our findings are hypothesis driving and need to ultimately be reexamined in a larger study with higher statistical power. If verified, potassium's low-risk profile, electrophysiological effects and few contraindications could make it an adjunct or alternative to acute short-term conventional therapies used for conversion of ROAF.

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Declarations of interest

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ahj.2019.12.014.

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III

Circulation Research

ORIGINAL RESEARCH

Hypokalemia Promotes Arrhythmia by Distinct Mechanisms in Atrial and Ventricular Myocytes

Kiarash Tazmini,* Michael Frisk,* Alexandre Lewalle, Martin Laasmaa, Stefano Morotti, David B. Lipsett, Ornella Manfra, Jonas Skogested, Jan M. Aronsen, Ole M. Sejersted, Ivar Sjaastad, Andrew G. Edwards, Eleonora Grandi, Steven A. Niederer, Erik Øie, William E. Louch

RATIONALE: Hypokalemia occurs in up to 20% of hospitalized patients and is associated with increased incidence of ventricular and atrial fibrillation. It is unclear whether these differing types of arrhythmia result from direct and perhaps distinct effects of hypokalemia on cardiomyocytes.

OBJECTIVE: To investigate proarrhythmic mechanisms of hypokalemia in ventricular and atrial myocytes.

METHODS AND RESULTS: Experiments were performed in isolated rat myocytes exposed to simulated hypokalemia conditions (reduction of extracellular $[K^+]$ from 5.0 to 2.7 mmol/L) and supported by mathematical modeling studies. Ventricular cells subjected to hypokalemia exhibited Ca^{2+} overload and increased generation of both spontaneous Ca^{2+} waves and delayed afterdepolarizations. However, similar Ca^{2+}-dependent spontaneous activity during hypokalemia was only observed in a minority of atrial cells that were observed to contain t-tubules. This effect was attributed to close functional pairing of the Na^+-K^+ ATPase and Na^+-Ca^{2+} exchanger proteins within these structures, as reduction in Na^+ pump activity locally inhibited Ca^{2+} extrusion. Ventricular myocytes and tubulated atrial myocytes additionally exhibited early afterdepolarizations during hypokalemia, associated with Ca^{2+} overload. However, early afterdepolarizations also occurred in untubulated atrial cells, despite Ca^{2+} quiescence. These phase-3 early afterdepolarizations were rather linked to reactivation of nonequilibrium Na^+ current, as they were rapidly blocked by tetrodotoxin. Na^+ current-driven early afterdepolarizations in untubulated atrial cells were enabled by membrane hyperpolarization during hypokalemia and short action potential configurations. Brief action potentials were in turn maintained by ultra-rapid K^+ current (I_{Kur}); a current which was found to be absent in tubulated atrial myocytes and ventricular myocytes.

CONCLUSIONS: Distinct mechanisms underlie hypokalemia-induced arrhythmia in the ventricle and atrium but also vary between atrial myocytes depending on subcellular structure and electrophysiology.

VISUAL OVERVIEW: An online visual overview is available for this article.

Key Words: arrhythmia ■ calcium signaling ■ electrophysiology ■ hypokalemia ■ ion channel

Meet the First Author, see p 808

Hypokalemia is a common electrolyte disturbance, which is present in over 20% of hospitalized patients.[1] Defined as serum $[K^+]$ <3.6 mmol/L, hypokalemia is associated with in-hospital mortality rates that are 10-fold higher than that of the entire hospitalized population.[2] The increased mortality is linked to a 5-fold elevated incidence of ventricular fibrillation in patients with hypokalemia compared with patients with normokalemia.[3] Patients with hypokalemia also have a higher risk of atrial fibrillation; an association that is independent of age, sex, serum $[Mg^{2+}]$, and other potential confounders.[4] Understanding the proarrhythmic role of hypokalemia in

Correspondence to: William E. Louch, PhD, Institute for Experimental Medical Research, Oslo University Hospital Ullevål, Kirkeveien 166, NO-0407 Oslo, Norway. Email w.e.louch@medisin.uio.no

*K.T. and M.F. contributed equally to this article.

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For Sources of Funding and Disclosures, see page 904.

ORIGINAL RESEARCH

Novelty and Significance

What Is Known?

- Hypokalemia is a common electrolyte disturbance particularly in hospitalized patients and is associated with higher mortality rates as well as elevated risk of both ventricular and atrial fibrillation.
- In ventricular fibrillation, the association with hypokalemia has been attributed to the direct effects of reduced extracellular potassium levels on ventricular myocytes, as it promotes development of calcium overload and associated electrical instability (early and delayed afterdepolarizations).
- It has been unclear whether a similar mechanistic relationship exists in atrial myocytes, linking hypokalemia to increased risk of atrial fibrillation.

What New Information Does This Article Contribute?

- We observed that calcium overload and accompanying calcium waves/delayed afterdepolarizations are robustly induced in hypokalemic ventricular cells, and in a subpopulation of atrial cells that contain t-tubules; membrane invaginations where calcium extrusion is slowed during hypokalemia.
- However, in untubulated atrial cardiomyocytes, hypokalemia induces early afterpolarizations due to sodium current reactivation, enabled by brief action potentials and membrane hyperpolarization.
- This important mechanistic distinction seems to hinge on the exclusive presence of ultra-rapid K^+ current (I_{Kur}) in untubulated atrial cells, which maintains brief action potential configuration.

Why do hypokalemic individuals have a higher risk of developing atrial fibrillation? Our results show that lowered extracellular potassium levels in the physiological range predispose atrial myocytes to electrical instability. We demonstrate two distinct mechanisms, depending on the presence or absence of t-tubules in these cells. Since t-tubule density varies across the atrium, our findings suggest that there may be complex regional differences in arrhythmia generation during hypokalemia. Furthermore, the realization that untubulated atrial myocytes develop over-activity by a mechanism which is distinct from the ventricle suggests that ventricular and atrial fibrillation may be differentially targeted by tailored treatments. Indeed, hypokalemic individuals with atrial fibrillation may constitute a unique patient pool that would benefit from personalized therapy. Given the atria-specific role of I_{Kur}, our results suggest that existing I_{Kur} inhibitors may be of unrealized benefit in this group of patients. Alternatively, potassium infusion may be considered as recent results indicate that this treatment can revert hypokalemic atrial fibrillation patients to sinus rhythm. These findings support a growing appreciation of the role of potassium homeostasis in maintaining electrical stability of both the ventricles and atria.

Nonstandard Abbreviations and Acronyms

AP	action potential
CaMKII	Ca^{2+}-calmodulin kinase II
DAD	delayed afterdepolarization
EAD	early afterdepolarization
IKur	ultra-rapid K^+ current
NCX	Na^+-Ca^{2+} exchanger
NKA	Na^+, K^+-ATPase
SR	sarcoplasmic reticulum

these 2 conditions thus has important implications for preventative treatment in a large number of patients.

The arrhythmogenic effects of hypokalemia have been suggested to result from an increased propensity for ectopic (triggered) activity. While the underlying mechanisms in atrial cardiomyocytes are unclear, considerable evidence from ventricular myocytes has linked triggered activity during hypokalemia to dysregulated cellular Ca^{2+} homeostasis. Initially, reduction in extracellular K^+ levels ($[K^+]_o$) decreases the magnitude of Ca^{2+} transients, as rapid hyperpolarization of the cell membrane augments driving force for Na^+-Ca^{2+} exchanger (NCX)-mediated Ca^{2+} extrusion and temporarily reduces sarcoplasmic reticulum (SR) Ca^{2+} content and release.[5,6] However, lowered K^+ levels also inhibit the Na^+, K^+-ATPase (NKA), reducing NCX-mediated Ca^{2+} extrusion and leading to progressive cellular Ca^{2+} overload.[7–10] Thus, the overall pattern of change in Ca^{2+} transients is biphasic, as the eventual increase in SR Ca^{2+} content at steady state produces larger Ca^{2+} transients than are present in normokalemic conditions.[6] Spontaneous Ca^{2+} release from the overloaded SR is removed from the cell by NCX, resulting in a phasic depolarization classified as an early afterdepolarization (EAD) or delayed afterdepolarization (DAD), depending on whether it occurs during the action potential (AP) or between beats. EADs are additionally promoted by prolongation of the AP during hypokalemia, which occurs as repolarizing K^+ currents are inhibited by lowered $[K^+]_o$.[7] The longer AP allows recovery of L-type Ca^{2+} channels from inactivation, and recent evidence has suggested that activation of CaMKII (Ca^{2+}-calmodulin kinase II) by rising Ca^{2+} levels may additionally favor L-type channel reactivation.[11]

Although hypokalemia is also associated with atrial fibrillation, it is uncertain if and how hypokalemia promotes triggered activity in atrial cardiomyocytes. Myocyte structure and Ca^{2+} homeostasis are fundamentally different in ventricular and atrial myocytes, as atrial cells generally exhibit a low, but variable density of t-tubules and associated Na^+ and Ca^{2+} handling proteins.[12,13] Thus, it is not intuitive that exposure of atrial cells to hypokalemic conditions will result in Ca^{2+} overload in a manner resembling ventricular cells. Furthermore, the AP is much briefer in atrial cells, which might deter EAD generation due to delayed repolarization and reactivation of L-type Ca^{2+} channels. However, recent work has indicated that cells with abbreviated APs may be susceptible to phase-3 EADs driven by nonequilibrium reactivation of Na^+ current (I_{Na}).[14,15] It is unknown if hypokalemia would facilitate this mechanism.

In the present work, we identify distinct mechanisms which lead to triggered activity in ventricular and atrial cardiomyocytes during hypokalemia. We show that Ca^{2+} overload and accompanying Ca^{2+} waves/DADs are robustly induced in hypokalemic rat ventricular cells, and in a subpopulation of atrial cells which contain t-tubules. These effects are attributed to a close functional pairing of NKA and NCX proteins within these structures. While EADs are induced by both Ca^{2+} waves and L-type channel reactivation in ventricular cells, we show that brief APs in untubulated hypokalemic atrial cells promote phase-3 EADs during hypokalemia driven by nonequilibrium I_{Na} reactivation.

METHODS

The data that support the findings of this study are available from the corresponding author upon reasonable request.

A detailed methods description and Major Resources Table are provided in the Data Supplement.

This study was approved by the Norwegian Animal Research Authority (FDU application number 7786) under the Norwegian Animal Welfare Act and conformed with Directive 2010/63/EU of the European Parliament.

Recordings of Ca^{2+} Transients and Waves

Myocytes were isolated from the left ventricle and both left and right atrium of male Wistar rats, as described previously.[12] Cells were then loaded with 20 μM fluo-4 AM (Molecular Probes, Eugene, OR) for 10 minutes, plated on laminin-coated coverslips, and placed in a superfusion chamber mounted on an inverted microscope. Characterization of whole-cell Ca^{2+} transients was performed during field-stimulation at 1 Hz. In an initial 2-minute control period, myocytes were superfused at 37°C with a solution containing (in millimolar): NaCl 140, KCl 5.0, $MgCl_2$ 0.5, NaH_2PO_4 0.4, $CaCl_2$ 1.0, Hepes 5, D-glucose 5.5, pH adjusted to 7.4 with NaOH. Hypokalemia was then simulated by rapidly reducing $[K^+]_o$ from 5.0 to 2.7 mmol/L via a rapid-solution changer for the ensuing 3 minutes. In follow-up experiments, the incidence of Ca^{2+} waves was investigated during a 30-second pause in the stimulation. SR Ca^{2+} content was estimated by rapid application of 10 mmol/L caffeine (Sigma-Aldrich) and measuring the magnitude of the resulting Ca^{2+} transient. Ca^{2+} transients elicited by 1 Hz field stimulation during continuous caffeine superfusion were employed to examine rates of Ca^{2+} extrusion.[16]

T-Tubule Imaging in Isolated Cardiomyocytes

Quantification of t-tubule structure in isolated ventricular and atrial myocytes was performed by staining cells with 10 μmol/L di-8-ANEPPS (Invitrogen, Paisley, United Kingdom) or with CellMask Orange (1:1000 dilution; Thermo Fisher Scientific, Waltham, MA; C10045). For each cell, t-tubule density was determined by thresholding the image intensity of the entire cell by the Otsu method, using an automated algorithm in ImageJ (National Institutes of Health). The t-index[17] was then calculated for the myocyte interior, defined as the percentage of the cellular cross-sectional area, excluding the nucleus, occupied by above-threshold pixels. Cells exhibiting a t-index of ≥ 2% were defined as being tubulated.[12] Detubulation was performed using a protocol similar to that described by Kawai et al.[18]

Monitoring of Intracellular Na^+

Intracellular Na^+ levels were assessed in myocytes loaded with SBFI AM (Thermo Fischer) and 0.15% Pluronic F-127 for 45 minutes.[16,19] Cardiomyocytes were field stimulated and superfused with the same normokalemic and hypokalemic solutions as used in the fluo-4 experiments (1 Hz pacing, 37°C).

Immunocytochemistry and Imaging

Isolated cardiomyocytes were seeded on coated coverglasses, before fixation with 4% paraformaldehyde, and permeabilization with 0.5% Triton X-100 (Sigma-Aldrich). The following primary and secondary antibodies were used at the indicated dilutions: NCX (Swant, R3F1, 1:100), NKA-α1 (Merck Millipore, 05-369, 1:100), NKA-α2 (Merck Millipore, 07-674, 1:100), F(ab')2-goat anti-mouse IgG (H+L) secondary antibody (Alexa Fluor 488, Thermo Fisher, A-11017, 1:200), and F(ab')2-goat anti-mouse IgG (H+L) secondary antibody (Alexa Fluor 546, Thermo Fisher, A-11071, 1:200). Cells were imaged on an LSM-800 confocal microscope (Carl Zeiss AG, Oberkochen, Germany) in Airyscan mode.

Immunocytochemistry was additionally performed on rapidly excised ventricular and atrial tissue, frozen in Tissue Tek O.C.T. compound (Sakura Fintek, Torrance, CA). Ten micrometer tissue sections were transferred to microscope slides, fixed in 4% paraformaldehyde, and permeabilized with 0.5% Triton X-100 (Merck). Subsequent antibody labeling and Airyscan imaging were performed as described above for isolated cardiomyocytes. T-tubules were labeled in intact cardiac tissue using caveolin-3 antibody (Abcam, ab2912) at 1:100 dilution. For all employed immunolabels, imaging was performed with secondary antibodies alone to exclude the influence of nonspecific fluorescence.

Patch-Clamp Experiments

Both current- and voltage-clamp experiments were performed using 1 to 2 MΩ pipettes, an Axoclamp-2B amplifier (Axon Instruments, Foster City, CA), and pCLAMP software (Axon Instruments). APs were recorded in bridge-mode

and elicited by 3 ms supra-threshold current steps at 1 Hz. Effects of inhibition of ultra-rapid K^+ current (I_{Kur}) on AP configuration were assessed during rapid application of 50 µmol/L 4-aminopyridine (4-AP). This concentration has been previously shown to almost completely block I_{Kur} while having minimal influence on other currents such as transient outward K^+ current (I_{TO}).[20,21] EADs were defined as positive voltage deflections during the downstroke of AP repolarization with a minimum amplitude of 2 mV. DADs were defined as minimum 2 mV depolarizing deflections from resting potential. AP duration was measured as time from the upstroke to 25%, 50%, and 75% repolarization (APD_{25}, APD_{50}, APD_{75}), and full duration was defined as repolarization to 5 mV above resting membrane potential.

Membrane currents were recorded in discontinuous voltage-clamp, using a switching rate of 8 kHz. NKA currents were recorded based on a described protocol,[6] using hyperpolarizing voltage ramps from +70 to −120 mV, and defined as the reduction in current when extracellular K^+ was rapidly removed (illustrated in Online Figure IA and IB). Steady-state K^+ currents were measured at the end of 500 ms depolarizing voltage steps from −70 mV to a range of potentials (illustrated in Online Figure IIA). In further experiments, the contribution of I_{TO} to steady-state current was inhibited using a 1 second inactivating voltage step from −70 mV to +50 mV, before test steps to a range of potentials (illustrated in Online Figure IIIA, described in study by Wang et al[22]). NCX activity was examined using the tail current elicited by repolarization following a 100 ms voltage step from −45 to 0 mV (illustrated in Online Figure IV).[6]

Western Blotting and PCR Analyses

Expression of KCNA5/K_v1.5 was examined by qPCR and Western blotting, as described in study by Røe et al[23] and Lipsett et al[24], respectively.

Mathematical Modeling

A computational simulation of the time-dependent effects of hypokalemia on Na^+ and Ca^{2+} homeostasis was performed by adapting the model of Terkildsen et al[25] wherein a set of ordinary differential equations describes the electrophysiology of the rat ventricular cardiomyocyte. For the purpose of this study, crosstalk between NCX and NKA activity was simulated by including a new dimensionless parameter NCX_{rev} to scale the intracellular Na^+ concentration (Na_i) sensed by the exchanger:

$$I_{NCX} = \frac{g_{NCX}\left(NCX_{rev} \times e^{\eta FV/(RT)} Na_i^3 Ca_o - e^{(\eta-1)FV/(RT)} Na_o^3 Ca_i\right)}{\left(Na_o^3 + K_{mNa}^3\right)\left(Ca_o + K_{mCa}\right)\left(1 + k_{sat} e^{(\eta-1)FV/(RT)}\right)}$$

Increasing NCX_{rev} thus effectively augmented the proportion of exchanger activity operating in reverse mode, as expected when NCX is localized closer to NKA and higher local Na^+ levels. Hypokalemia experiments were simulated by instantaneously changing $[K^+]_o$ from 5.0 to 2.7 mmol/L. After each modification of the model, steady state was reached by integrating the model equations over 10 000 beats.

Computational analysis of atrial myocyte electrophysiology was performed using our established human atrial cell model,[26] recently updated with a Markov model of I_{Na}.[14] Experimental voltage recordings were used as inputs in AP-clamp simulations to reveal underlying dynamics of Na^+ and Ca^{2+} currents. Model code is available for download at: https://somapp.ucdmc.ucdavis.edu/Pharmacology/bers/ or http://elegrandi.wixsite.com/grandilab/downloads.

Statistics

All data were tested for normality of distribution using a Shapiro-Wilk test. Normally distributed data were compared with Student *t*-test or ANOVA with Bonferroni correction for multiple comparisons, as appropriate. Non-normal distributions were examined with a Wilcoxon signed-rank test, Mann-Whitney rank-sum test, or Kruskal-Wallis analysis on ranks with Dunn correction for multiple comparisons. Two-factor comparisons were performed by 2-way ANOVA with Bonferroni correction; results of post hoc comparisons are presented in the Online Tables. Differences in proportions were determined by *z*-test. *P* values <0.05 were considered statistically significant. All data were analyzed by Sigmaplot software (Systat Software, Chicago) and are presented as mean±SE.

RESULTS

Effects of Hypokalemia on Ca^{2+} Transients and Waves in Ventricular and Atrial Cardiomyocytes

Effects of hypokalemia on isolated rat ventricular and atrial cardiomyocytes were simulated by lowering $[K^+]_o$ from 5.0 to 2.7 mmol/L for 3 minutes, during continuous 1 Hz pacing. In agreement with previous work,[6] we observed that ventricular myocytes exhibited a biphasic change in Ca^{2+} transient amplitude (Figure 1A). Lowering of $[K^+]_o$ was associated with an initial depression of Ca^{2+} transients, followed by a rising phase which ultimately yielded larger transients than present in normokalemia. This second phase of the response was associated with an increased incidence of spontaneous Ca^{2+} waves when the stimulation was paused (Figure 1B).

More variable effects of hypokalemia were observed in atrial cardiomyocytes; while some of these cells (13 of 31 cells) exhibited a biphasic response similar to that observed in ventricular cells, other cells showed only a monophasic decline, with a steady-state reduction in Ca^{2+} transient amplitude (Figure 1A). This variability in the response to lowered $[K^+]_o$ was comparable in cells isolated from the left and right atria, as biphasic responses were observed in 59% and 38% of cells, respectively. In keeping with observations in ventricular cells, atrial cells which displayed a biphasic response leading to larger Ca^{2+} transients exhibited an increased incidence of spontaneous Ca^{2+} waves (Figure 1B). However, Ca^{2+} wave frequency was not increased in atrial cells which exhibited a monophasic reduction in Ca^{2+} transients.

The Biphasic Response to Hypokalemia Is Dependent on T-Tubules

As recent reports have shown that t-tubule organization is variable between individual atrial cells,[12,27–29] we

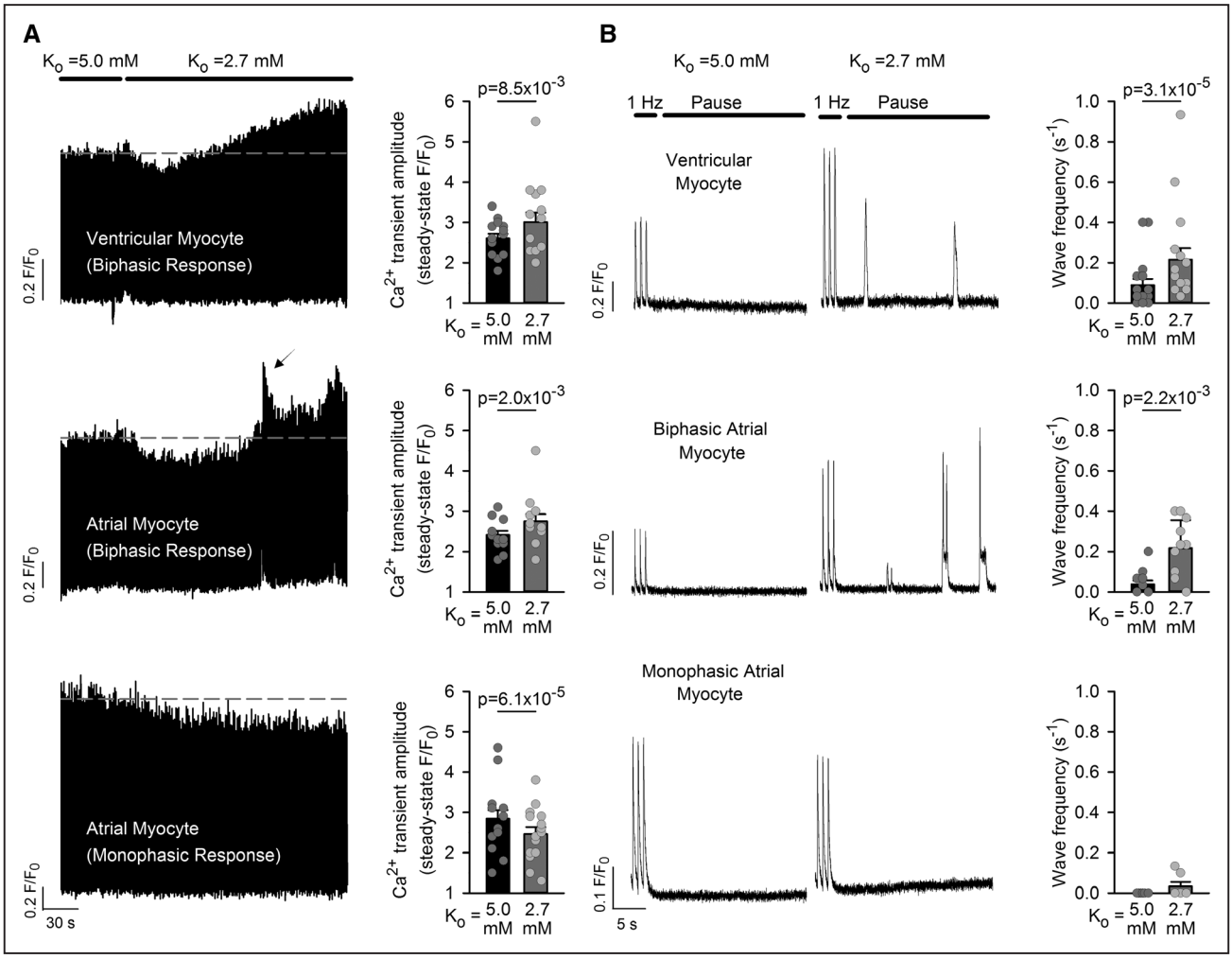

Figure 1. Hypokalemia promotes a steady-state increase in Ca^{2+} transients and Ca^{2+} waves in ventricular myocytes and a subpopulation of atrial myocytes.

A, In field-stimulated ventricular cells, rapidly lowering $[K^+]_o$ from 5.0 to 2.7 mmol/L produced an initial depression of Ca^{2+} transient magnitude. A secondary rising phase followed which ultimately yielded larger Ca^{2+} transients compared with control conditions (right, n=15 cells, 8 hearts). A similar biphasic response to hypokalemia was observed in some atrial cardiomyocytes (13 of 31 cells, 10 hearts), with associated over-activity (arrow). Other atrial cells exhibited only a monophasic reduction in Ca^{2+} transient amplitude. **B**, Ca^{2+} waves were assessed during pauses in the electrical stimulus. Ventricular cells and those atrial cells which exhibited a biphasic response demonstrated an increased frequency of Ca^{2+} waves during hypokalemia. For Ca^{2+} wave measurements, n_{cells}=17, 7, 12; n_{hearts}=10, 5, 11 in ventricular, biphasic atrial, and monophasic atrial populations. Statistics: Wilcoxon signed-rank test.

investigated whether such differences could account for the differing effects of hypokalemia on Ca^{2+} transients and waves. Imaging in intact tissue and isolated cells showed that while all ventricular myocytes exhibited a high density of well-organized t-tubules, atrial tissue contained distinct populations of tubulated and untubulated myocytes (Figure 2A and 2B). In those atrial cells that were tubulated, t-tubule density remained well below that of ventricular cells (Figure 2C), and tubules were generally disorganized in appearance. Paired imaging of t-tubules and Ca^{2+} revealed that only tubulated atrial myocytes showed a biphasic response to hypokalemia (n_{cells}=12), while untubulated cells exhibited a monophasic, steady-state reduction in Ca^{2+} transient amplitude (n_{cells}=7; Figure 2D). Furthermore, experimentally detubulating ventricular cells by osmotic shock reproduced the monophasic decline in Ca^{2+} transients observed in untubulated atrial cells (t-tubule density: 18.2±0.7% in control versus 3.4±0.3% in detubulated, n_{cells}=40 control versus 40 detubulated, P=1.81×10^{-22} by paired t-test; Ca^{2+} transient amplitude: F/F_0=2.35±0.19 in normokalemia versus 2.01±0.13 at 3-minute hypokalemia, n_{cells}=10, P=5.12×10^{-3} by Wilcoxon signed-rank test). These data support that the presence of t-tubules promotes the proarrhythmic biphasic response of Ca^{2+} transients to hypokalemia.

T-Tubular NKA-NCX Crosstalk Drives Ca^{2+}-Dependent Arrhythmogenesis

Previous work in ventricular myocytes suggested that reduced activity of the NKA during hypokalemia promotes intracellular Na^+ accumulation sensed by NCX,

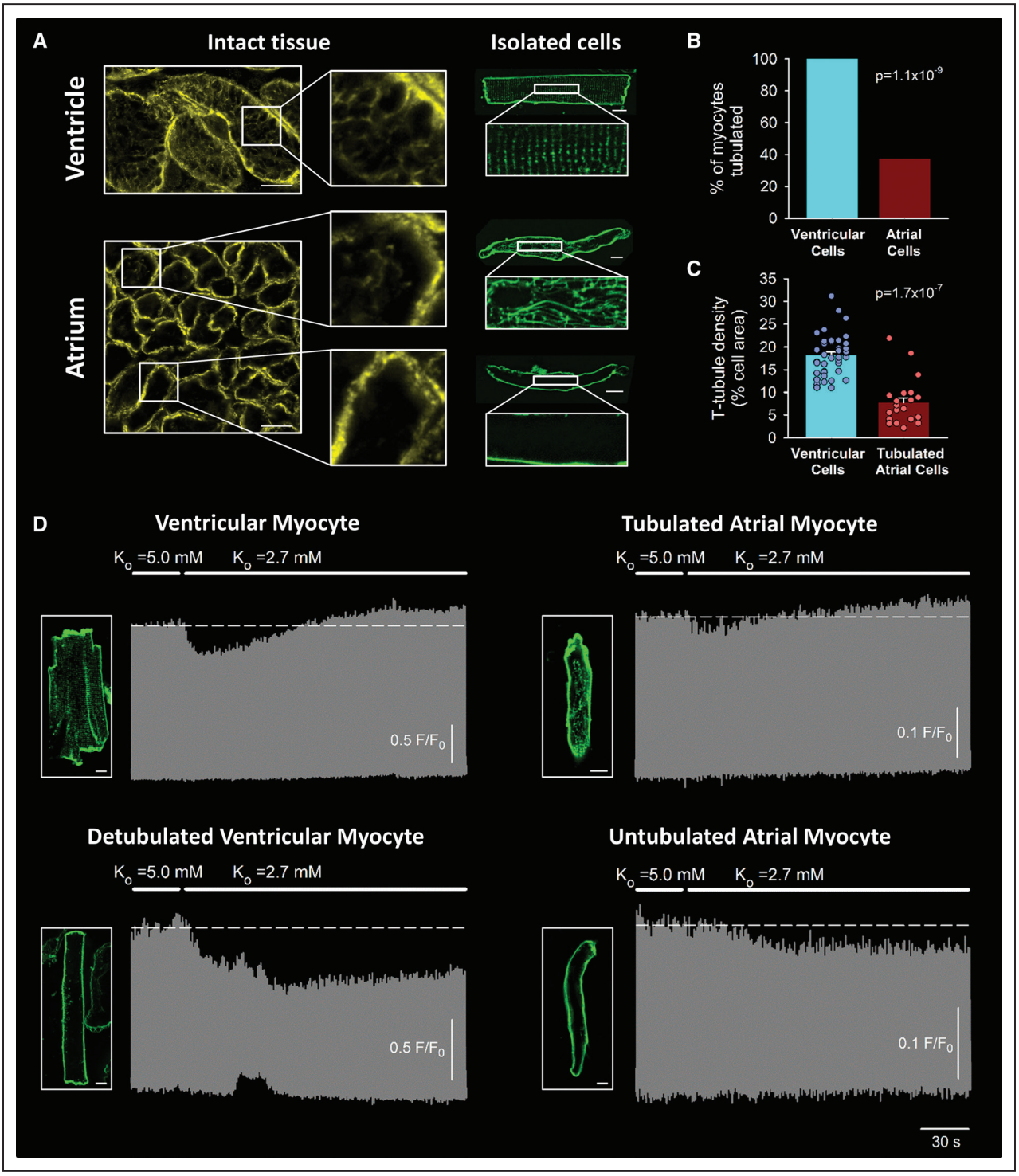

Figure 2. The biphasic Ca^{2+} transient response to hypokalemia requires t-tubules.
A, T-tubule staining in intact tissue and isolated cells (caveolin-3 and di-8-ANEPPS, respectively) revealed a dense and well-organized t-tubule network in ventricular cells (scale bars=10 μm). T-tubules were only observed in approximately one-third of atrial cells (**B**, 21 of 56 cells, 4 hearts), and when present, these tubules were less well organized and at lower density (**C**) than in ventricular cells (40 cells, 3 hearts). **D**, Paired imaging of t-tubules and Ca^{2+} revealed that only tubulated atrial cells exhibited a biphasic response to hypokalemia (n=12 cells, 10 hearts), while untubulated cells exhibited a monophasic decline in Ca^{2+} transient amplitude (n=7 cells, 5 hearts). A similar monophasic decline in Ca^{2+} transients was reproduced in experimentally detubulated ventricular myocytes (n=10 cells, 3 hearts). Statistics: (**B**): *z*-test; (**C**): Mann-Whitney rank-sum test.

leading to reduced Ca^{2+} extrusion and gradual loading of the SR with Ca^{2+}.[6,7] While both NKA and NCX are highly localized in t-tubules in the ventricle, the distribution of these transporters in atrial cells is unknown. Immunolabeling and Airyscan imaging of intact tissue and isolated cells confirmed the presence of the α_1 NKA isoform in

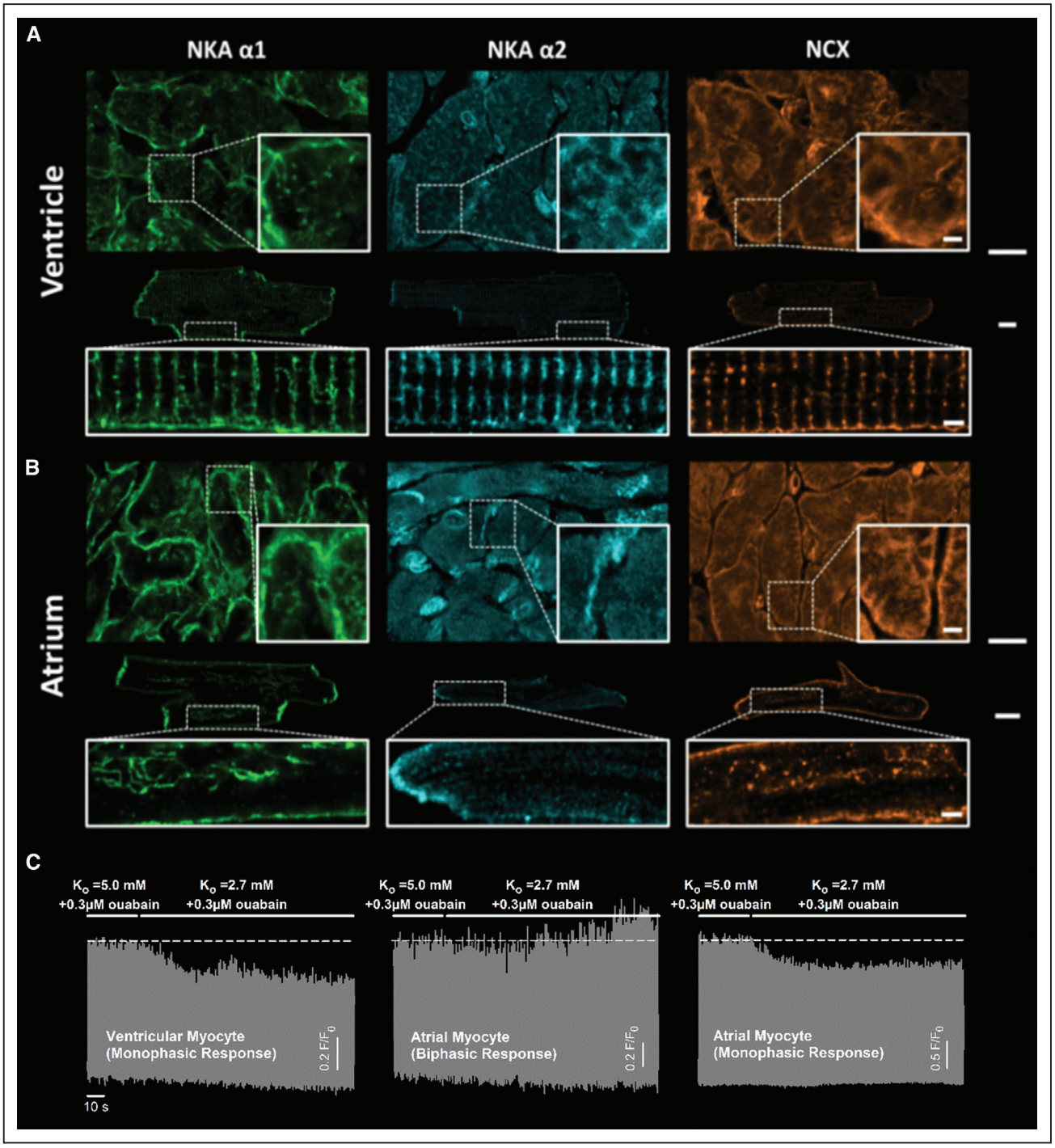

Figure 3. The NKA (Na^+, K^+-ATPase) and Na^+-Ca^{2+} exchanger (NCX) are robustly expressed within the t-tubules of both ventricular and atrial cardiomyocytes.

A, Immunolabeling in intact tissue and isolated ventricular cells showed a robust expression of the α_1 NKA isoform in both the surface membrane and t-tubules, while the α_2 isoform was preferentially expressed in t-tubules. **B**, In atrial cells, NKA α_1 was expressed in both the surface membrane and, when present, the t-tubules. NKA α_2 expression was low in atrial cells, never present in t-tubules, and appeared limited to the intercalated disks. NCX (right) was robustly expressed in both the surface membrane and t-tubules of ventricular and atrial cells. **C**, In ventricular cells, preferential blockade of NKA α_2 with 0.3 μmol/L ouabain[19] inhibited the biphasic response of Ca^{2+} transients to hypokalemia (5 of 5 cells). In atrial cells, both biphasic (7 cells) and monophasic responses (3 cells) continued to be observed in the presence of low-dose ouabain, in agreement with limited α_2 expression in these cells. Scale bars in (**A**) and (**B**)=10 μm in zoomed-out images, 2 μm in enlargements.

both the surface membrane and t-tubules of ventricular cells and showed that this isoform was similarly localized in tubulated atrial cells (Figure 3A and 3B). Of note, untubulated atrial cells also expressed α_1 NKA in the surface membrane. The α_2 NKA isoform was robustly present in ventricular cells, with particular prevalence within

t-tubules, as reported previously (Figure 3A).[19,30] However, NKA α_2 expression was very low in both tubulated and untubulated atrial myocytes; staining was limited to the intercalated disk regions adjoining neighbouring cells, and no expression was observed in t-tubules when they were present (Figure 3B). This finding suggests that the presence of the α_2 NKA isoform in t-tubules is not a requirement for a biphasic response to hypokalemia. Indeed, preferential blockade of this isoform with low-dose ouabain (0.3 µmol/L, see study by Swift et al[19]) inhibited the biphasic response in ventricular cells but not in tubulated atrial cells (Figure 3C).

Measurements of NKA activity also did not identify differences between tubulated and untubulated cells which could account for distinct responses to hypokalemia. NKA current, measured as the K^+-sensitive current obtained during hyperpolarizing voltage ramps (protocol illustrated in Online Figure I), was similarly reduced during hypokalemia in ventricular, tubulated, and untubulated atrial cells (Figure 4A). This modest inhibition of NKA activity was insufficient to elevate global cytosolic Na^+ levels during 3 minutes of hypokalemia, as assessed by experiments with the Na^+-sensitive dye SBFI (Figure 4B, protocol illustrated in Online Figure VB). Maintenance of global $[Na^+]$ during hypokalemia paralleled a similar lack of increase in resting $[Ca^{2+}]$ in both ventricular and atrial myocytes (resting $F_{Ko=2.7}$/resting $F_{Ko=5.0}$=1.02±0.02 in ventricle, 1.03±0.01 in atria, n_{cells}=15, 30; P=0.542, 0.054 by Wilcoxon signed-rank test, paired t-test). Experiments with low-dose ouabain, as described in Figure 3C, also failed to increase global $[Na^+]$, despite a demonstrated ability of SBFI to detect concentration changes as small as 1 mmol/L (Online Figure VA).

Since NKA function alone could not account for the key role of t-tubules in promoting Ca^{2+} overload during hypokalemia, we next examined NCX localization and function. As expected, immunolabeling in intact ventricular tissue and isolated cells revealed dense NCX localization in surface membrane and t-tubules (Figure 3A). Similar staining was observed in atrial cells, with robust staining in the t-tubules, when they were present (Figure 3B). NCX activity was assessed by fitting the decay phase of the Ca^{2+} transients stimulated in the continuous presence of caffeine. Ventricular cells exhibited slowed Ca^{2+} extrusion by NCX during hypokalemia (Figure 4C, left, P=0.016 versus K_o=5.0 mmol/L by Wilcoxon signed-rank test). A similarly slowed time course of NCX-mediated Ca^{2+} removal was observed in patch-clamped ventricular myocytes, measured during the tail current following triggering of L-type Ca^{2+} current (Online Figure IV). This finding suggests that although no change in global cytosolic Na^+ levels was detected, NKA inhibition during hypokalemia leads to Na^+ accumulation which is sensed by t-tubular NCX, perhaps due to close co-localization of the proteins. A resulting reduction in NCX-mediated Ca^{2+} removal during hypokalemia was associated with elevation of SR Ca^{2+} content (Figure 4D, left, protocol illustrated in Online Figure VIA), providing the basis for the observed steady-state increase in Ca^{2+} transients and Ca^{2+} waves in ventricular cells. Similar observations were made in tubulated atrial cells, as reduced Ca^{2+} removal by NCX during hypokalemia (Figure 4C) was also associated with elevation of SR Ca^{2+} content (Figure 4D). In untubulated atrial cells, however, NCX-mediated Ca^{2+} removal was not slowed at the conclusion of the hypokalemic period. Any local elevation of Na^+ was therefore not sensed by NCX, while augmented Ca^{2+} removal by NCX, particularly during early hypokalemia, lead to reduced SR Ca^{2+} content (Figure 4D), small steady-state Ca^{2+} transients (monophasic response, Figure 1A), and low incidence of Ca^{2+} waves (Figure 1B).

We further investigated the role of t-tubular NKA-NCX crosstalk in driving Ca^{2+} overload during hypokalemia by employing a mathematical model of the rat ventricular cardiomyocyte (adapted from study by Terkildsen et al[25]). In agreement with observations in cellular experiments, reducing $[K^+]_o$ from 5.0 to 2.7 mmol/L in the model produced a biphasic response of both SR Ca^{2+} content and Ca^{2+} transient magnitude (Figure 5A). However, under baseline conditions, the time course of the rising phase of the response was markedly slower than that observed experimentally, perhaps reflecting the lack of accommodation for protein colocalization in the model. To simulate a closer relationship between NKA and NCX with a shared local pool of Na^+, the fraction of NCX operating in reverse-mode (NCX_{rev}) was increased in a stepwise manner. This intervention resulted in a stepwise augmentation and acceleration of the biphasic response (Figure 5A and 5B) and slowing of the decay phase of the Ca^{2+} transient (Figure 5D), as the modest rise in cytosolic Na^+ levels induced by NKA inhibition was sensed by NCX. The necessity for such sensing was further demonstrated by removing reverse-mode NCX function in the model, which resulted in a monophasic decline in Ca^{2+} transient magnitude and no change in Ca^{2+} decay kinetics (Figure 5A and 5D), resembling responses in untubulated myocytes. Sensitivity analyses indicated that the biphasic response could not be similarly inhibited by reducing other t-tubular ion fluxes (Na^+ and L-type Ca^{2+} currents; Figure 5C), further supporting a cooperative and central role of NKA and NCX in driving Ca^{2+} overload. A schematic summarizing these findings is shown in Figure 8.

EADs Are Driven by Distinct Mechanisms in Subpopulations of Atrial Cardiomyocytes

The above data indicate that the presence of t-tubules predisposes ventricular cardiomyocytes and tubulated atrial myocytes to developing Ca^{2+} waves during hypokalemia. While spontaneous Ca^{2+} release can drive DADs from resting potential and spontaneous APs, these events may also drive EADs if they occur during the repolarizing phase of the AP. Indeed,

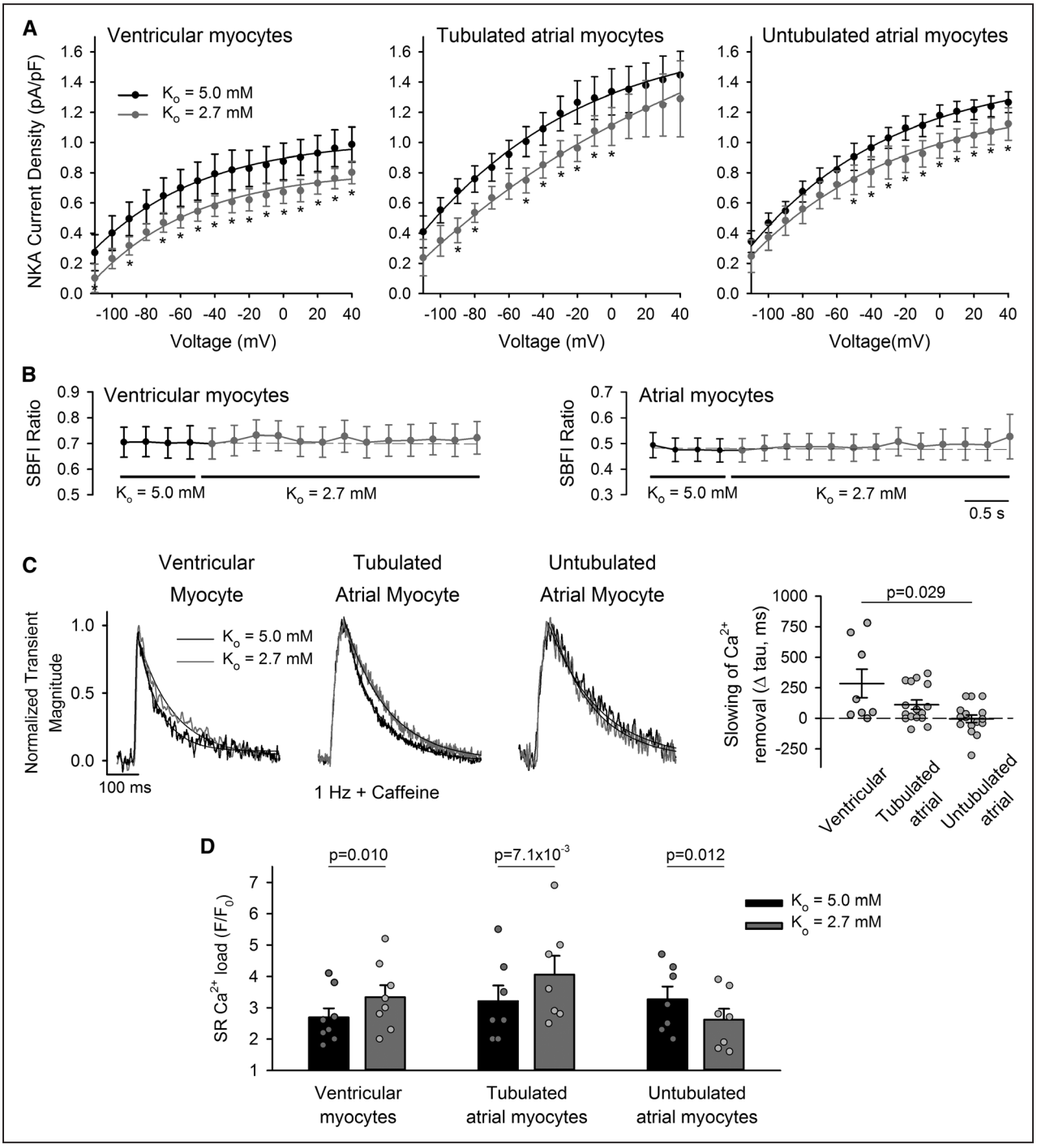

Figure 4. Decreased Na⁺, K⁺-ATPase (NKA) activity during hypokalemia inhibits Ca^{2+} extrusion by t-tubular Na^+-Ca^{2+} exchanger (NCX), elevating sarcoplasmic reticulum (SR) Ca^{2+} content.

A, NKA activity was measured during hyperpolarizing voltage ramps and calculated as the K^+-sensitive current (see Online Figure I for protocol and representative traces). Hypokalemia induced a modest and similar reduction in NKA current in ventricular myocytes and atrial cells, regardless of the presence of t-tubules (n_{cells}=10, 7, 9; n_{hearts}=4, 4, 5 in ventricular, tubulated atrial, untubulated atrial cells). **B**, SBFI experiments revealed no change in global cytosolic [Na^+] during the protocol (n_{cells}=12, 8; n_{hearts}=4, 4 for ventricular, atrial cells). **C**, However, tubulated cells showed slowed Ca^{2+} removal by NCX during hypokalemia, as indicated by the declining phase of Ca^{2+} transients stimulated in the continuous presence of 10 mmol/L caffeine (change in tau values shown at right, n_{cells}=8, 16; n_{hearts}=3, 5, in ventricular, tubulated atrial cells, P=0.016, 0.011 vs K_o=5.0 by Wilcoxon signed-rank test). No change in Ca^{2+} removal rate was observed in untubulated atrial cells (n_{cells}=16; n_{hearts}=5; P=0.912). Transient magnitude during K_o=2.7+caffeine: F/F_0=1.46±0.07, 1.66±0.12, 1.57±0.08 in ventricular, tubulated, and atrial cells. **D**, Ventricular cells and tubulated atrial cells exhibited increased SR Ca^{2+} content during hypokalemia, as assessed by the magnitude of caffeine-induced Ca^{2+} release (n_{cells}=8, 7, 7; n_{hearts}=6, 6, 5 in ventricular, tubulated atrial, untubulated atrial cells; representative traces illustrated in Online Figure VI). Statistics: (**A**): 2-way repeated measures ANOVA with Bonferroni correction (see Online Table II for full results); (**B**): Kruskal-Wallis test (difference in medians: P=1.000, 1.000 in ventricular, atrial cells); (**C**): Kruskal-Wallis test with Dunn correction (difference in medians: P=0.023); (**D**): paired t-test. *P<0.05 vs K_o=5.0.

ORIGINAL RESEARCH

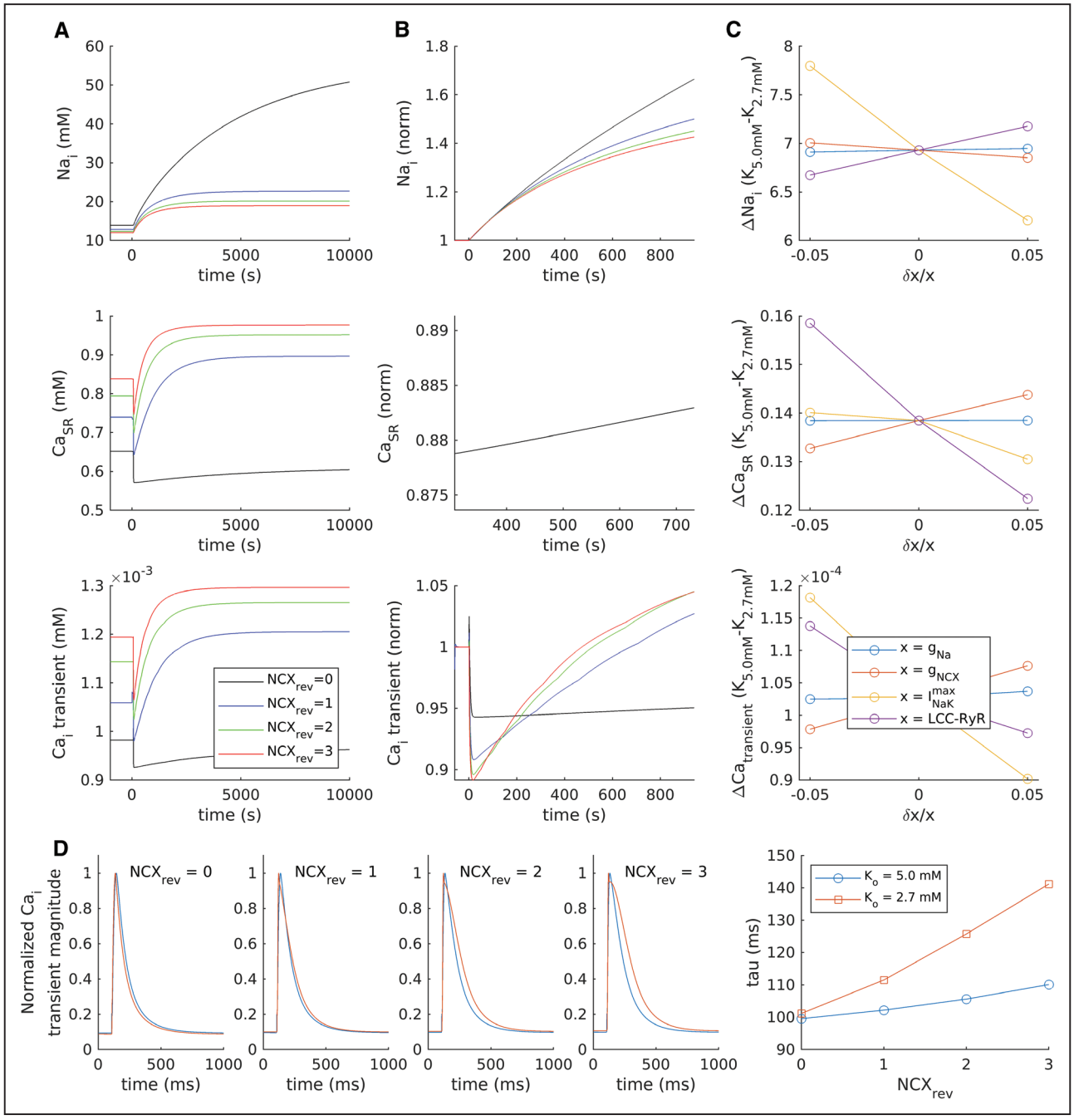

Figure 5. Close Na⁺, K⁺-ATPase (NKA)-Na⁺-Ca²⁺ exchanger (NCX) crosstalk drives the biphasic Ca²⁺ transient response to hypokalemia.

A mathematical model of the rat ventricular cardiomyocyte was employed to investigate the contribution of various ion channels and transporters to changes in cellular [Na⁺], SR Ca²⁺ content, and Ca²⁺ transients. **A**, With baseline model settings (blue lines), reducing $[K^+]_o$ from 5.0 to 2.7 mmol/L triggered a modest accumulation of intracellular [Na⁺], and a biphasic response of SR Ca²⁺ content and Ca²⁺ transients which occurred over a markedly longer time course than that observed experimentally. To simulate greater crosstalk between NCX and NKA, the fraction of NCX operating in reverse mode (NCX_{rev}) was increased in a step-wise manner, which produced an augmented and accelerated biphasic response (see also enlargement of the first 1000 s in [**B**], presented normalized to $[K^+]_o$=5.0). Removing the NCX_{rev} contribution prevented any secondary rise in Ca²⁺ transients, in resemblance to experiments in untubulated cells. **C**, Sensitivity analyses were performed to examine the effects of modulating individual ion fluxes±5%. Reducing t-tubule-associated fluxes such as I_{Na} (g_{Na}) or the number of L-type-RyR units (LCC-RYR) did not inhibit the biphasic response, while changing NCX and NKA fluxes had proportionally opposite effects. **D**, In addition to augmenting the biphasic response to hypokalemia, increasing NCX_{rev} slowed Ca²⁺ transient decay at steady state.

in field-stimulated ventricular cells, we observed that hypokalemia induced Ca²⁺ oscillations during the late phase of the Ca²⁺ transient which appeared to be consistent with EADs (Figure 6A, arrows in top). These events were even more frequent in atrial cells (68% of cells versus 33% ventricular cells; P=0.026 by z-test),

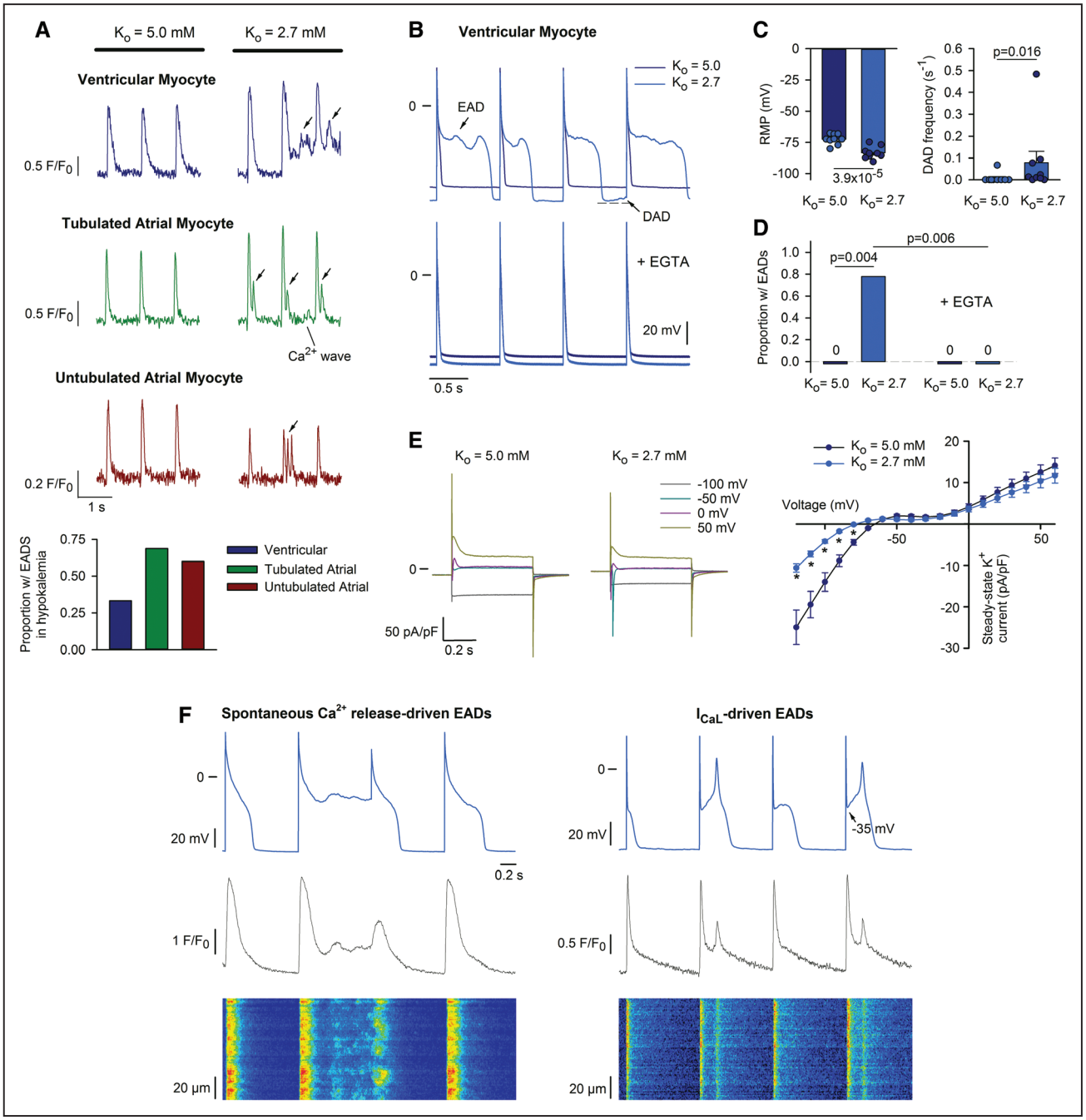

Figure 6. Early afterdepolarization (EAD) generation during hypokalemia: mechanisms in ventricular cells.
A, In electrically stimulated ventricular and atrial myocytes, Ca^{2+} oscillations were commonly observed during the late phase of the Ca^{2+} transient, consistent with EAD generation (arrows, n_{cells}=24, 16, 10 in ventricular, tubulated, untubulated atrial cells during 3-min hypokalemia). **B**, Patch-clamp recordings of action potentials (APs) confirmed the occurrence of EADs in ventricular cells during hypokalemia (7 of 9 cells from 5 hearts), which were prevented when Ca^{2+} was buffered by EGTA in the patch pipette (lower, 8 of 8 cells from 4 hearts). **C**, Mean changes in resting membrane potential (RMP) and delayed afterdepolarization (DAD) incidence (n_{cells}=9 from 5 hearts). **D**, Proportion of cells exhibiting EADs. **E**, Steady-state K^+ currents were reduced at negative potentials during hypokalemia (n_{cells}=8, 7 in normo-, hypokalemia from 4, 4 hearts). **F**, Pairing AP recordings with Ca^{2+} imaging (fluo-4, confocal line-scans) revealed EADs associated with spontaneous Ca^{2+} release (left) or reactivation of L-type Ca^{2+} current (right). Statistics: (**A**): *z*-test; (**C**): paired *t*-test for RMP, Wilcoxon signed-rank test for DAD frequency; (**D**): *z*-test; (**E**): 2-way repeated measures ANOVA with Bonferroni correction (see Online Table III for full results). *P<0.05 vs K_o=5.0.

and were common in untubulated atrial myocytes (Figure 6A, bottom) despite an absence of signs of Ca^{2+} overload and waves in these cells (described in Figure 1B).

We employed the patch-clamp technique to investigate the underlying mechanisms. In ventricular cells, current-clamp recordings (Figure 6B) confirmed that hypokalemia promoted marked hyperpolarization of resting membrane

potential and a robust increase in the occurrence of both DADs and EADs (Figure 6C and 6D). In agreement with previous work,[7] EADs manifested as stereotypical oscillations during the plateau phase of the prolonged AP during hypokalemia (AP duration=108±18, 162±34 ms in K_o=5.0, 2.7, n=9, P=4.2×10^{-3} by Wilcoxon signed-rank test). AP prolongation was linked to decreased steady-state K^+ currents at negative potentials, consistent with reduction of I_{K1} (Figure 6E; protocol presented in Online Figure IIA). Inclusion of 60 µmol/L EGTA in the patch pipette to buffer intracellular Ca^{2+} fully prevented EAD generation in ventricular myocytes (Figure 6B, lower panel, Figure 6D), indicating that these events are Ca^{2+} dependent. The Ca^{2+} dependence of EADs was further illustrated by pairing AP recordings with Ca^{2+} imaging by confocal linescans. Spontaneous Ca^{2+} release events observed during repolarization were temporally associated with the upstroke of EADs (Figure 6F, left). In other cases, EADs were associated with L-type Ca^{2+} channel re-opening, as evidenced by takeoff potentials clearly within the range for L-type activation, and a uniform increase in Ca^{2+} across the cell (Figure 6F, right).

Distinct mechanisms of EAD generation were observed in tubulated and untubulated atrial myocytes. Both types of atrial cells demonstrated significant membrane hyperpolarization during hypokalemia (tubulated cells: −11.5±1.2 mV, n=21; untubulated cells: −11.5±0.8 mV, n=48; P=3.38×10^{-9} and P=6.46×10^{-18}, respectively, by paired t-test). As observed in ventricular myocytes, tubulated atrial cells exhibited an increased incidence of DADs during hypokalemia (0.047±0.007 events/s versus 0 in K_o=5.0 mmol/L, P=0.044 by paired t-test), and EADs associated with spontaneous Ca^{2+} release events (Figure 7A). Buffering of intracellular Ca^{2+} with EGTA significantly reduced the occurrence of these EADs (Figure 7B, top, Figure 7C). In contrast, untubulated atrial myocytes showed no significant increase in DAD frequency during hypokalemia (0.063±0.021 events/second versus 0 in K_o=5.0 mmol/L, P=0.069 by paired t-test), which paralleled measurements of Ca^{2+} wave frequency (Figure 1B). However, EADs were frequent in untubulated cells, even after treatment with EGTA to further ensure Ca^{2+} quiescence (Figure 7B, bottom, Figure 7C). Importantly, the EADs observed in these cells were triggered during phase-3 of the AP as the membrane potential rapidly repolarized (mean time to initiation=38±1 ms following AP peak), from membrane potentials below the activation range for L-type Ca^{2+} current (mean take-off potential=−50.9±0.4 mV, n=128 events from 10 cells). Rather, we observed that phase-3 EADs in untubulated atrial myocytes were rapidly inhibited by application of 1 µmol/L tetrodotoxin, consistent with triggering by I_{Na} (Figure 7E, upper). We observed that these EADs were also inhibited by rapid application of 10 mmol/L caffeine to inhibit Ca^{2+} release during the AP (Figure 7E, lower). This finding is in agreement with previous work indicating that inward NCX current generated by Ca^{2+} extrusion during the Ca^{2+} transient can collaborate with nonequilibrium I_{Na} to drive phase-3 EADs.[14,15]

Triggering of phase-3 EADs by I_{Na} requires brief AP configuration, which allows rapid recovery of Na^+ channels from inactivation.[14,15] Indeed, untubulated atrial myocytes, where I_{Na}-driven EAD generation was most prominent, exhibited significantly shorter APs than their tubulated counterparts (Figure 7D; Online Figure VII). Rapid AP repolarization in untubulated cells was linked to larger outward K^+ current, in comparison with tubulated atrial cells (Figure 7F; protocol described in Online Figure IIA). This difference remained present during hypokalemia and also when a voltage-clamp protocol was employed to inactivate I_{TO} (Figure 7G, upper; protocol described in Online Figure III). However, application of 50 µmol/L 4-AP, which selectively inhibits ultra-rapid K^+ current (I_{Kur}),[20,21] significantly reduced current only in untubulated atrial cells (Online Figure IIB and IIC). Remaining 4-AP-insensitive current was similar in tubulated and untubulated cells (Figure 7G, bottom, Online Figure IIIC), supporting that the larger outward K^+ current observed in untubulated atrial myocytes is due to the exclusive presence of I_{Kur} in these cells. In keeping with this finding, 4-AP application was also observed to rapidly prolong AP repolarization in untubulated atrial myocytes, both in normo- and hypokalemic conditions (Figure 7H; Online Figure IID), but not ventricular cells (APD_{50}=34.7±11.6, 29.3±7.1 ms in K_o=2.7, K_o=2.7+4-AP, n=8, P=0.41 by paired t-test).

The above findings suggest that the presence of I_{Kur} and a brief AP increases Na^+ channel availability in untubulated hypokalemic atrial cells, allowing for phase-3 EADs driven by nonequilibrium reactivation of the fast I_{Na}. To further examine this hypothesis, we introduced representative AP waveforms into a mathematical model of the human atrial cardiomyocyte.[14] The model confirmed that the upstroke of the EAD was initiated by nonequilibrium I_{Na} reactivation and was paralleled by a small inward NCX current (Figure 7I). These I_{Na}-driven events are only possible when AP configuration is very brief, and I_{Na} availability is greatest (Online Figure VIII). Indeed, we observed that prolonging AP repolarization in the model inhibited the generation of I_{Na}-driven EADs (Online Figure IX). The model additionally demonstrated that hyperpolarization of the resting membrane potential during hypokalemia is also critical, as it further increases Na^+ channel availability (Online Figure VIII). These findings support that the brief and hyperpolarized AP configuration is key to induction of phase-3 EADs in untubulated atrial myocytes during hypokalemia, while tubulated atrial and ventricular cells are susceptible to Ca^{2+}-dependent EADs during phase 2. These mechanisms are summarized in Figure 8.

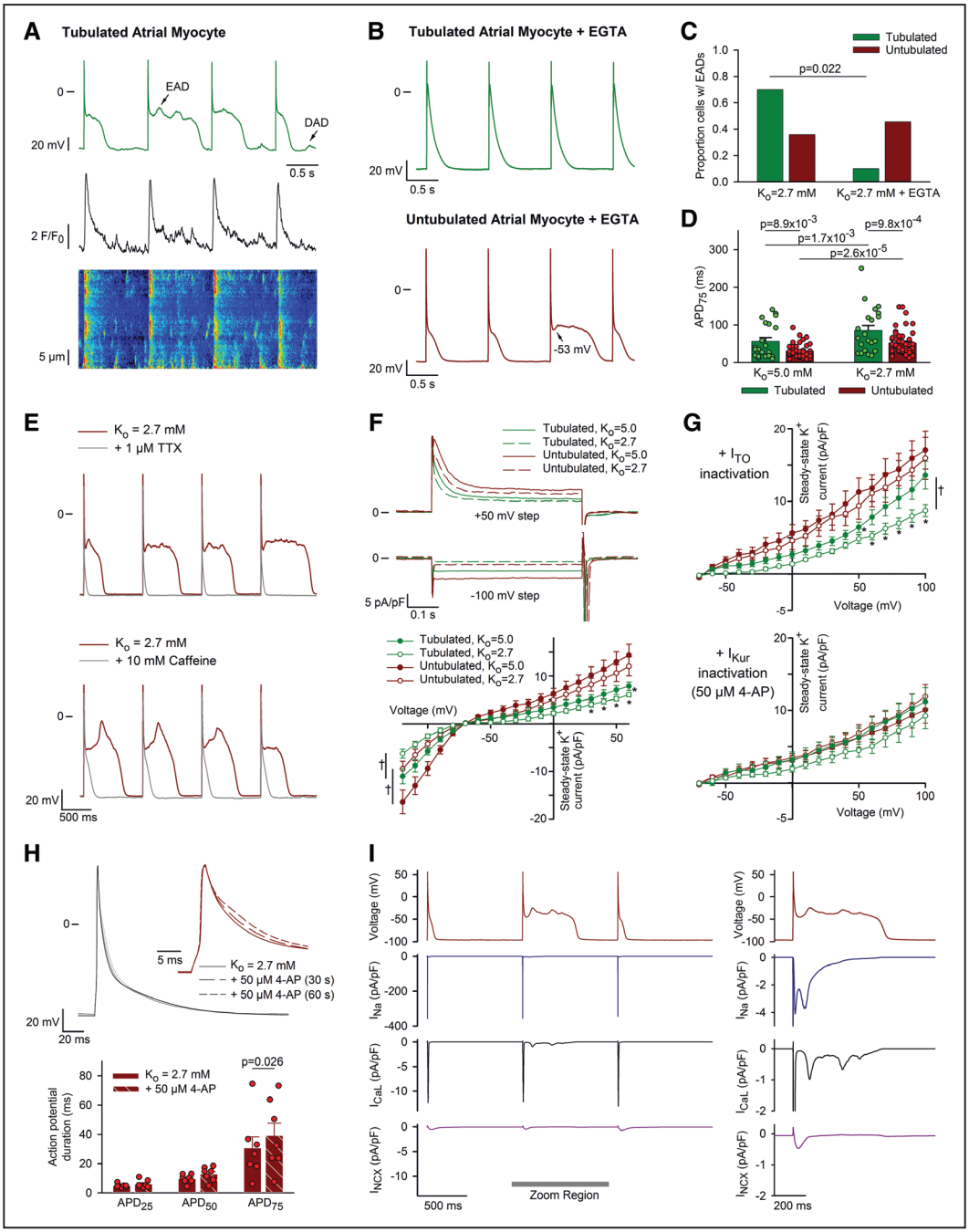

Figure 7. I_{Kur} maintains brief action potential (AP) configuration in untubulated atrial myocytes, promoting early afterdepolarization (EADs) driven by nonequilibrium I_{Na} during hypokalemia.

A, In tubulated atrial myocytes, hypokalemia-induced EADs were frequently associated with Ca^{2+} waves. **B**, Buffering of intracellular Ca^{2+} with patch pipettes containing 60 μmol/L EGTA reduced EAD incidence in tubulated cells. However, in untubulated cells, EADs remained present and were observed to be triggered during rapid AP repolarization from negative potentials (arrow). **C**, Incidence of EADs in tubulated and untubulated atrial myocytes (−EGTA: n_{cells}=10, 14 from 7, 8 hearts; +EGTA: n_{cells}=10, 11 from 6, 9 hearts). **D**, AP configuration in tubulated and untubulated atrial cells (n_{cells}=21, 48 from 12, 20 hearts). **E**, EADs observed in hypokalemic, untubulated atrial myocytes were inhibited by rapid application of 1 μmol/L TTX (top) or 10 mmol/L caffeine (bottom). **F**, Short AP configuration in untubulated atrial myocytes was associated with larger outward steady-state K^+ current than in tubulated cells (protocol described in Online Figure IIA; n_{cells}=11, 22 in tubulated, untubulated from 6, 11 hearts). **G**, Top: steady-state current remained larger in untubulated cells after inhibition of I_{TO} (1 s prepulse to +50 mV, see Online Figure III; n_{cells}=9, 9 in tubulated, untubulated from 5, 6 hearts), but not following addition of 50 μmol/L 4-AP to inhibit I_{Kur} (bottom, K_o=5.0: n_{cells}=6, 8 from 5, 4 hearts; K_o=2.7: n_{cells}=6, 6 from 4, 3 hearts). **H**, I_{Kur} inhibition prolonged APD_{75} in untubulated atrial cells during hypokalemia (n_{cells}=7 from 3 hearts). **I**, Inclusion of AP waveforms in a mathematical model of the human atrial cardiomyocyte linked EADs triggered during Ca^{2+} quiescence to reactivation of nonequilibrium I_{Na}, parallel involvement of a small, forward-mode NCX current (I_{NCX}), and subsequent recruitment of L-type Ca^{2+} current. Statistics: (**C**): *z*-test; (**D**, **F**, **G**, **H**): 2-way ANOVA with Bonferroni correction. **D**, Difference in means: P=1.19×10^{-3} for tubulated vs untubulated, P=1.77×10^{-7} for K_o=5.0 vs K_o=2.7. **H**, Difference in means: P=0.114. Full results of (**F**) and (**G**) are presented in Online Tables IV and V. *P<0.05 vs untubulated and †P<0.05 vs K_o=5.0.

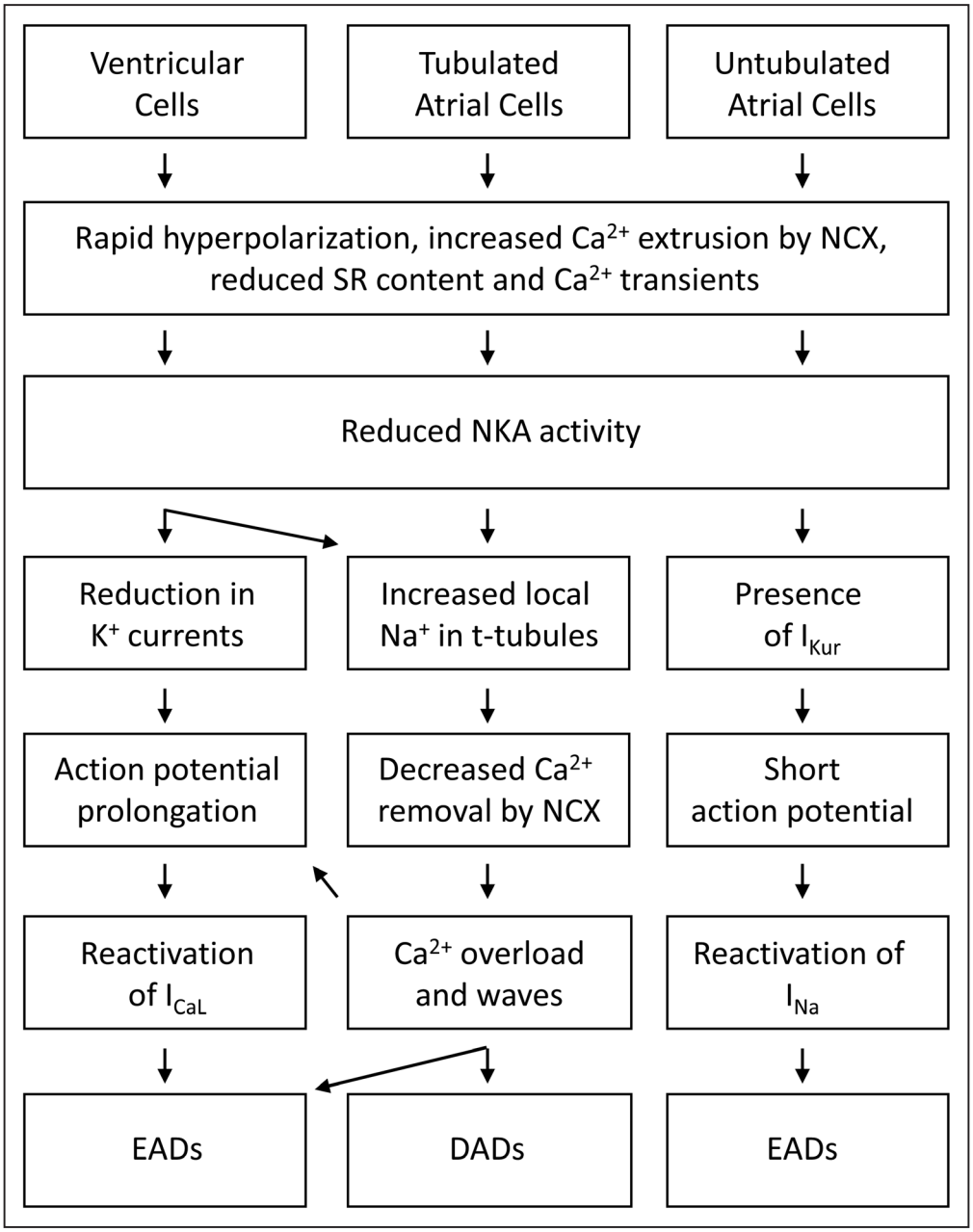

Figure 8. Schematic overview.
Distinct mechanisms were observed to promote arrhythmogenesis in ventricular and atrial cardiomyocytes. DAD indicates delayed afterdepolarization; EAD, early afterdepolarization; NCX, Na^+-Ca^{2+} exchanger; and NKA, Na^+, K^+-ATPase.

DISCUSSION

We have presently identified distinct mechanisms which promote triggered activity in ventricular and atrial myocytes exposed to hypokalemia. We observed that hypokalemia induces progressive Ca^{2+} overload and associated Ca^{2+} waves and DADs, both in ventricular cells and in a subpopulation of atrial cells which contain t-tubules. This effect is attributed to a functional coupling of NKA and NCX proteins within these structures, where inhibited Na^+ extrusion by NKA slows Ca^{2+} removal by NCX and elevates SR Ca^{2+} content. Although untubulated atrial cells were not susceptible to DADs during hypokalemia, EADs were common. These events were found to be driven by nonequilibrium I_{Na}, enabled by membrane hyperpolarization and a brief AP configuration which yielded rapid recovery of Na^+ channels from inactivation. Prompt AP repolarization in untubulated atrial myocytes was linked to the presence of I_{Kur}; a current observed to be absent in tubulated atrial cells. This mechanism is distinct from that which occurs in hypokalemic ventricular cells, where AP prolongation predisposes for EADs driven by reactivation of L-type Ca^{2+} current. Despite these differing mechanisms, our results show that lowered $[K^+]_o$

markedly increases susceptibility to afterdepolarizations in both cell types; actions which may contribute to an increased incidence of atrial and ventricular arrhythmias previously demonstrated in patients with hypokalemia.[3,4]

Both atrial and ventricular cells show an early reduction in Ca^{2+} transient magnitude when $[K^+]_o$ is lowered, as the resting membrane potential becomes rapidly hyperpolarized.[5,6,8,31] This hyperpolarization favors forward-mode NCX function, leading to an initial reduction in SR Ca^{2+} content and release.[5,6] The subsequent rising phase of the biphasic response to hypokalemia is also dependent on NCX activity, as NKA inhibition slows removal of Ca^{2+} by NCX, leading to a progressive increase in SR Ca^{2+} content and release. We presently show that this secondary response requires t-tubules, as it was absent in untubulated atrial cells and detubulated ventricular cells (Figure 2D). It should be noted that we were not able to maintain viable atrial cells during the detubulation procedure, which is why experiments were instead performed with paired t-tubule imaging.

Previous work in ventricular cardiomyocytes has shown that NKA and NCX have higher activity in t-tubules than the surface sarcolemma, and that these currents balance each other within the two locations.[19,32–34] Our data support this view, as NKA and NCX activity were paired in the different cell types; overall activity of both transporters was the largest in ventricular cells, smallest in untubulated atrial cells, and intermediate in tubulated atrial cells (Online Figures IC and VIB). However, mathematical modeling showed that the mere presence of NKA and NCX at higher densities is not sufficient to elicit a biphasic response to hypokalemia (Figure 5C). In fact, altering the activity of the 2 transporters in parallel has opposing effects which cancel out. We instead show that there must be a close functional pairing of NKA and NCX within t-tubules, which enables sensing of a shared local Na^+ pool. Indeed, tubulated cells exhibited progressive Ca^{2+} gain during hypokalemia without a detectable increase in global $[Na^+]$ measured by SBFI; a dye which we observed can report changes in $[Na^+]$ as small as 1 mmol/L (Online Figure VA).[35] This effect did not occur in untubulated atrial cells despite a similar reduction in NKA current (Figure 4A). Furthermore, we observed that preferential blockade of NKA activity in t-tubules using low-dose ouabain[19] did not increase global $[Na^+]$ (Online Figure VC) but inhibited the biphasic response in ventricular cells (Figure 3C). This suggests that ouabain-induced Na^+ accumulation was sufficient to favor nearby reverse-mode NCX activity at baseline, limiting further Ca^{2+} entry during hypokalemia. In the absence of a shared local Na^+ pool, mathematical modeling in fact predicted opposite effects of NKA blockade (Figure 5C). However, NCX-NKA crosstalk could be simulated by increasing the fraction of NCX operating in reverse mode, and this effectively slowed Ca^{2+} extrusion and augmented Ca^{2+} gain during hypokalemia (Figure 5A, 5B, and 5D).

Others have previously reported a close functional pairing of NCX and NKA, reliant on a shared local Na^+ domain.[19,36,37] A common explanation for these observations is that there is restricted diffusion of Na^+ around NKA, creating a subsarcolemmal fuzzy space with higher local Na^+ levels.[19,33,36,38,39] It has been suggested that the α_2 NKA isoform is particularly important in regulating $[Na^+]$ within this microdomain and thereby fine-tunes cardiac contractility.[19,40] However, recent super-resolution imaging data indicate that α_2 is not positioned in closer proximity to ryanodine receptors than the α_1 isoform.[30] Instead, the authors proposed that the preferential localization of α_2 within the t-tubules of ventricular cells is simply sufficient to enable local control of Na^+ levels near NCX. Our present findings support this view, as imaging data in ventricular cells also showed preferential positioning of α_2 within t-tubules (Figure 3A), and since α_2 blockade inhibited NCX-mediated Ca^{2+} gain during hypokalemia (Figure 3C). However, we observed that even in the absence of the α_2 isoform, the presence of α_1 in atrial t-tubules was sufficient to mediate NKA-NCX crosstalk, and a biphasic response to hypokalemia was observed which persisted in the presence of low-dose ouabain (Figure 3C). Thus, our data support that NKA-NCX crosstalk is facilitated within t-tubules, but is not limited to the NKA α_2 isoform.

In addition to driving Ca^{2+} waves and DADs, Ca^{2+} overload during hypokalemia has important implications for EAD generation. Ca^{2+} waves occurring during the repolarizing phase of the AP are a well-established driver of EADs, and we presently confirmed the robust presence of such events in hypokalemic ventricular and tubulated atrial cells. Previous work in ventricular myocytes has implicated a key involvement of CaMKII activation in triggering these events, as Ca^{2+}-dependent activation of the kinase enhances late I_{Na} and Ca^{2+} current in a positive feedback loop.[11] The increase in these currents prolongs the AP, enabling recovery of L-type Ca^{2+} channels from inactivation. In keeping with this mechanism, we observed L-type current-driven EADs in hypokalemic ventricular cells, characterized by uniform patterns of Ca^{2+} release across the cell and take-off potentials within the activation range of the current (Figure 6F). We further observed that EADs were absent in ventricular cells patch-clamped with EGTA-containing internal solution (Figure 6B and 6D). Reduction in several K^+ currents is also reported to contribute to AP prolongation during hypokalemia.[7] Indeed, we observed a marked reduction in K^+ currents at negative potentials consistent with reduced I_{K1} and a tendency toward reduced current at positive potentials (Figure 6E). Thus, there are complex changes in Ca^{2+} handling and electrophysiology which promote EAD generation in hypokalemic ventricular cells, as summarized in Figure 8.

In contrast to EADs observed in hypokalemic ventricular cells and tubulated atrial cells, these events

remained present in untubulated atrial cells when cytosolic Ca^{2+} was buffered by EGTA (Figure 7B and 7C). Both our experimental and modeling data indicated that these EADs were generated during phase-3 of the AP when membrane hyperpolarization and short AP duration increased Na^+ channel availability and enabled recruitment of nonequilibrium current (Figure 7I; and Online Figures VIII and IX). Others have previously shown that short APs, such as those present in mouse ventricular cells[15] or human atrial cells,[14,41] are similarly prone to EADs triggered by nonequilibrium I_{Na}, under conditions where I_{Na} availability is comparable to that presently observed (Online Figure VIIID). In keeping with a key role of I_{Na} in triggering phase-3 EADs, we observed that these events were rapidly blocked by tetrodotoxin treatment (Figure 7E). Notably, previous work has shown that NCX-mediated Ca^{2+} removal and current during the peak of the Ca^{2+} transient can be synchronized with reactivation of I_{Na}, leading to synergistic depolarization and triggering of a phase-3 EAD.[14,15] Our present work supports this view, since inhibition of triggered Ca^{2+} release by caffeine treatment blocked phase-3 EAD generation experimentally (Figure 7E). Our AP clamp simulations also showed that a small inward NCX current was temporally aligned with nonequilibrium I_{Na} (Figure 7I).

Further investigation indicated that the brief AP was maintained in untubulated atrial cells by the presence of I_{Kur}; a current which activates rapidly upon depolarization and is resistant to inactivation. Indeed, rapid application of 50 µmol/L 4-AP, which selectively blocks I_{Kur},[20,21] resulted in abrupt protraction of early AP repolarization (Figure 7H; Online Figure IID). Interestingly, I_{Kur} was observed to be absent in tubulated atrial and ventricular myocytes (Online Figure IIB and IIC), despite robust expression of $K_v1.5$ in both the atria and ventricles (Online Figure X). The reason for this apparent mismatch between channel expression and current in ventricle is unclear but seems to be in keeping with previous observations in rat myocardium (reviewed in study by Ravens et al[42]). Previous work has indicated that expression of $K_v1.5$ channels is largely restricted to the intercalated disk region of ventricular myocytes, while in atrial myocytes there is also significant localization longitudinally along the surface membrane.[43,44] It is unclear if these channels have a different function at these sites, distinct from carrying I_{Kur}.[43] In contrast, the presence of $K_v1.5$ and I_{Kur} in atrial myocytes is well established, and there has been considerable recent interest in employing I_{Kur} blockade for the treatment of atrial fibrillation, aimed at prolonging the AP and refractory period and reducing the risk of reentry (reviewed in study by Ravens[45]). Our results suggest that I_{Kur} blockade may be particularly beneficial in patients with hypokalemic atrial fibrillation, where brief APs are expected to favor both triggered and reentrant arrhythmia.

Taken together, the present results suggest that while ventricular myocytes are susceptible to Ca^{2+} overload-induced afterdepolarizations during hypokalemia, such overactivity in atrial cells is limited to those cells which contain t-tubules. We expect this mechanism of EAD and DAD generation to be particularly prominent in the atrial epicardium, where cells are the most frequenty tubulated.[12] Untubulated myocytes, which are predominantly localized in the endocardium,[12] are nevertheless prone to EADs during Ca^{2+} quiescence, due to the presence of I_{Kur} and short AP configurations. We speculate that elevated risk of atrial fibrillation in patients with hypokalemia[4] may reflect these proarrhythmic mechanisms. Such a cause-and-effect relationship seems to be supported by recent clinical results showing that K^+ infusion can revert patients with hypokalemic atrial fibrillation to sinus rhythm.[46] These findings underscore an emerging role for K^+ homeostasis in maintaining electrical stability of both the ventricles and atria.

ARTICLE INFORMATION

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Affiliations

From the Institute for Experimental Medical Research, Oslo University Hospital (K.T., M.F., M.L., D.B.L., O.M., J.S., J.M.A., O.M.S., I.S., W.E.L.) and KG Jebsen Center for Cardiac Research (M.F., M.L., O.M., I.S., W.E.L.), University of Oslo, Norway; Department of Internal Medicine, Diakonhjemmet Hospital, Oslo, Norway (K.T., E.Ø.); Division of Imaging Sciences and Biomedical Engineering, King's College London, United Kingdom (A.L., S.A.N.); Department of Pharmacology, School of Medicine, University of California Davis (S.M., A.G.E., E.G.); and Bjørknes College, Oslo, Norway (J.M.A.).

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Disclosures

None.

Supplemental Materials

Expanded Methods
Online Figures I–X
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