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ARRHYTHMOGENIC EFFECT OF EXTRACELLULAR K^+-DEPLETION IS PREVENTED BY THE TRANSVERSE-AXIAL TUBULAR SYSTEM IN A VENTRICULAR CARDIAC CELL MODEL

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A b s t r a c t

In this work, we studied the role of the transverse-axial tubular system (TAT-system) in arrythmogenesis of ventricular cardiac cells under conditions of simulated hypokalaemia (low $[K^+]_e$). We used the model of a mammalian ventricular myocyte that integrated the quantitative description of electrical activity of surface and tubular membranes and dynamic changes in intracellular ion concentrations. To maintain potassium homeostasis, an energy-dependent K^+ extrusion pump was incorporated into the model. According to predictions provided by the model, the TAT-system protects the cell against arrhythmogenesis due to the enhancement of a potassium concentration gradient between tubular and extracellular spaces at low levels of $[K^+]_e$. The energy-dependent K^+ extrusion pump maintains tubular $[K^+]$ at a level higher than the overall $[K^+]_e$. This makes the activation of tubular K^+-conductances responsible for action potential repolarisation and resting voltage.

K e y w o r d s

Ventricular cell, Low external $[K^+]$, Tubular system, Quantitative modelling

INTRODUCTION

The sarcolemma of a ventricular heart muscle cell has a large number of invaginations that form a complex system of tubules *(1, 2)* termed transverse-axial tubular system (TAT-system). This system serves to transmit the cardiac action potential from the surface membrane deep into the cellular interior, thereby ensuring rapid and synchronous contraction of sarcomeres. The recent experimental evidence *(3, 1, 4)* suggests that the electrophysiological properties of cardiac cells might also be significantly affected by accumulation and/or depletion of ions in the restricted space of TAT-system. In particular, the preferential localisation of K^+-channels in the TAT-system *(4)* may underlie activity-dependent accumulation of K^+-ions in the lumen of the TAT-system. This phenomenon may become more significant when the extracellular concentration

of potassium ($[K^+]_e$) is decreased, as occurs in the pathological situation of hypokalaemia. The question whether and how the TAT-system would modulate the development of potentially lethal arrhythmias related to hypokalaemia *(5, 6, 7)* has so far not been answered. In the present study, we addressed this question by examining the behaviour of a quantitative model of a mammalian ventricular cardiac cell containing the TAT-system.

METHODS

The general approach is based on numerical reconstruction of the electrical activity of a ventricular cell, using a mathematical model *(Fig. 1)* that was mainly designed on the basis of data from the guinea pig *(8)*, according to the quantitative description proposed by *Luo and Rudy in 1994 (9)*. It includes formulations of the following ionic currents in surface (s) and tubular (t) membranes: fast sodium currents ($I_{Na,s}$ $I_{Na,t}$), calcium currents through L-type channels ($I_{Ca,s}$, $I_{Ca,t}$), delayed rectifier potassium currents ($I_{K,s}$, $I_{K,t}$), plateau potassium currents ($I_{Kp,s}$, $I_{Kp,t}$), inward rectifying potassium currents ($I_{K1,s}$, $I_{K1,t}$), nonspecific calcium-activated currents ($I_{ns(Ca),s}$, $I_{ns(Ca),t}$), background calcium currents ($I_{Cab,s}$ $I_{Cab,t}$), background sodium currents ($I_{Nab,s}$ $I_{Nab,t}$), sodium-calcium exchange currents ($I_{NaCa,s}$, $I_{NaCa,t}$), sodium-potassium pump currents ($I_{NaK,s}$, $I_{NaK,t}$) and calcium pump currents ($I_{pCa,s}$, $I_{pCa,t}$). Changes in intracellular ionic concentrations were also calculated. To maintain potassium homeostasis, the model was completed by additional electroneutral K-pump fluxes ($J_{pK,s}$, $J_{pK,t}$), as postulated by *Nordin (10)*. The current densities, conductivities or permeabilities of individual transporters were set according to previously published data *(4, 11–14)*. The partial models of surface membrane and tubular membrane electrical activity were interconnected through the total series resistance of TAT-system *(8)*. The intracellular space contained subsarcolemmal fuzzy space, uptake and release compartments of the sarcoplasmic reticulum and Ca^{2+}-buffering by calmodulin and troponin.

The geometric parameters of the TAT-system were set according to a recent quantitative microscopic analysis of rat ventricular myocytes *(2)*. These parameters comprise the density of tubules at the cell surface (27.78×10^6 tubules/cm^2) and the average radius of tubules ($r_t=127\times10^{-7}$ cm) and their length ($l_t=10$ μm). Time constants of ion diffusion between TAT-system and extracellular space were set to 64 ms for K^+ and Na^+-ions and 250 ms for Ca^{2+}-ions according to the data from guinea pig ventricular cells *(15)*.

RESULTS AND DISCUSSION

To explore the role of the TAT-system in ventricular cell arrhythmogenesis under the conditions of low $[K^+]_e$, comparative simulations at different frequencies of stimulation were performed on a model either with or without the TAT-system. At a stimulation of 1 Hz, a sudden decrease of $[K^+]_e$ from its physiological value (5.4 mM) to values ranging between 2.2 mM and 1.8 mM resulted in a transient development of early afterdepolarisations in the model without TAT-system *(Fig. 2B)* but not in the model including TAT-system *(Fig. 2A)*. Similar differences in behaviour of the models were observed at a stimulation frequency of 2 Hz. However, they appeared in a lower range of $[K^+]_e$ between 1.9 mM and 1.5 mM.

When stimulated at higher frequencies (above 2 Hz), both models were more resistant to generation of afterdepolarisations upon lowering $[K^+]_e$. This was

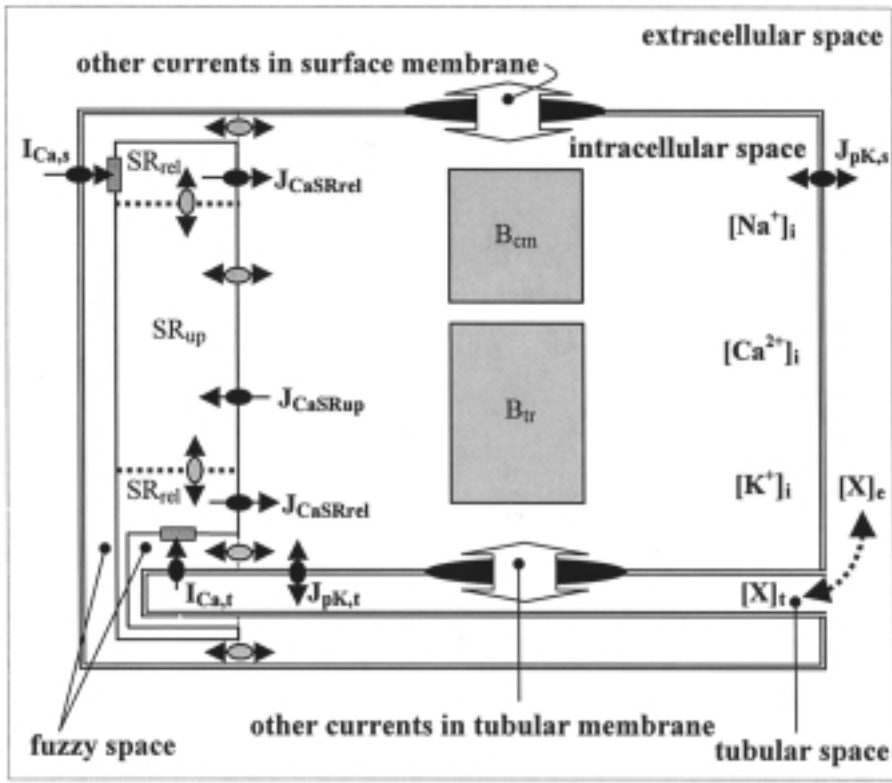

Fig. 1.
Schematic diagram of the ventricular cell model. The description of electrical activity of surface (s) and tubular (t) membranes comprises calcium current (I_{Ca}), other currents as defined in *(9)*, and electroneutral K-pump flux (J_{pK}). The intracellular space contains Ca^{2+}-uptake (SR_{up}) and Ca^{2+}-release (SR_{rel}) compartments of the sarcoplasmic reticulum and Ca^{2+}-buffering by calmodulin (B_{cm}) and troponin (B_{tr}). The symbols $J_{CaSRrel}$ and J_{CaSRup} denote Ca-release and Ca-uptake by SR (the sarcoplasmic reticulum), respectively. The small grey rectangles in SR_{rel} membrane denote ryanodine receptors. The arrows with a grey ellipse denote Ca^{2+}-diffusion and the dashed arrow between tubular and extracellular spaces represents diffusion of ions between these two spaces. X stands for Na^+, K^+ and Ca^{2+}.

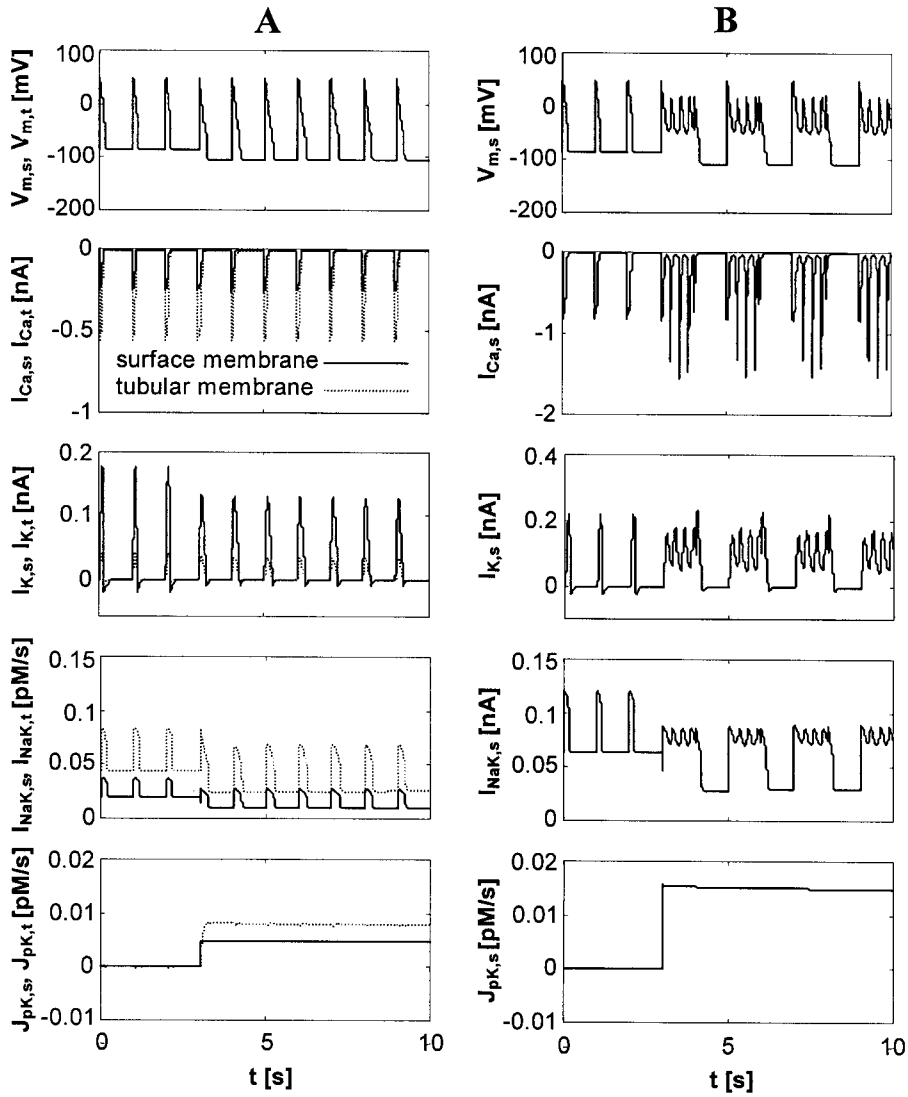

Fig. 2.
Comparison of responses of the model including TAT-system (A) and the model without TAT-system (B) to stimulation (64 pA/pF, 1 ms) at 1 Hz before and after a sudden decrease of $[K^+]_e$ from 5.4 mM to 2 mM in time t =3 s. The traces depict: surface and tubular membrane action potentials ($V_{m,s}$, $V_{m,t}$), calcium currents through L-type channels ($I_{Ca,s}$, $I_{Ca,t}$), delayed rectifier potassium currents ($I_{K,s}$, $I_{K,t}$), sodium-potassium pump currents ($I_{NaK,s}$, $I_{NaK,t}$) and potassium electroneutral pump fluxes (J_{pKs}, J_{pKt}).

caused by an increase of repolarizing currents I_K, I_{NaK}, $I_{ns(Ca)}$, and by shortening of the depolarising current I_{Ca}. However, even under these conditions, the two models exhibited significant differences. For example, at 3 Hz stimulation (not illustrated), the model without TAT-system continued to produce regular action potentials unless $[K^+]_e$ dropped below 1.4 mM, while the critical level of $[K^+]_e$ for the model including TAT-system was 1 mM. Below these critical values of $[K^+]_e$, both models exhibited pacemaker activity with a frequency higher than was the frequency of stimulation.

An analysis of the results showed that the model including TAT-system was more resistant to the development of early afterdepolarisations under low $[K^+]_e$. The main reason was a higher conductivity of tubular channels for repolarising currents $I_{K,t}$ and $I_{K1,t}$ as well as a higher activity of tubular Na/K-pump due to a higher tubular potassium concentration ($[K^+]_t$) in comparison with $[K^+]_e$. The relative enhancement of $[K^+]_t$ *(Fig. 3A)* was predominantly caused by an increase of $J_{pK,t}$ and might be observed during several minutes (after decrease of $[K^+]_e$) until the intracellular potassium concentration ($[K^+]_i$) reached a new steady level and $J_{pK,t}$ (dependent on $[K^+]_e$ and $[K^+]_i$) were diminished. The changes in tubular calcium concentration ($[Ca^{2+}]_t$) accompanying the electrical activity of the cell *(Fig. 3A)* were mainly induced by the inward current I_{Ca}. Their relative magnitude (~ 10 %) was not sufficient to significantly affect cellular arrhythmogenic properties under low $[K^+]_e$. As to the tubular sodium concentration $[Na^+]_t$, its relative changes (~ 0.6 %), mainly induced by the inward depolarising current $I_{Na,t}$, appeared to be negligible. Regarding the intracellular ionic concentrations, they exhibited significant changes during pacing at low $[K^+]_e$ *(Fig. 3)*. $[K^+]_i$ decreased in both models, but with a smaller slope in the model including TAT-system. This was predominantly caused by a lower magnitude of tubular potassium flux $J_{pK,t}$ resulting from a higher level of $[K^+]_t$ in comparison with diminished $[K^+]e$. The increase in intracellular sodium concentration ($[Na^+]_i$) was mainly caused by the inhibition of $I_{NaK,s}$ and $I_{NaK,t}$ at low $[K^+]_e$. However, due to an increase in $I_{NaK,s}$ and a partial decrease in $I_{Nab,s}$ during a train of spontaneous afterdepolarisations *(Fig. 2B)*, the growth of $[Na^+]_i$ was remarkably reduced. As for the intracellular calcium transients ($[Ca^{2+}]_i$), they were evoked not only by regular excitations but also by spontaneous electrical activity, as observed in the model without TAT-system *(Fig. 3B)*. The increase of peak value of Ca^{2+}-transients corresponded to the increase of Ca^{2+}-load in the sarcoplasmic reticulum during a train of previous afterdepolarisations.

The generation of repetitive afterdepolarisations by the model without TAT-system was regularly initiated by one stimulation pulse (64 pA/pF, 1 ms) and was subsequently interrupted shortly after the next stimulation pulse *(Fig. 2B)*. The main cause of this interruption lay in a cumulative enhancement of $I_{K,s}$ during the stimulation pulse and, consequently, in the triggering of all-or-none

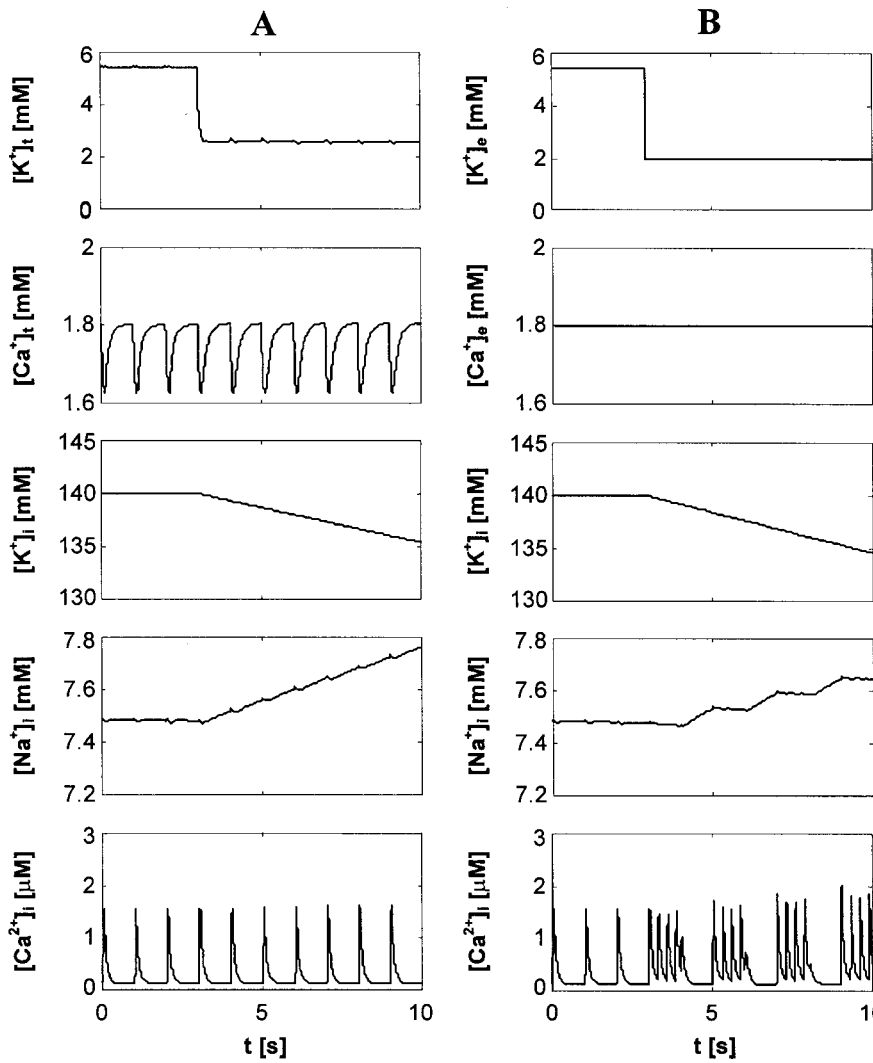

Fig. 3.
Comparison of ionic concentration changes in extracellular ($[K^+]_e$, $[Ca^{2+}]_e$), tubular ($[K^+]_t$, $[Ca^{2+}]_t$) and intracellular ($[K^+]_i$, $[Na^+]_i$, $[Ca^{2+}]_i$) compartments during the electrical activity of the ventricular cell model characterised in *Fig. 2*. A: Model including the TAT-system. B: Model without the TAT-system.

repolarisation. These features correspond to the dual stability of membrane voltage and the associated early afterdepolarisations, as reported for the ventricular cardiac muscle in low $[K^+]_e$ *(5–7)*.

In the present model, none of the tubular ionic concentrations exhibited any substantial cumulative increase or decrease even at a high frequency of stimulation (above 3 Hz). The present setting of time constants for ionic diffusion (64 ms for K^+ and Na^+-ions, 250 ms for Ca^{2+}-ions) as well as a single compartment formulation of the TAT-system certainly underestimated the magnitude of depletion/accumulation phenomena and the related antiarrhythmogenic property of the TAT-system.

In conclusion, our results predict a significant role for the TAT system in protection against cellular arrythmogenesis. It appears that, under the conditions of low $[K^+]_e$, the most important component responsible for antiarrhythmogenic properties of the TAT-system is the energy-dependent electroneutral K^+-transport predicted by the model of *Nordin (10)* and recently identified, as H^+/K^+-ATPase, in experiments on cardiac cells *(16, 17)*. Whether the TAT-system will also have a protective role in other proarrhythmogenic conditions remains to be investigated.

A c k n o w l e d g e m e n t s

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TRANSVERSÁLNĚ-AXIÁLNÍ TUBULÁRNÍ SYSTÉM SNIŽUJE ARYTMOGENNÍ ÚČINKY NÍZKÉ EXTRACELULÁRNÍ DRASLÍKOVÉ KONCENTRACE U MODELU KOMOROVÉ SRDEČNÍ BUŇKY

S o u h r n

Předmětem této práce je kvantitativní analýza vlivu transverzálně-axiálního tubulárního systému (TAT-systému) na arytmogenezi u komorové srdeční buňky v podmínkách nízké koncentrace extracelulárních iontů draslíku ($[K^+]_e$). Použitý model spojuje kvantitativní popis elektrické aktivity povrchové a tubulární membrány buňky s popisem změn intracelulárních a tubulárních iontových koncentrací. Výsledky výpočtového modelování ukázaly, že TAT-systém může mít významné antiarytmogenní účinky, které jsou důsledkem zvýšení rozdílu draslíkových koncentrací mezi extracelulárním a tubulárním prostorem při snížení $[K^+]_e$. Hlavním transportním mechanismem zodpovědným za toto zvýšení je podle modelu draslíková elektroneutrální ATP-pumpa v tubulární membráně.

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