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

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Abstract
In this work, we studied the role of the transverse-axial tubular system (TAT-system) in arrhythmogenesis 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.

Key words
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 (I, 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 (INaₛ, INaₜ), calcium currents through L-type channels (ICaₛ, ICaₜ), delayed rectifier potassium currents (IKₛ, IKₜ), plateau potassium currents (IKₚₛ, IKₚₜ), inward rectifying potassium currents (IK₁ₛ, IK₁ₜ), nonspecific calcium-activated currents (I NS(Ca)ₛ, I NS(Ca)ₜ), background calcium currents (ICabₛ, ICabₜ), background sodium currents (INabₛ, INabₜ), sodium-calcium exchange currents (INaCaₛ, INaCaₜ), sodium-potassium pump currents (INaKₛ, INaKₜ) and calcium pump currents (ICaPₛ, ICaPₜ). Changes in intracellular ionic concentrations were also calculated. To maintain potassium homeostasis, the model was completed by additional electroneutral K-pump fluxes (JPKₛ, JPKₜ), 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 Ca²⁺-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 (27.78×10⁶ tubules/cm²) and the average radius of tubules (rₜ=127×10⁻⁷ cm) and their length (lₜ=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 Ca²⁺-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 ($I_{Ca,t}$), other currents as defined in (9), and electroneutral K-pump flux ($J_{pK,t}$). 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 $J_{CaSRel}$ 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 ($J_{pK,s}$, $J_{pK,t}$).
caused by an increase of repolarizing currents \( I_{K} \), \( I_{NaK} \), \( I_{m(Ca)} \), 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 \( I_{K,1,t} \) 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, [Ca²⁺]e), tubular ([K⁺]t, [Ca²⁺]t) and intracellular ([K⁺]i, [Na⁺]i, [Ca²⁺]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 arrhythmogenesis. 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.

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TRANSVERSÁLNÉ-AXIÁLNÍ TUBULÁRNÍ SYSTÉM SNIŽUJE ARYTMOGENNÍ ÚČINKY NÍŽKÉ EXTRACELULÁRNÍ DRASLÍKOVÉ KONCENTRACE U MODELU KOMOROVÉ SRDEČNÍ BUŇKY

Souhrn

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.
REFERENCES


