Mechanistic Investigation of the Causes of Cellular K^+ Loss during Acute Myocardial Ischemia: A Simulation Study

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Abstract

During acute myocardial ischemia, extracellular K^+ accumulation has been proved to induce reentrant arrhythmias. Despite the important of this phenomenon, the causes of cellular K^+ loss remain still unknown.

The aim of this work was to investigate the simultaneous effect of the activation of the ATP-sensitive K^+ current ($I_{K(ATP)}$), the inhibition of Na^+/K^+ pump current (I_{NaK}) and the appearance of an ischemic Na^+ inward current (I_{ONa}) on $[K^+]_o$ in the absence of coronary flow using computer modelling.

Our results show that the simultaneous activation of these three mechanisms produces a fast increase of $[K^+]_o$ during the first 11.6min which is mainly due to the enhancement of K^+ efflux. Then, $[K^+]_o$ stabilizes in a plateau level of 15.5 mmol/L. This biphasic time course is qualitatively and quantitatively similar to the increase of $[K^+]_o$ experimentally observed.

1. Introduction

The first minutes after the interruption of coronary flow in a cardiac tissue are the most dangerous because of the frequent appearance of reentrant arrhythmias. The lack of oxygen and glucose produce important alterations in cellular metabolism and in the permeability of cell membrane. As a consequence, ionic concentrations also change and, in particular, during the first minutes of myocardial ischemia, K^+ ions accumulate in the extracellular space [1]. It is known that extracellular K^+ accumulation is strongly related to electrophysiological changes that have been proved to be in the origin of reentrant arrhythmias [2].

Because of the importance of this phenomenon, several investigation groups have tried to characterize the increase of $[K^+]_o$. Their results show that extracellular K^+ concentration ($[K^+]_o$) increases during 10-15 minutes from its normoxic value to a plateau level of 10-18mmol/L depending on experimental conditions [3]. Then, $[K^+]_o$ reaches a plateau phase during which it remains constant for several minutes. Finally, a slower second rise takes place which corresponds to the late phase of myocardial ischemia.

However, the precise mechanisms that make cardiomyocyte lose K^+ after the interruption of coronary flow are still unknown. Experimental reports propose three mechanisms as the most probable among the possible causes of the first increase of $[K^+]_o$: the activation of ATP-sensitive K^+ current ($I_{K(ATP)}$), the inhibition of Na^+/K^+ pump current (I_{NaK}) and the appearance of an ischemic Na^+ current (I_{ONa}) [1, 3]. These three mechanisms have been incorporated to Luo-Rudy phase II action potential model [4] in order to investigate their effect in the $[K^+]_o$ of one cardiomyocyte in the absence of coronary flow.

2. Methodology

Because of its ability to simulate the electrophysiological behavior of ventricular myocytes, a modified version of Luo-Rudy phase II action potential model [4] has been used to investigate the effect of several factors of myocardial ischemia in one cardiomyte.

To this aim, a formulation of $I_{K(ATP)}$ was incorporated into the model [5]. To simulate its activation during ischemia, the fraction of opened ATP-dependent K^+ channels (f_{ATP}) was increased in a linear manner from zero to a final value of 0.8% during 14 minutes in the absence of coronary flow.

Inhibition of Na^+/K^+ pump current was simulated by substituiting the maximal current (I_{NaKMAX}) by the following mathematical expression:

$$I_{NaKMAX}(t) = I_{NaKMAX(INI)} \cdot (1 - f_{INHIB}(t))$$

where $I_{NaKMAX(INI)}$ is the normoxic value of I_{NaKMAX} (that is 2.75μA/μF [4]) and f_{INHIB} is the inhibition degree of Na^+/K^+ pump. In accordance with experimental observations [6], f_{INHIB} progressively increased from zero to its final value 35%.

During myocardial ischemia, the accumulation of substances such as lysophophatidylcholine could lead to the alteration of permeability of cell membrane to Na^+ ions [7, 8]. As a consequence, a Na^+ leak current appears

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which in our simulations has been represented as the new Na^+ current I_{ONa}. The amplitude of this current was linearly increased from zero to its final value –1.2 μA/μF. In accordance with experimental results, its activation begins two minutes later than the changes in $I_{K(ATP)}$ and in I_{NaK} [8].

And finally, the absence of ionic flow between the interstitial space (cleft) and the bulk extracellular medium was simulated by steeply increasing the time constant for diffusion of ions (τ_{diff}) from its normoxic value 1000ms to infinite. In order to stabilize ionic concentrations, τ_{diff} was increased one minute before the beginning of alterations in $I_{K(ATP)}$ and in I_{NaK}.

In order to investigate the effect of simulated ischemia in K^+ fluxes, the number of K^+ ions transported into and out of the cell, n_{IKout} and n_{INaK} respectively, was monitored using the following expressions:

$$n_{IKout}(t) = \int_0^t I_{Kout} d\tau$$

$$n_{INaK}(t) = \int_0^t I_{NaK} d\tau$$

where I_{Kout} is the total outward K^+ current.

Then, to determine if the activation of ischemic mechanisms modifies K^+ fluxes, we calculated the ratio between the number of K^+ ions transported across the cell membrane during simulated ischemia with respect to the amount of K^+ transported during normoxia was calculated using the variables $Rn_{IKout}(t)$ and $Rn_{INaK}(t)$ which were defined as:

$$Rn_{IKout}(t) = \frac{n_{IKout}(t) - n_{IKout}(60s)}{n_{IKoutNORM}(t) - n_{IKoutNORM}(60s)}$$

$$Rn_{INaK}(t) = \frac{n_{INaK}(t) - n_{INaK}(60s)}{n_{INaKNORM}(t) - n_{INaK\,NORM}(60s)}$$

where $n_{IKoutNORM}(t)$ and $n_{INaKNORM}(t)$ are, respectively, $n_{IKout}(t)$ and $n_{INaK}(t)$ under normoxic conditions but in the absence of coronary flow.

The model used in the simulations considers the three following compartments: the intracellular space, the interstitial extracellular clefts and a bulk extracellular medium in which concentrations were assumed to be constant. Dynamic changes in the extracellular (cleft) concentration of each ionic specie S ($[S]_o$) are simulated with the equation [5]:

$$\frac{d[S]_o}{dt} = -\frac{A_m}{V_{cleft}F} I_{S,tot} - \frac{[S]_o - [S]_{bulk}}{\tau_{diff}}$$

where A_m is the area of the myocyte, V_{cleft} is the volume of the interstitial cleft (per cell), F is the Faraday constant, $I_{S,tot}$ is the total ionic current associated to ion S, τ_{diff} is the time constant for diffusion of S from the interstitial clefts to the bulk extracellular medium, and $[S]_{bulk}$ is the S concentration in the bulk.

The frequency of stimulation was 90 beats per minute that corresponds to a basic cycle length (BCL) of 666ms. The model was written in ACSL language. The nonlinear system of different equations was solved using the Gear stiff method. A maximum time step of 0.01ms was allowed.

3. Results

As related above, three mechanisms ($I_{K(ATP)}$, I_{NaK} and I_{ONa}) have been proposed to be at least in part responsible for cellular K^+ loss during ischemia. In precedent studies of our group, it has been shown that none of this mechanisms could explain on its own the extracellular K^+ accumulation observed during the first minutes of myocardial ischemia [5, 10, 11, 12]. In this work, computer simulations have been carried out to determine the effect of the simultaneous activation of these three mechanisms in $[K^+]_o$.

Figure 1 depicts the time course of $[K^+]_o$ during 14 minutes of simulated ischemia. In the absence of coronary flow, the activation of the $I_{K(ATP)}$ together with the inhibition of Na^+/K^+ pump current produces a fast increase of $[K^+]_o$ which is enhanced by the activation of I_{ONa} two minutes later. This first rise lasts 11.6min and therafter $[K^+]_o$ stabilizes remaining constant in a plateau level of 15.5 mmol/L during the rest of the 14 minutes simulated.

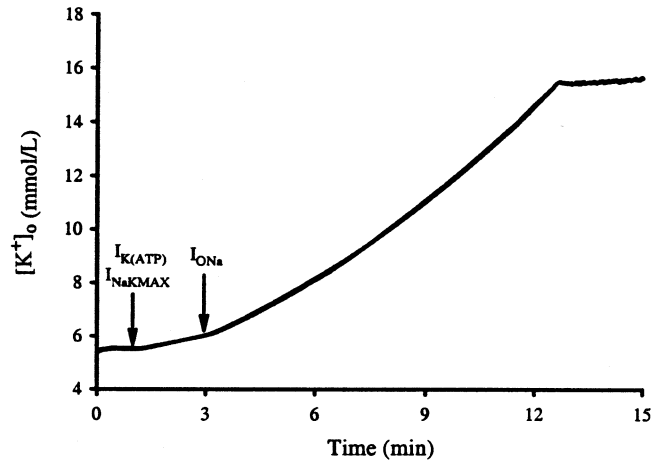

Figure 1. Extracellular K^+ accumulation during acute myocardial ischemia. The arrows indicate when the activation of each mechanism begins.

In order to further investigate cellular K^+ loss during ischemia, the variables Rn_{IKout} and Rn_{INaK} have been monitored during 14 minutes of simulated ischemia. As explained in Methodology section, these two parameters represent the ratio between the number of K^+ ions

transported across the cell membrane during simulated ischemia with respect to the amount of K^+ transported during normoxia. Figure 2 shows the time course of these two variables under the conditions of simulated ischemia described in Methodology section.

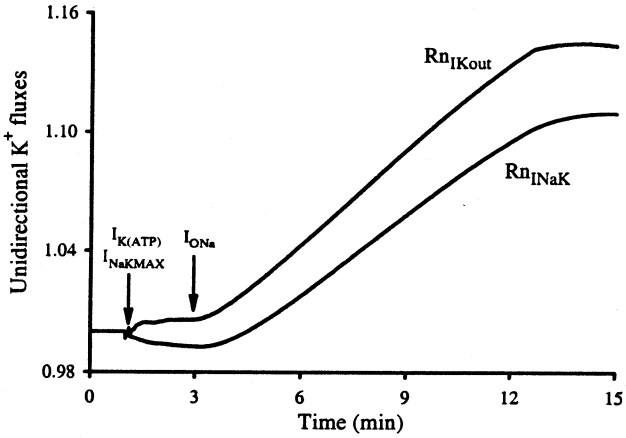

Figure 2. Effect of ischemic mechanisms in undirectional K^+ fluxes.

As it can be observed, the simultaneous effect of the alterations in $I_{K(ATP)}$ and I_{NaKMAX} produces a slight increase of Rn_{IKout}. Two minutes later, the beginning of the activation of I_{ONa} provokes a faster increase of Rn_{IKout} that denotes an important enhancement of outward K^+ efflux. During the whole simulation, Rn_{IKout} is higher than one, proving that the concomitant effect of the three mechanisms in the absence of coronary flow increases unidirectional outward K^+ flux. Thereafter, three minutes before the end of the simulation, the slope of the curve stabilizes and remains constant in its final value 1.15.

The time courses of Rn_{IKout} and Rn_{INaK} are very similar even if there are some differences. In these sense, during the first two minutes of simulated ischemia the linear decrease of I_{NaKMAX} reduces the ability of Na^+/K^+ pump to transport K^+ ions into the cell. Thereafter, the activation of I_{ONa} is followed by a progressive increase of Rn_{INaK} up to 1. As for unidirectional K^+ efflux, during the last minutes of the simulation, this variable stabilizes in a final value of 1.10.

4. Discussion

Experimental reports have proved that extracellular K^+ accumulation is pivotal in the genesis of reentrant arrhythmias during the early phase of myocardial ischemia [2]. The importance of this phenomenon has involved a large number of investigators in the determination of the mechanisms responsible for cellular K^+ loss [1, 3]. Even if the precise mechanisms are still unknown, their results suggest that the alteration of three mechanisms of ionic transport (opening of K_{ATP} channels, inhibition of Na^+/K^+ pump activity and altered Na^+ fluxes) could play an important role in the ischemic increase of $[K^+]_o$.

Precedent studies from our group [10, 11, 12] have shown that the separate activation of only one of these mechanisms is not able to cause the increase of $[K^+]_o$ experimentally observed. However, as it can be observed in Figure 1 their simultaneous alteration leads to a progressive rise of $[K^+]_o$ followed by a plateau phase during which $[K^+]_o$ remains almost constant. Qualitatively, this biphasic time course of $[K^+]_o$ is typical of the first 10-15 minutes of acute myocardial ischemia [3]. Quantitatively, the rate of rise of $[K^+]_o$ and the plateau level are in the range of those measured in cardiac tissues of pig, rabbit and guinea-pig. Consequently, the concomitant effect of the progressive change of $I_{K(ATP)}$, I_{NaKMAX} and I_{ONa} in the absence of coronary flow accurately reproduces the extracellular K^+ accumulation observed during acute mycardial ischemia.

The opening of K_{ATP} channels represents an increase of K^+ permeability of cell membrane that could enhance K^+ efflux and extracellular K^+ accumulation. However, precedent works [12] show that the single activation of $I_{K(ATP)}$ produces a final extracellular K^+ accumulation of 0.8mmol/L. A possible explanation of this small effect is that the opening of K_{ATP} channels increases K^+ efflux across $I_{K(ATP)}$ but also produces a faster repolarization of action potential. As a consequence, action potential duration is shortened and the time during which other K^+ currents are activated is reduced. Furthermore, the $I_{K(ATP)}$ enhancement of g_K not only produces the shortening of APD but also a reduction of K^+ driving force that would restrain outward K^+ fluxes. Thus, extracellular K^+ accumulation during ischemia could only be explained if this self-limiting effect of g_K is counteracted by an inward current.

The nature of this inward current is debated but experimental evidences have suggested that altered Na^+ fluxes could contribute to extracellular K^+ accumulation. In this sense, the accumulation of substances like lysophosphatidylcoline has been proved to profoundly modify the characteristics of Na^+ channels [7, 8]. Their action during ischemia could result in a net Na^+ inward current that could contribute to the increase of $[Na^+]_i$ [9]. Simultaneously, this depolarizing current would enhance K^+ driving force and K^+ efflux during the early phase of myocardial ischemia as suggested in experimental reports [13].

In accordance with this hypothesis, our results show that an inward current is necessary to explain the ischemic K^+ accumulation in the extracellular space. The activation of I_{ONa} counteracts the effects of increasing f_{ATP} on APD and on K^+ driving force and facilitates cellular K^+ loss across $I_{K(ATP)}$ and other K^+ channels. Then, the concomitant effect of the opening of K_{ATP} channels and the activation of a Na^+ inward current

provokes a fast increase of unidirectional outward K^+ flux.

In addition to the increase of K^+ efflux, a decrease of Na^+/K^+ pump ability to transport K^+ ions would contribute to their accumulation in the extracellular space. In this sense, experimental reports suggest that the pump could be partially inhibited by several ischemic factors like accumulation of inorganic phosphates, decreased intracellular pH and reduced intracellular ATP levels [6]. However, other studies have shown that K^+ influx persists and even is enhanced after the interruption of coronary flow [14].

Our results could explain this apparent discrepancy because, even if the I_{NaKMAX} is linearly decreased, K^+ influx is only slightly reduced during the first minutes of simulated ischemia. Once the activation of I_{ONa} begins, the increase of $[Na^+]_i$ and also of $[K^+]_o$ is sufficient to enhance I_{NaK} despite the reduction of I_{NaKMAX}. Thus, in agreement with experimental observations, Na^+/K^+ pump activity could play an important role on the onset of the plateau phase.

5. Conclusions

The biphasic time course of extracellular K^+ accumulation observed during the first 14 minutes of simulated ischemia is quantitative and qualitatively in accordance with the experimental data obtained during acute myocardial ischemia.

These results support the hypothesis that extracellular K^+ accumulation could be caused by the concomitant effect of opening of K_{ATP} channels, Na^+/K^+ pump inhibition and a net Na^+ inward current in the absence of coronary flow. Cellular K^+ loss results from an increase of unidirectional K^+ efflux which is counteracted by an enhancement of Na^+/K^+ current, possibly produced by changes in ionic concentrations such as $[K^+]_o$ and $[Na^+]_i$.

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