Modulation of L-type calcium current by internal potassium in guinea pig ventricular myocytes

Klaus W. Linz, Rainer Meyer *

Physiological Institute, University of Bonn, Wilhelmastraße 31, D-53111 Bonn, Germany

Received 10 January 1996; accepted 22 August 1996

Abstract

Objectives: The early phase of myocardial ischemia is characterized by a considerable K⁺ efflux from cardiac myocytes, causing decreasing internal ([K⁺]ᵢ) and increasing external ([K⁺]ₒ) K⁺ concentrations. The change in [K⁺]ᵢ and [K⁺]ₒ is one of the factors thought to initiate the ischemia-induced changes in electrical activity. Nevertheless, little is known about the influence of [K⁺]ᵢ and [K⁺]ₒ on the L-type calcium current. Methods: The whole-cell patch-clamp technique combined with an internal perfusion system was used to test possible actions of altered [K⁺]ᵢ and [K⁺]ₒ on L-type current carried by Ca²⁺ and Ba²⁺ in isolated guinea pig ventricular myocytes. Results: Changing the [K⁺]ᵢ in the range of 110–170 mM revealed a sigmoidal concentration–response relationship between the L-type current and [K⁺]ᵢ. The maximum change in current amplitude was more than 40% with a half-saturation concentration of 136 mM which is near the physiological [K⁺]ᵢ. Ca²⁺ influx during action potential clamp increased by approximately 42% after raising [K⁺]ᵢ from 130 to 170 mM. Internal perfusion with Cs⁺ demonstrated that Cs⁺ is less effective than K⁺ in regulating the L-type current. By using ATP-analogues, [K⁺]ᵢ was shown to affect the L-type channel in a phosphorylation-independent way. Changes in [K⁺]ₒ only modulated the L-type current via alterations in [K⁺]ᵢ. Conclusions: The decrease in [K⁺]ᵢ during early ischemia is, per se, sufficient to reduce the L-type current by up to 15%, thereby decreasing the action potential duration, and Ca²⁺ influx into the cells. This may act in addition to well-known mechanisms such as changes in internal pH and falling ATP levels, which influence the L-type current. Moreover, the phenomenon may complicate the interpretation of electrophysiological measurements of L-type current under conditions where [K⁺]ᵢ is not precisely controlled.

Keywords: Myocardial ischemia; Calcium current, L-type; Potassium concentration, external; Potassium concentration, internal; Guinea pig, ventricular myocytes; Patch-clamp

1. Introduction

Myocardial ischemia results in marked alterations in electrical and metabolic activity of cardiac myocytes (for review, see [1]). One of the most striking metabolic changes is a dramatic K⁺ efflux from the cells. This efflux starts a few seconds after coronary occlusion and is probably secondary to the efflux of anions from the cells [1,2]. Within the initial 15 min of ischemia the extracellular K⁺ activity is increased from about 4 up to 15 mM [3,4]; simultaneously the internal K⁺ activity (aKᵢ) decreases by 3–4 mM [4] up to 13.5 mM [5]. These alterations in internal and external [K⁺] are accompanied by a depolarization of the membrane to about −60 mV [6], a decrease in action potential amplitude and upstroke velocity, and a reduction in action potential duration until the cells become inexcitable after about 15 min (for review, see [7]). The shortening of the action potential duration, first described by Trautwein et al. [8], has been attributed to the abolition of the plateau phase [9]. At present, the decrease in action potential duration is thought to be mainly due to an increased K⁺ conductance, and the activation of an ATP-dependent K⁺ current [7,10,11]. An additional contribution of a decrease in L-type Ca²⁺ current (Iₐ,Cₐ,L) is also taken into account [11–13], as the amplitude of Iₐ,Cₐ,L is depressed by hypoxia [5,14]. Possible cellular mechanisms leading to this down-regulation of Iₐ,Cₐ,L are a decrease in
cytosolic pH [15,16] and a fall in intracellular ATP concentration ([ATP]) [11,17]. Only effects on pH seem to be of physiological significance in early ischemia, since [ATP] remains unchanged during the first 10 min [18]. Surprisingly, the very fast and dramatic changes in intracellular and extracellular K+ have not been considered as other possible factors modulating \( I_{\text{Ca,L}} \). Therefore, the effect of altering internal and external K+ concentrations on \( I_{\text{Ca,L}} \) was investigated in the present study. We found that the \( I_{\text{Ca,L}} \) amplitude is modulated by changes in the internal K+ concentration ([K+]\(_i\)). A possible physiological function during early ischemia is discussed. In addition, possible methodological complications arising from this phenomenon are described.

2. Methods

2.1. Cell preparation

Guinea pig ventricular myocytes were isolated by enzymatic dispersion as previously described in detail [19]. Briefly, the heart from male guinea pigs, weighing 250–400 g, was perfused in a Langendorff apparatus with the following solutions (cf. Section 2.4 for composition): (1) Ca\(^{2+}\)-free Tyrode’s solution (5 min); (2) high K+ solution (HPS; 5 min); (3) HPS with trypsin (0.625 mg/ml, Boehringer, Mannheim, FRG; 10 min); (4) HPS with collagenase (type I; 1 mg/ml; Sigma, St. Louis, MO, USA; 10–15 min). Isolated, cells were stored in oxygenated standard Tyrode’s solution at 4°C and used within 6–8 h. The investigation conforms with the guide for care and use of laboratory animals according to the Deutsches Tierschutzgesetz.

2.2. Recording methods

Membrane potential and whole-cell currents were recorded at 35 ± 1°C using the patch-clamp technique [20]. Patch pipettes (2–4 MΩ) were pulled from borosilicate glass (Hillegersberg, Malsfeld, FRG for standard recordings, or Corning #7052; World Precision Instruments, Sarasota, FL, USA, for recordings with internally perfused electrodes). Membrane currents were recorded with a single-electrode continuous-voltage clamp amplifier (L/M EPC 7, List Medical Electronic, Darmstadt, FRG). Control experiments were carried out with a single-electrode switching amplifier (SEC-IL, NPI Electronic, Tamm, FRG). Cell capacitance and series resistance were compensated using the circuitry of the amplifier. Prior to cell attachment, the junction potential of the electrode was adjusted to zero (see also the next section). Voltage-clamp protocols, data acquisition, and data storage were accomplished with a custom-made program or the pClamp 6.0 software (Axon Instruments, Foster City, CA, USA).

Action potentials were elicited by suprathreshold current pulses (0.5–1.0 nA; 0.2 or 1 Hz) applied by the patch electrode. Action potential duration was determined at 50 and 90% of repolarization (APD\(_{50}\) and APD\(_{90}\)) using averaged action potentials of 10 consecutive stimulations. During voltage clamp measurements holding potential was –80 mV, unless otherwise indicated. The fast Na+ current and the T-type calcium current were inactivated by a 200 ms lasting pre-clamp in the range of –40 to –35 mV preceding each test clamp. In some experiments Na+ currents were prevented by substituting NaCl with choline chloride in the Tyrode solution. In most voltage clamp experiments square pulses were applied as command voltage although, in some, digitized action potentials (action potential clamp [21,22]) were used. The L-type Ca\(^{2+}\) current was determined in four different ways: (1) as Ca\(^{2+}\) antagonist-sensitive current, (2) as current flow of Ba\(^{2+}\), (3) as current difference between the peak inward current and the current after 100 ms, and (4) as absolute inward current after block of Na+ and K+ conductances. In the case of rectangular pulses, Ca\(^{2+}\) currents measured in different test solutions at the same cell were normalized to the Ca\(^{2+}\) current amplitude recorded at a membrane potential of +10 mV in standard external (4 mM K+) and internal (130 mM K+) solutions, since it was not possible to test more than two [K+]\(_i\) on one cell. Current–voltage relations were fitted by the following equation:

\[
I_{\text{Ca,L}} = \frac{G_{\text{max}}(V - V_{\text{rev}})}{1 + \exp\left[\frac{V_{1/2} - V}{k}\right]}
\]

where \(G_{\text{max}}\) = maximum conductance, \(V_{\text{rev}}\) = reversal potential, \(V_{1/2}\) = potential of half-maximum activation, and \(k\) = slope of activation curve.

All values are reported as means ± s.e.m.; \(n\) = number of cells. Error bars in figures represent s.e.m. The significance of relative changes in data was tested using unpaired (effects monitored at different samples of cells) or paired (effects monitored at the same cell) \(t\)-test. Differences with \(P < 0.05\) were regarded as significant.

2.3. Internal perfusion system

For measurements with varying intracellular ion concentrations a custom-modified patch electrode perfusion system (NPI Electronic, Tamm, FRG) was employed. The commercially available system consists of a vacuum generator (2 PK, ALA Scientific Instruments, Westbury, NY, USA), connected to the patch electrode, and a thin quartz-glass pipette, which is inserted into the patch electrode. Its end is about 75 μm away from the tip of the patch electrode. The quartz-glass pipette is connected to a small vessel with the second perfusion solution. Applying negative pressure to the patch electrode will suck solution into it via the quartz-glass pipette. This system of Tang and co-workers [23,24] was further developed from an open system to a closed one. In this closed system positive or
negative pressure is always applied to both the patch electrode and the perfusion pipette. This construction improves formation of the ‘giga seal’ and avoids any uncontrolled solution changes during the measurement. Only in case of intentional solution change are different pressures applied. For optical control, the second solution usually contained 0.5 mM phenol red which did not produce obvious changes in membrane currents ($n = 12$). Negative pressure of 2.6–3.3 kPa was applied continuously throughout the measurements to minimize changes in series resistance due to variations in pressure. The efficiency of internal cell dialysis was determined as shift in the reversal potential of the inward rectifying current ($I_{K1}$) after changing the $K^+$ concentration of the pipette solution by internal perfusion (Fig. 2). Upon an increase in $K^+$ from 130 to 150 or 170 mM the reversal potential of $I_{K1}$ is shifted by $-4.5 \pm 1.3$ mV ($n = 4$) or $-7.2 \pm 0.7$ mV ($n = 21$), respectively. These values are in good agreement with the shifts in $K^+$ equilibrium potential calculated by the Nernst equation. Assuming a constant external $K^+$ concentration ($[K^+]_o$) of 4 mM, these are $-3.82$ (150 mM) and $-7.17$ mV (170 mM). According to Kameyama et al. [25] diffusion of small ions from the pipette solution into cells of the dimensions of cardiac myocytes occurs within less than 5 min. This is consistent with the observation that effects here were regularly observed within 2–8 min after the new solution had reached the tip of the pipette (optical control). The variance in time for diffusional exchange is most probably due to variations in the access resistance in different experiments [26]. Variations of the internal Cl$^-$ concentration can cause changes in the surface potential at the chlorided silver wire connecting the patch-pipette to the preamplifier. To avoid this artifact, the second pipette solution was only sucked into the tip of the pipette (i.e., the foremost 5–10 mm). Since the chlorided silver wire was positioned at a distance of more than 25 mm from the tip, changes in electrode surface potentials could be excluded within the time course of the experiment. Nevertheless, a change in electrode potential due to an elevated internal Cl$^-$ concentration at the silver wire will lead to a simultaneous outward shift of the holding current and the current at the end of the 400 ms lasting test clamps to $+10$ mV. This was not observed during the measurements. In addition, experiments with aspartate instead of chloride provided comparable results, indicating that the measurements were not influenced by a change in surface potential (Table 1).

Changes of the ion concentration in the pipette may influence pipette access resistance. However, changing the internal KCl concentration in the range used in this study produced only minor changes in pipette access resistance ($\approx 2\%$). Artificial effects due to these changes could be excluded since similar results were obtained in measurements where $[K^+]_o$ was raised by addition of K-salts to the internal solution and in experiments where $[K^+]_o$ was raised by equimolar substitution of KCl for CsCl.

2.4. Solutions

The standard Tyrode solution was composed of (mM): NaCl, 135; KCl, 4; CaCl$_2$, 1.8; MgCl$_2$, 1; glucose, 11; HEPES, 2 (pH 7.2 with NaOH), bovine serum albumin (1 g/l) and trypsin inhibitor (Sigma; 0.0167 g/l). The Ca$^{2+}$-free Tyrode solution used for cell preparation contained 2.6 mM EGTA instead of CaCl$_2$. The HPS consisted of (mM): NaCl, 4; KCl, 10; K-glutamate, 130; CaCl$_2$, 0.025; MgCl$_2$, 1; HEPES, 4 (pH 7.2 with KOH); glucose, 10.

The external solutions for patch-clamp measurements were based on standard Tyrode’s solution. Appropriate amounts of KCl were added for variations of $[K^+]_o$. In some experiments CaCl$_2$ was substituted by an equimolar amount of BaCl$_2$. To block the L-type Ca$^{2+}$ current 100 $\mu$M Cd$^{2+}$ or 1 $\mu$M P200-110 (Isradipine; Research Biochemicals International, Natick, MA, USA) was used. PN200-110 was added from stock solution (10 mM in ethanol), which was prepared freshly every day and kept dark. For $\beta$-stimulation of the cells the Tyrode’s solution contained 1 $\mu$M isoprenaline (N-isopropyl-$\beta$-noradrenaline-hydrochloride; Fluka, Buchs, Switzerland). Isoprenaline–Tyrode’s solution was prepared freshly every 1–2 h by addition of crystalline isoprenaline. To inhibit Na$^+$ and K$^+$ currents in some experiments, the external solution consisted of (mM): choline chloride, 135; KCl, 4; CaCl$_2$, 1.8; BaCl$_2$, 2; MgCl$_2$, 1; 4-aminopyridine (4-AP), 2; glucose, 11; HEPES, 2 (pH 7.2 with KOH).

The standard internal solution contained (mM): KCl, 130; EGTA, 0.2; HEPES, 10 (pH 7.2 was obtained by addition of approximately 5 mM NaOH). For measurements with the patch-pipette perfusion system, the internal solutions were based on (mM): NaCl, 5; KOH, 40; Mg-ATP (Sigma), 2.5; EGTA, 10; HEPES, 10 (pH 7.2 with HCl). Appropriate amounts of KCl or K-aspartate were added to obtain final K$^+$ concentrations of 110, 130, 140, 150, and 170 mM. K$^+$-free internal solutions were made using Cs-salts instead of K-salts. In some experiments Mg-ATP was replaced by 5 mM adenosine 5'-O-(3-thiotriphosphate) (ATP$_7$yS; Sigma) or 5 mM of S'-adenylylimidodiphosphate (AMP-PNP; Sigma). In experiments in which Na$^+$ and K$^+$ currents were blocked, the internal solutions were Na$^+$-free, and additionally contained 20 mM tetraethylammonium chloride (TEA). In this case, solutions with different KCl concentrations were made iso-osmotic by addition of sucrose.

3. Results

3.1. Effects of $[K^+]_o$ on action potential duration and steady-state $K^+$ currents

Under control conditions (i.e., normal Tyrode’s solution in the bath and a pipette solution with 130 mM K$^+$), the APD$_{50}$ at 0.2 Hz was 259.9 ± 26.1 ms ($n = 6$) and the APD$_{90}$ 316.3 ± 28.3 ms ($n = 6$). After increasing [K$^+$],
from 130 to 170 mM by perfusing the patch pipette, the
APD50 and APD90 increased by 15% and 10%, respec-
tively (Fig. 1A, Table 1). A contribution of run-down
phenomena can be excluded, since the action potential
duration continuously shortens as \( I_{\text{calc}} \) decreases and
potassium outward currents increase [27]. The observed
prolongation of the action potential could be explained by
(1) an increase in \( I_{\text{calc}} \), leading to a longer plateau phase,
and (2) a decrease in K\(^+\) conductance also prolonging the
plateau and/or slowing the repolarization phase. To distin-
guish between these possibilities, we studied the influence
of changes in [K\(^+\)]\(_i\) on the steady-state K\(^+\) currents and on
the \( I_{\text{calc}} \).

Steady-state K\(^+\) currents were investigated by applica-
tion of slow voltage ramps ascending from –100 to +50
mV (slope: 15 mV/s). Raising [K\(^+\)]\(_i\) from 130 to 170 mM
changed the N-shaped current–voltage relation only at
negative clamp potentials (Fig. 2). The reversal potential
was shifted by about –7 mV (see Section 2), the outward
current was significantly decreased at membrane potentials
between –30 and –65 mV (–40 mV: \( P = 0.02 \)), and significantly increased between –65 mV and the reversal potential (–70 mV: \( P = 0.04 \); –80 mV: \( P = 0.01 \)). As it is difficult to estimate whether these
changes in K\(^+\) currents are sufficient to cause the prolon-
gation of the action potential or not, action potentials were
recorded after blocking \( I_{\text{calc}} \) by adding 100 \( \mu \text{M} \) Cd\(^{2+}\) or
1 \( \mu \text{M} \) PN200-110 to the external solution (Fig. 1B). This
shortened the APD50 by 52.9 ± 1.9% and the APD90 by
39.1 ± 1.0% (n = 5) in the case of Cd\(^{2+}\), and in the case
of PN200-110 by 37.7 ± 2.9% and 31.9 ± 3.0% (n = 9).
In the absence of the \( I_{\text{calc}} \), the plateau and the repolariza-
tion phase of the action potential are mostly determined by
changes in K\(^+\) conductances. Accordingly, changes in
action potential duration induced by altered K\(^+\) conduct-
ances should be even more pronounced in this case.
However, increasing the [K\(^+\)]\(_i\) from 130 mM to 170 mM
did not increase the action potential duration in cells where
the \( I_{\text{calc}} \) was blocked with Cd\(^{2+}\) or PN200-110 (Table 1).
Thus, changes in K\(^+\) conductance are not responsible for
the observed prolongation in action potential duration.

| Effect of increasing the internal K\(^+\) concentration on action potential duration, L-type calcium current amplitude during rectangular voltage-clamp pulses, and Ca\(^{2+}\)- influx during action-potential clamp: comparison of different recording conditions (i.e., different internal and external solutions) |
|-------------------------------------------------|-------------------------------------------------|
| **Recording conditions** | **External solution** | **Change (%) mean (s.e.m.)** | **n** |
| Internal solution | **Action potential recording** | | |
| 130 to 170 mM K\(^+\) (KCl) | Standard Tyrode’s solution | APD50 | 14.6 (0.8) * * |
| | Tyrode’s solution; 100 \( \mu \text{M} \) Cd\(^{2+}\) | APD50 | –0.03 (1.4) |
| | Tyrode’s solution; 1 \( \mu \text{M} \) PN200-110 | APD50 | –3.8 (1.5) * |
| | | APD90 | +9.9 (1.1) * * |
| | | APD90 | –0.6 (1.1) |
| | | APD90 | –3.0 (1.2) * |
| | | n | 6 |
| | | | 5 |
| | | | 9 |
| **Rectangular voltage-clamp pulse (+10 mV)** | **L-type current amplitude** | | |
| 130 to 170 mM K\(^+\) (KCl) | Standard Tyrode’s solution | +26.1 (2.8) * * |
| 130 to 170 mM K\(^+\) (K-Asp) | Standard Tyrode’s solution | +24.0 (4.6) * * |
| 130 to 170 mM K\(^+\) (\( I_{\text{calc}} \) blocked) | Na-free Tyrode’s solution (\( I_{\text{calc}} \) blocked) | +27.7 (7.5) * |
| 130 mM Cs\(^+\) to 130 mM K\(^+\) | Standard Tyrode’s solution | +17.7 (2.7) * |
| | | n | 8 |
| | | | 6 |
| | | | 5 |
| | | | 10 |
| **Action potential clamp** | **Ca\(^{2+}\)- influx** | | |
| 130 to 170 mM K\(^+\) (\( I_{\text{calc}} \) blocked) | Na-free Tyrode’s solution (\( I_{\text{calc}} \) blocked) | +42.3 (4.5) * |
| | | n | 5 |

The quantity of change is given as percent increase (+) or decrease (−). * \( P < 0.05 \); ** \( P < 0.01 \).
3.2. Effects of [K\textsuperscript{\textdagger}]\textsubscript{i} on L-type current

3.2.1. [K\textsuperscript{\textdagger}]\textsubscript{i}-induced changes in L-type current

As a second possible reason for the prolongation of the action potential, the influence of [K\textsuperscript{\textdagger}]\textsubscript{i} on the amplitude of the L-type current was examined (Fig. 3). Under control conditions the I\textsubscript{Ca,L} elicited by a test clamp to 10 mV had a mean amplitude of $-11.8 \pm 0.4$ pA/pF ($n = 35$) (Fig. 3A). An elevation of [K\textsuperscript{\textdagger}]\textsubscript{i} from 130 to 150 mM induced a rise of I\textsubscript{Ca,L} by about 25\% (Table 2). Using Ba\textsuperscript{2+} as charge carrier (I\textsubscript{Ba,L}), the same elevation of [K\textsuperscript{\textdagger}]\textsubscript{i} increased the current by more than 30\% (Fig. 3B, Table 2) starting at $-11.9 \pm 0.5$ pA/pF ($n = 16$). At the higher [K\textsuperscript{\textdagger}]\textsubscript{i}, the time to peak and the half-time of inactivation were reduced for both I\textsubscript{Ca,L} and I\textsubscript{Ba,L} (Table 2).

To rule out effects of increased internal osmolarity on the L-type current due to elevated internal KCl concentrations, the cells were dialysed with a solution of unchanged K\textsuperscript{+} concentration which additionally contained 40 or 80 mM sucrose. This increase in osmolarity which was equivalent to that induced by addition of 20 or 40 mM KCl did not produce any visible change in I\textsubscript{Ca,L} ($n = 8$).

Changes in Na\textsuperscript{+} or K\textsuperscript{+} currents could lead to artifacts when I\textsubscript{Ca,L} is determined as current difference between the peak current and the outward current at 100 ms after onset of the test clamp. To exclude this, we performed control experiments in which K\textsuperscript{+} conductances were blocked by Ba\textsuperscript{2+}, TEA, and 4-AP, and in which Na\textsuperscript{+} conductances were inhibited by substitution of Na\textsuperscript{+} with choline. The original recordings in Fig. 4A–D show that the effect of raising [K\textsuperscript{\textdagger}]\textsubscript{i} on I\textsubscript{Ca,L} was also observed under these conditions. Increasing [K\textsuperscript{\textdagger}]\textsubscript{i}, iso-osmotically from 130 to 170 mM elevated I\textsubscript{Ca,L} during a clamp step to 10 mV to the same extent as without blocking agents (Table 1). At the end of each experiment, I\textsubscript{Ca,L} was blocked by application of 100 \\mu M Cd\textsuperscript{2+}. Time-dependent changes in current were then no longer observed (i.e., the only time-dependent current was I\textsubscript{Ca,L}).

The time course of I\textsubscript{Ca,L} during an action potential is quite different from the time course during rectangular voltage pulses [21,22,28–30]. Following the upstroke of the action potential there is only a relatively small inward-directed current peak. After partial inactivation, the I\textsubscript{Ca,L}...
reactivates and finally inactivates at the end of the plateau [22,29,30]. Therefore, we examined the effects of changing [K+] on I_{ca,L} elicited by digitized action potentials as command voltage. Fig. 4E shows original recordings of the I_{ca,L} after block of Na+ and K+ currents during action potential clamp experiments. These measurements demonstrate that raising [K+], from 130 to 170 mM leads to a marked increase in I_{ca,L} during the action-potential plateau. The inward flow of Ca2+ increases by 42% from 234.4 ± 12.43 to 333.6 ± 17.64 fC/pF (c.f. Table 1).

3.2.2. Concentration dependence

A series of voltage clamp experiments with square pulses was carried out to establish a concentration–response relation between [K+] and I_{ca,L}. All experiments were designed to induce an increase in I_{ca,L} (i.e., [K+] was always raised) to avoid the artificial influence of run-down of L-type channel activity [27]. Fig. 5A shows current-voltage relations recorded at different [K+]. Upon a rise in [K+], from 130 to 150 or 170 mM a gradual increase in I_{ca,L} at membrane potentials between 0 and +60 mV was observed. The reversal potential (67.3 ± 0.6 mV; n = 7) was not affected by the change in [K+], indicating a minor influence of the K+ conductance of the

![Figure 4](image_url)

**Fig. 4.** Original recordings of L-type Ca2+ current (I_{ca,L}) after inhibition of Na+ currents by substitution of Na+ with choline and block of K+ currents by Ba2+, TEA, and 4-AP. Panels A–D: I_{ca,L} elicited by rectangular voltage pulses to the clamp potentials (V_{clamp}) indicated above the traces. Panel E: Action potential clamp using the command voltage plotted in the upper trace. The digitized action potential was modified by addition of a 200 ms lasting pre-clamp to −37 mV to inactivate the T-type calcium current. Increasing the internal potassium concentration ([K+]i) iso-osmotically from 130 mM (○) to 170 mM (▲) raises the I_{ca,L} amplitude. After block of I_{ca,L} by 100 μM Cd2+ (■) time-dependent changes in current cannot be recorded any longer.

![Figure 5](image_url)

**Fig. 5.** Quantification of the effect of alterations in the internal K+ concentration ([K+]i) on the L-type Ca2+ current (I_{ca,L}). **Panel A:** Graphs showing current–voltage relations of I_{ca,L} recorded at different [K+]. Amplitude values were normalized to the I_{ca,L} measured at [K+] = 130 mM and a clamp potential of +10 mV. Increasing [K+] from 130 (○: n = 7) to 150 (▲: n = 4) and 170 mM (■: n = 3) led to a gradual increase in I_{ca,L}. **Panel B:** Concentration–response curve of the [K+] effect. Amplitude values obtained at different [K+] following clamp pulses to +10 mV are plotted against the respective [K+]. Amplitude values were normalized to control recordings at [K+] = 130 mM. For clear presentation [K+] values are plotted on a linear abscissa. Error bars indicate s.e.m. In some cases the error bars are smaller than the symbols.
L-type channel. The concentration–response relation of the 
$I_{Ca,L}$ to [K+]i, shows a sigmoidal dependence of the current amplitude at +10 mV against the applied [K+]i, (Fig. 5B). Fitting of the data points reveals a half-saturation concentration of 135.9 ± 1.0 mM and a Hill coefficient of 21.7 ± 1.3. The maximum increase in $I_{Ca,L}$ amplitude was calculated as 43.6%.

It is noteworthy that the effect of [K+]i on the $I_{Ca,L}$ amplitude was enhanced in the presence of isoprenaline. After β1-stimulation by 1 μM isoprenaline, an increase of [K+]i, from 130 to 150 mM raised the $I_{Ca,L}$ amplitude by about 35% (Table 2) which is significantly higher (P = 0.048) than the 25% in the absence of isoprenaline. β1-Stimulation thus intensified the stimulatory effect of [K+]i, by more than a third.

3.2.3. Influence of other ions

To exclude effects of Cl− ions on the $I_{Ca,L}$ amplitude, we performed measurements where K-aspartate instead of KCl was used to increase the [K+]i. The amount of increase in current amplitude following an elevation of [K+]i, was similar for both anions (P = 0.7; Table 1).

In electrophysiological investigations of $I_{Ca,L}$, Cs ions are commonly used as a substitute for K+. This raises the question whether Cs+ is able to substitute for K+ in regulating the L-type current amplitude. Fig. 6 shows the time course of the $I_{Ca,L}$ amplitude during repetitive voltage clamp pulses. In the course of this experiment the internal solution was changed iso-osmotically from a K+-free solution containing 130 mM Cs+ to control solution with 130 mM K+. The recording was started about 5 min after establishment of the whole cell configuration, when the depolarization of the membrane potential due to the diffusion of Cs+ into the cell reached a steady state of −42.1 ± 1.0 mV (n = 12). In most experiments, the run-down of $I_{Ca,L}$ was markedly enhanced following perfusion with Cs+-rich pipette solution. After perfusion of the cell with an internal solution containing 130 mM K+, the L-type current amplitude increased by 18% (Table 1). This suggests that Cs+ cannot replace K+ in modulating $I_{Ca,L}$, or has at least a different effective concentration range. Substituting internal K+ with Cs+ reduced the L-type current amplitude to a value slightly below the one measured in the presence of [K+]i = 110 mM, emphasizing the saturation characteristic of the concentration–response relation.

3.3. Effects of [K+]o on L-type current

3.3.1. [K+]o-induced changes in L-type current

Since during ischemia [K+]o increases simultaneously with the fall in [K+]i, the influence of the [K+]o on currents through L-type channels was tested also. With Ba2+ as charge carrier, an elevation in [K+]o, from 4 to 16 mM neither led to any change in amplitude and voltage dependence of the L-type current (Fig. 7A) nor did it modify its time course of activation or inactivation (Table 2).

The situation became completely different when Ca2+ was used as charge carrier. A rise in [K+]o, from 4 to 8, 16 or 30 mM led to a progressive increase in $I_{Ca,L}$ amplitude (Fig. 7B) and an acceleration of the activation and inactivation time course (Table 2). It is possible that the increase in $I_{Ca,L}$ with increasing [K+]o might be superimposed upon a change in K+ currents leading to false interpretation of Ca2+ current measurements. To exclude this possi-
bility, the dihydropyridine-sensitive current was determined by addition of 1 μM PN200-110 (Fig. 7C). Since the blocking effect of PN200-110 is not fully reversible, it was not possible to determine the dihydropyridine-sensitive current at two different [K+]i's in the same cell. To overcome this difficulty, we always measured the control current at [K+]o = 4 mM and in addition the PN200-110-sensitive current at a [K+]o of either 4 or 16 mM of an individual cell. All amplitude data were normalized to the maximum amplitude of the Ic,a,L determined under control conditions in each cell. As can be seen in Fig. 7C, it is in fact the dihydropyridine-sensitive current that was increased after the rise in [K+]o. Similar to the observations with varying [K+], the amplitude of the Ic,a,L depends in a sigmoidal way on the [K+]o (Fig. 7D), with half-saturation at 9.5 ± 0.7 mM and a Hill coefficient of 1.2 ± 0.1. The maximum increase in amplitude was calculated to be 22.1%. After β1-stimulation by external application of 1 μM isoprenaline the [K+]i-induced increase in Ic,a,L was significantly enhanced from 16 to 36% (Table 2, c.f. also Fig. 10).

The explanation for the obvious discrepancy between the observed effect of an increase in [K+]o on the L-type current carried by either Ba2+ or Ca2+ might be that it is rather [K+]i, than [K+]o, which influences Ic,a,L. Since the cells were clamped to a holding potential of −80 mV between the single test pulses, one might expect a continuous net K+ influx over time at [K+]o > 4 mM, leading to a progressive increase in [K+]i. This explanation was tested by application of slow voltage ramps (slope: 15 mV/s) during the increase in [K+]o (Fig. 8). After raising [K+]o from 4 to 16 mM, the reversal potential was shifted to less negative values and the inward rectifying K+ current (Ic,IK) was increased due to a drop in membrane resistance [31]. If the cell is voltage-clamped to −80 mV under these conditions, the [K+]i, starts to increase due to the marked K+ influx. This change in [K+]i, could be seen as a shift in reversal potential in negative direction. When Ba2+ is used as charge carrier for the L-type current, Ic,IK is blocked by Ba2+. This might explain the lack of an effect of changing [K+]o on Ic,Ba,L, since under these conditions an influx of K+ via Ic,IK would not happen.

3.3.2. [K+]o-induced changes in [K+].

The next set of experiments was done to correlate the changes in [K+]o and [K+], with the changes in Ic,a,L. To obtain an index of changes in [K+]o and [K+], the amplitude of the Ic,IK was determined at the end of the 200 ms lasting pre-clamp to potentials between −40 and −35 mV. At these potentials a rise in [K+]o from 4 to 16 mM (indicated by the dotted vertical line) Vhold was turned from −80 to −50 mV, a value near the newly achieved reversal potential. By subsequent changes in Vhold, as indicated by the horizontal bars, transmembrane fluxes of K+ were evoked, leading to changes in Ic,IK and Ic,a,L. Similar results were obtained in 5 (A) and 4 (B) preparations.

Fig. 8. Steady-state current–voltage relations recorded from one cell, using slow voltage ramps. The curves were recorded successively at an external K+ concentration ([K+]o) of 4 mM (○), and 1 min (■) and 10 min (▲) after increasing [K+]o to 16 mM, respectively. The holding potential for the time between the single voltage ramps was −80 mV. Similar results were obtained in 8 cells.
gradual rise in [K+]i. After returning to control solution, Ic, decreased to its control value, again showing a biphase time course. In contrast, the increase and decrease in Ic, amplitude exhibited only monophasic time courses which proceeded simultaneously with the slow increase or decrease in Ica, reaching their steady states at almost the same time. Therefore, it seems likely that it is not [K+]i, but the subsequent change in [K+]i, which modulates Ic, amplitudes. If this is true, it should be possible to induce changes in Ic and Ica, amplitudes by varying the holding potential applied between test pulses. In the experiment shown in Fig. 9B, the holding potential was shifted to −50 mV simultaneously with the rise in [K+]i. Since −50 mV is close to the reversal potential achieved with the increased level of [K+]i (see Fig. 8), K+ fluxes during the time between test pulses were prevented. As a consequence, neither the slow change in Ic, nor any alteration of Ica, could be observed. After switching the holding potential to a value positive to the reversal potential, a K+ efflux was induced, leading to a decrease in both Ic, and Ica. Holding the cell at −80 mV, which caused a K+ influx, reversed both processes. If the holding potential was switched to −40 mV, the subsequent K+ efflux again reduced Ica.

3.4. Molecular mechanism of [K+]i action on Ica

There are at least three different possibilities for the action of [K+]i on the current through L-type channels: (1) direct interaction of the intracellular K+ with the channel proteins, (2) interaction with the enzymes responsible for the phosphorylation/dephosphorylation process of the channels, and (3) modulation of other processes regulating the L-type channels. We tried to check the involvement of the phosphorylation/dephosphorylation process in the [K+]i-dependent regulation of the L-type current. Experiments were carried out with the ATP-analogues ATPyS and AMP-PNP. ATPyS is used by protein kinase A (PKA) for a long-lasting thio-phosphorylation of its substrates [32,33]. In contrast, AMP-PNP cannot be hydrolyzed by PKA to phosphorylate the channel protein [25,32].

In these experiments [K+]i was not raised by internal perfusion, but increased indirectly by holding the cells at −80 mV after elevating [K+]i, from 4 to 16 mM. Fig. 10 shows an example of a cell dialysed with ATPyS. The cell was kept for approximately 5 min at free-running membrane potential to allow the diffusion of ATPyS into the cytosol. Then, the cell was clamped to the holding potential of −80 mV and pulsed repetitively to +10 mV at 0.1 Hz. During this time the amplitude of Ica increased due to progressive thio-phosphorylation of L-type channel proteins [33]. β1-Stimulation by 1 μM isoprenaline further increased the Ica amplitude by approximately a factor of 3 [34]. The change in steady-state current at −35 mV from an outward to an inward directed current observed with isoprenaline can be explained by an isoprenaline-activated Cl− current [35,36]. After wash-out of isoprenaline the stimulatory effect did not reverse, indicating thio-phosphorylation of the channel proteins. Subsequent elevation of [K+]i from 4 to 16 mM caused the already described biphasic change in steady-state current at −35 mV, due to the drop in membrane resistance and the slow rise in [K+]i produced by K+ influx. Simultaneously with the second phase of steady-state current increase, the Ica amplitude increased 43.9 ± 4.8% (n = 14; P < 0.0001) above the isoprenaline-stimulated value. This effect was almost completely reversed by changing [K+]i back to 4 mM. As in measurements where [K+]i, was directly varied (see above), β1-stimulation intensifies the effect of [K+]i on Ica, leading to a more pronounced increase in current (cf. Fig. 7D, Table 2). β1-Stimulation in the presence of ATPyS is supposed to lead to a long-lasting thio-phosphorylation of the entire L-type channel population of a cell [33], and therefore the additional K+-induced rise in Ica may not be explained by further phosphorylation, especially since it is reversible within a short time. Investigations of AMP-PNP-dialysed cells supported this. In such cells, β1-stimulation with 1 μM isoprenaline did not increase the Ica amplitude significantly (16.3 ± 7.0%; n = 3; P = 0.14) in contrast to approximately 200% without AMP-PNP. Nevertheless, indirectly increasing [K+]i, again led to a rise in Ica, amplitude by 15.6 ± 4.2% (n = 5; P = 0.02) which is comparable to the rise in the absence of AMP-PNP and isoprenaline (cf. Fig. 7D).

Fig. 10. Effect of β1-stimulation and increase in external K+ concentration ([K+]o) on L-type Ca2+ current (Ica, •) and steady-state current (Ic, ○) in an ATPyS dialysed cell. Ica, and Ic, were measured during repetitive voltage-clamp pulses to +10 and −35 mV, respectively. The horizontal bars indicate the time during which 1 μM isoprenaline or 16 mM [K+]i was applied. In 14 cells similar results were obtained. Reversibility was tested in 4 cells. The inset shows original current traces recorded at the times indicated in the graph. The dotted line indicates the zero current level.
4. Discussion

4.1. Modulation of L-type Ca\(^{2+}\) current by \([K^+]_o\)

4.1.1. Possible sources of artifacts

The results presented in this study demonstrate that the amplitude of \(I_{\text{Ca,L}}\) is regulated by \([K^+]_o\), following a sigmoidal concentration–response relation. An important question is whether alterations in other current systems or other influences on the \(I_{\text{Ca,L}}\) could artificially mimic this result. The contamination of the observed effects by changes in other current systems was excluded in control experiments. The Na\(^+\) current and Na/Ca exchange current were inhibited by exchanging all NaCl against choline chloride, the T-type Ca\(^{2+}\) current was inactivated by a pre-clamp, and the K\(^+\) currents were blocked by 4-AP, Ba\(^{2+}\) and TEA. Effects of the anions increased simultaneously with \([K^+]_o\), on the \(I_{\text{Ca,L}}\) were ruled out by the use of different potassium salts and experiments in which \([K^+]_o\), was increased by substituting Cs\(^+\) with K\(^+\).

Another difficulty might have arisen from a change in internal osmotic pressure, since the L-type channel may be stretch-sensitive which was rejected for guinea pig ventricular myocytes [37] but was demonstrated for rabbit atrial myocytes [38]. In our control experiments, osmotic changes were excluded by addition of sucrose (Fig. 4) or by iso-osmotic substitution of \([K^+]_o\), for \([Cs^+]_o\), (Fig. 6). Moreover, internal perfusion with a solution of unchanged \([K^+]_o\), containing 40 or 80 mM sucrose did not alter \(I_{\text{Ca,L}}\). The differences between our findings and the results of Matsuda and co-workers [38] may be due to species and tissue differences, and to different experimental conditions. Additionally, the time course of activation and inactivation was increased by an increase in \([K^+]_o\), (e.g., Fig. 3), whereas it remained unchanged when \(I_{\text{Ca,L}}\) rose due to mechanical stretch [38].

As \(I_{\text{Ca,L}}\) can be depressed by increasing internal Ca\(^{2+}\) [39], additional artifacts could arise from variations in \([Ca^{2+}]_i\), induced by changes in \([K^+]_o\) as well as \([K^+]_e\). Therefore, the \([Ca^{2+}]_o\), was buffered by 10 mM EGTA. Nevertheless, reduction of \([K^+]_o\), will reduce the Na/K-pump activity and increase the intracellular Na\(^+\) concentration, thereby possibly leading to a rise in \([Ca^{2+}]_i\), via Na/Ca exchange [40]. However, according to Lee and Fozzard [41] only a reduction of \([K^+]_o\), below 4 mM will cause a small decrease in the internal Na\(^+\) activity \((a_{\text{Na}})\), while elevations of \([K^+]_o\) above that value will not alter \(a_{\text{Na}}\) significantly. These results have been confirmed by measurements of the Na\(^+\) equilibrium potential in sheep Purkinje fibers [42]. For the concentration range tested in the present study (\([K^+]_o\) = 2–30 mM), any changes in \([Ca^{2+}]_i\), would only be expected with \([K^+]_o\) = 2 mM and would have been negligible anyway, because the suppression of \(I_{\text{Ca,L}}\) obtained by reduction of \([K^+]_o\), was smaller than that obtained by reducing \([K^+]_e\), directly.

4.1.2. Correlation between \([K^+]_o\), \([K^+]_e\), and \(I_{\text{Ca,L}}\)

Between 110 and 170 mM \([K^+]_o\), the amplitude of the \(I_{\text{Ca,L}}\) exhibits a characteristic sigmoidal concentration–response curve. In contrast, the \([K^+]_o\) had no direct influence on the L-type current amplitude. The increase in \(I_{\text{Ca,L}}\) induced by a rise in \([K^+]_o\), could be attributed to a secondary increase in \([K^+]_e\), due to a K\(^+\) influx driven by a \(V_{\text{f,old}}\) negative to \(E_K\). Changes in \([K^+]_o\), produced in this way again showed the sigmoidal concentration–response relationship between \([K^+]_o\), and \(I_{\text{Ca,L}}\), now expressed as a function of \([K^+]_e\). The maximum increase in \(I_{\text{Ca,L}}\) observed under these experimental conditions is about 20% which is markedly lower than the 44% increase measured when \([K^+]_o\), is directly varied. This discrepancy may be due to a saturation of \([K^+]_o\), at a value below 170 mM, if the rise in \([K^+]_o\), is induced by K\(^+\) influx. The rise in \([K^+]_o\), can be estimated from the shift in reversal potential induced by K\(^+\) influx. In the case of 16 mM \([K^+]_o\) (Fig. 8) the observed shift of –2.5 mV is consistent with an increase in \([K^+]_e\), from 130 to about 145 mM according to the Nernst equation. When directly varying \([K^+]_o\), this rise increases \(I_{\text{Ca,L}}\) by 15–20% (Fig. 5A), which is similar to that measured after a change in \([K^+]_o\), from 4 to 16 mM (Fig. 7D). There is an obvious difference in the Hill coefficients of the concentration–response relations of \([K^+]_o\), and \([K^+]_o\), vs. \(I_{\text{Ca,L}}\) of 22 compared to 1.2. This may be explained by the fact that multiplication of \([K^+]_o\), produces only a small change in \([K^+]_o\), (e.g., a 4-fold increase in \([K^+]_o\), raises \([K^+]_o\), by a factor of only 1.1).

4.1.3. Molecular mechanism

\(I_{\text{Ca,L}}\) was shown to depend steeply on \([K^+]_o\), as expressed by the rather high Hill coefficient of 22. This points to the involvement of an amplification mechanism via a cascade system. The most reasonable mechanism by which the \([K^+]_o\), might influence the L-type channel could involve the cAMP pathway. Surprisingly, the phosphorylation/dephosphorylation pathway does not seem to be involved since (1) the stimulatory effect of \([K^+]_o\), was not reduced, but enhanced after \(\beta_1\)-adrenergic stimulation, (2) even after thio-phosphorylation of the entire L-type channel population \(I_{\text{Ca,L}}\) could still be reversibly increased by a rise in \([K^+]_o\), and (3) inhibition of phosphorylation by AMP-PNP leads to an almost complete inhibition of \(\beta_1\)-stimulatory rise of the L-type current while the \([K^+]_o\)–mediated increase in \(I_{\text{Ca,L}}\) was not inhibited. This view is supported by the changes in activation and inactivation time course of \(I_{\text{Ca,L}}\) simultaneously to changes in current amplitude, because \(\beta_1\)-adrenergic stimulation does not alter the time course of L-type current [17,43–45]. Phosphorylation-independent regulation of L-type current has to be considered then like (1) modulation of metabolites such as MgATP [46] or lactate [47] which have been shown to regulate the L-type channel, or (2) a more direct action of \([K^+]_o\), on the L-type channel, although this would require...
postulation of multiple binding events. Further experiments are necessary to distinguish between these mechanisms.

While up to now a \([K^+]\)-dependent modulation of \(I_{Ca,L}\) has not been described, there are some reports about the alteration of L-type current by external Na ions (for review, see [48]). Both an increase [49] and a decrease [50,51] of \(I_{Ca,L}\) has been described in the case of lowering the external \(Na^+\) concentration, \([Na^+]_o\). Matsuoka et al. [52] reported a reduction in epinephrine-induced \(I_{Ca,L}\) after replacing extracellular \(Na^+\) with \(Li^+\). Harvey et al. [53] conclude that the \(Na^+\) sensitivity of \(\beta_1\)-adrenergically stimulated currents, like \(I_{Ca,L}\), results from changes in \([Na^+]_o\), following changes in \([Na^+]_o\). Although these findings show a high degree of complexity, the Na ions are thought to affect the phosphorylation or dephosphorylation process in all cases [49,53]. This is in contrast to the mechanism of the \([K^+]\)-mediated effect which does not seem to act on this pathway.

4.2. Could changes in \([K^+]_i\) give rise to false interpretation of experimental results?

As discussed above, a holding potential which is different from the \([K^+]\) equilibrium potential will induce changes in \([K^+]_i\), which in turn lead to changes in \(I_{Ca,L}\). Similar results have been obtained in frog ventricular myocytes by Schouten and Morad [54]. The authors attribute the so-called ‘slow inactivation’ to a voltage-dependent process, and exclude a \(K^+\)-dependent mechanism. Nevertheless, our experiments demonstrate that a decrease in \(I_{Ca,L}\) is induced by a potential dependent \(K^+\) efflux. An accelerated decrease in \(I_{Ca,L}\) amplitude after changing \(V_{\text{hold}}\) from \(-80\) to \(-40\) mV was also shown in guinea pig ventricular myocytes by Wang et al. [55]. Although the authors attribute this to the lack of \(Na^+\) influx at a \(V_{\text{hold}}\) = \(-40\) mV, it could also be explained by a reduction of \([K^+]_i\) due to \(K^+\) efflux, especially since in their study the use of \(V_{\text{hold}}\) = \(-80\) mV prevented the decrease of \(I_{Ca,L}\) more efficiently than internal perfusion of the cell with 10 mM \(Na^+\). Besides alterations in \([K^+]_i\), introduced by directly changing \(V_{\text{hold}}\) or \([K^+]_o\), even less obvious events may influence \(I_{Ca,L}\) (i.e., changes in \(K^+\) conductance). The activation of ATP-regulated \(K^+\) channels leads to a more than twofold increase in \(K^+\) outward current at \(-40\) mV in guinea pig ventricular cells [11]. If \(V_{\text{hold}}\) is at that voltage, a marked acceleration of the \(K^+\) efflux leading to a reduction of \(I_{Ca,L}\) has to be considered as the cell runs into an energy-deficiency state. These examples may illustrate that a \(V_{\text{hold}}\) of about \(-40\) mV which is commonly used in cardiac electrophysiology may lead to an underestimation of \(I_{Ca,L}\) due to a loss of \(K^+\).

We could also demonstrate that \(Cs^+\) is less effective or not able to substitute for \(K^+\) in modulating the L-type current. The substitution of the internal \(K^+\) for \(Cs^+\) may reduce \(I_{Ca,L}\) by 10 to 20\% (depending on the initial \([K^+]_i\)). A similar observation was also made by Matsuda and Noma [56] on guinea pig ventricular cells. After exchanging the internal \(K^+\)-rich solution for a \(Cs^+\)-rich one, the peak inward current was reduced by approximately 20\% (see their Fig. 2). However, they interpret this reduction in inward current as an overlap of the \(I_{Ca,L}\) with a ‘decaying outward current’ carried by \(K^+\) which is activated at low \([K^+]_i\). Dialysing myocardial cells with \(Cs^+\) reduces the \([Ca^{2+}]_i\) transients in guinea pig [57] as well as contractions in rabbit myocardial cells [58] by 40–50\%. Although Levi et al. [58] attribute this to interference of \(Cs^+\) with the \(Ca^{2+}\) release and re-uptake mechanism in the SR, a reduction of the \(I_{Ca,L}\) by about 18\% due to dialysis with \(Cs^+\) as shown here could, at least in part, explain these effects.

4.3. Physiological significance

The \([K^+]_i\) range affecting the amplitude of the L-type \(Ca^{2+}\) current has relatively narrow limits between 110 and 170 mM with a half-saturation concentration of approximately 136 mM. Taking into account a molar activity coefficient for cytoplasmic \(K^+\) between 0.613 and 0.73 [41,42], this \(EC_{50}\) value is in good agreement with physiological \(a_k\) measured by means of liquid ion exchange electrodes in myocardial tissue of different species [41,42,59]. Thus \([K^+]_i\) seems to be a suitable parameter for regulating the L-type \(Ca^{2+}\) current under physiological and pathophysiological conditions. The \(I_{Ca,L}\) during an action potential (Fig. 4E) as well as the action potential duration (Fig. 1) is to some extent regulated by \([K^+]_i\). According to this finding, modulatory effects of \([K^+]_i\), could be expected to occur in the working heart. For example, during the first 15 min of cardiac ischemia \(a_k\) may decrease by 3–4 mM [4]. In addition, measurements using liquid ion exchange microelectrodes have shown that \(a_k\) decreases by 5.3 mM [59] to 13.5 mM [5] in papillary muscle during hypoxia. Assuming a physiological \([K^+]_i\), of approximately 135 mM, a decrease in \(a_k\) of that order of magnitude is sufficient to reduce the \(I_{Ca,L}\) amplitude recorded during square pulses by approximately 7–15\%. In situ, during an action potential the decrease in \(Ca^{2+}\) influx will be about twice as much as demonstrated in the action potential clamp. Additional factors like shortening of the AP duration and \(\beta_1\)-stimulation will further enhance the regulatory action of \([K^+]_i\). In voltage clamp measurements, it has actually been shown that hypoxia reduces whole-cell \(Ca^{2+}\) conductance by 26\% in rat ventricular trabeculae [14]. This is consistent with findings of Wier [5]. However, in both studies investigations of the regulatory mechanism are missing. In addition to mediation by \([K^+]_i\), the action of changed intracellular pH or changed intracellular ATP levels on the \(I_{Ca,L}\) should of course be considered. The former decreases during ischemia as well as during hypoxia due to increased glycolytic activity [1,18]. Therefore, the raised intracellular proton activity will contribute to the reduction in \(I_{Ca,L}\) observed under these conditions (for review, see [48]). In contrast, an
action of a changed [ATP], seems to be less important since: (1) the [ATP], remains unchanged during the first 10 min of ischemia, as shown by NMR spectroscopic measurements in ferret hearts [18], and (2) a dose-dependent decrease of \( I_{\text{Ca,L}} \) was observed only if the [ATP], was reduced to less than 5 mM [11], which is well below the physiological value measured by Elliott et al. [18]. In addition to the pH-induced decrease in \( I_{\text{Ca,L}} \) the [K\(^+\)],-mediated reduction of \( I_{\text{Ca,L}} \) is a second mechanism leading to action potential shortening during early ischemia. Nevertheless, the action potential duration during early ischemia will mainly depend on the [K\(^+\)], [7] (i.e., the rising [K\(^+\)], will shorten the action potential markedly by increasing K\(^+\) conductance and thereby also reducing Ca\(^{2+}\) influx into the cell). Thus, there are at least three cardioprotective mechanisms in early ischemia which shorten the action potential and reduce the Ca\(^{2+}\) load of myocytes: (1) rising [K\(^+\)],, (2) falling pH, and (3) falling [K\(^+\)],. The importance of the falling [K\(^+\)], compared to the two other well-established mechanisms, however, cannot be judged from the experiments presented here.

5. Conclusion

Modulation of \( I_{\text{Ca,L}} \) by [K\(^+\)], might provide a mechanism for short-term regulation of the L-type current. Due to the relatively small effective concentration range and a half-saturation value near the physiological [K\(^+\)], small changes in [K\(^+\)], can induce large changes in \( I_{\text{Ca,L}} \) [K\(^+\)], can therefore serve as an additional regulatory mechanism independent of the cAMP pathway.

Acknowledgements

Part of this work was carried out while K.W.L. held a scholarship from the Graduiertenföderung of Northrhine-Westphalia. We are grateful to Dr. Jules C. Hancox for critically reading the manuscript and for helpful discussion of the results.

References


