Decreased inward rectifier current in adult rabbit ventricular myocytes maintained in primary culture: a single-channel study

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Abstract

Objective: Regulation of ion channel function in heart has been shown to be affected by changes in the cellular environment. Recently, it was shown that rabbit ventricular myocytes kept in primary culture, show a strong reduction in inward rectifier current (\(I_{K1}\)). The aim of the present study was to elucidate the mechanism underlying this decrease in \(I_{K1}\), using single-channel measurements. In addition, we studied the effects of primary culture on the ATP-regulated K\(^{+}\) (K\(_{\text{ATP}}\)) channel, also a member of the inwardly rectifying K\(^{+}\) channel family.

Methods: Adult rabbit ventricular myocytes were cultured for up to 3 days in Ham’s F-10 medium complemented with 1% rabbit serum and 5% glutamine. \(I_{K1}\) and K\(_{\text{ATP}}\) channel activity was studied in the inside-out patch configuration of the patch-clamp technique with equimolar K\(^{+}\) concentrations (140 mM K\(^{+}\)) on the intra- and extracellular side. Single channel characteristics were determined at various times during culture and compared to those present in freshly isolated myocytes.

Results: \(I_{K1}\) channels in freshly isolated myocytes (day 0) had a single-channel conductance of 56.1±2.5 pS (mean±SEM) and an open probability of 0.64±0.05 (mean±SEM). Neither the single-channel conductance nor the open probability \(P_o\) underwent significant changes during culture. The mean number of channels per patch, however, was drastically reduced from 1.2±0.3 (mean±SEM) at day 0 to 0.17±0.06 at day three. K\(_{\text{ATP}}\) channel density and open probability, on the other hand, were both increased with an optimum at day two. \(P_o\) increased from 0.27±0.06 at day 0 to 0.63±0.06 at day three. The mean number of channels per patch was 2.29±0.57 and 3.25±0.48 at days 0 and 3 respectively. The unitary current amplitude at -50 mV remained unchanged, suggesting no change in the K\(_{\text{ATP}}\) single-channel conductance.

Conclusions: The decrease in \(I_{K1}\), in rabbit ventricular myocytes as has been observed during primary culture is the result of a reduction in the number of active channels and not of altered kinetic or conductive channel properties. The increase in K\(_{\text{ATP}}\) channel activity under the same conditions suggests that gene expression of both channel types is differently regulated.

Keywords: Cell culture/isolation; Ion channels; K\(^{+}\)-channel; Membrane currents; Myocytes

1. Introduction

The inward rectifier current (\(I_{K1}\)) is abundantly present in the sarcolemma of cardiac ventricular myocytes and contributes to different phases of the ventricular action potential. Its large conductivity around the Nernst potential for potassium ions provides fast final repolarization and a stable resting membrane potential [1–3]. While its strong inward rectifying properties permit the existence of an extended plateau phase. Consequently, any change in this current will affect action potential duration and resting membrane potential, and thus also refractoriness and excitability [1–3]. Despite the continuous synthesis and degradation of ion channel proteins, the amplitude of \(I_{K1}\) remains relatively constant over time. However, regulation of \(I_{K1}\) channel function can be disturbed, as indicated by the changes in cardiac electrophysiology that develop under longlasting pathophysiologival conditions [4–6]. For instance, \(I_{K1}\) density is significantly reduced in cardiomyopathic hamsters [7], in dogs with pacing induced heart failure [8], in hypertrophied and failing rabbit hearts [9,10] and in patients with dilated or ischemic cardiomyopathy [11,12]. At present, little is known about the

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mechanisms involved in the long term regulation of ion channel function. Altered ionic currents may reflect a change in functional properties of the channel, or a change in channel density. In general, a change in current amplitude can be either due to altered ion channel kinetics, to a change in conductive properties, or to a change in the number of functional channels. Long lasting changes in mechanical load, electrical activation and neuro–humoral balance appear to be possible stimuli [4,6] or at least modulating factors for changes in ionic currents.

We were interested in the changes in inward rectifier ($I_{K1}$) channel function that occur during primary culture of ventricular myocytes, because a reduction in whole-cell $I_{K1}$ has also been demonstrated for ventricular myocytes maintained in primary culture [13,14]. Cell culture may serve as an in vitro system to investigate the mechanisms underlying regulation of ion channel function. It has the advantage that environmental factors can be controlled, whereas in animal models of chronic heart disease environmental factors may vary simultaneously, yielding a more complex situation for interpretation. The aim of the present investigation was to study $I_{K1}$ at the single-channel level at various days during primary culture of adult rabbit ventricular myocytes. Using the inside-out configuration of the patch–clamp technique, we determined open probability, single-channel conductance, and channel density to reveal the mechanism underlying the reduction in whole-cell $I_{K1}$. In addition, we studied the ATP-regulated potassium channel, another member of the same potassium channel superfamily [15], to test whether channel rundown is a general event during cell culture.

### 2. Methods

#### 2.1. Cell isolation and culture

Single rabbit ventricular myocytes were isolated by an enzymatic isolation procedure modified from Tytgat [16]. The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85–23). New Zealand white rabbits (1–2 kg) of either sex were anesthetized with 1 ml/kg intramuscular injected Hypnorm™ (10 mg/ml fluanison + 0.315 mg/ml fentanyl–citrate; Janssen Pharmaceutica). Additionally, 0.1 ml heparin sodium (5000 U/ml) was injected intravenously. The heart was rapidly removed and mounted on a constant-flow perfusion apparatus. The heart was retrogradely perfused through the aorta with solutions in the following sequence: (a) Normal Tyrode’s solution to wash out the blood for 5 min at a flow-rate of 20 ml/min. (b) $Ca^{2+}$-free Tyrode’s solution for 10 min at a flow-rate of 16 ml/min. (c) $Ca^{2+}$-free Tyrode’s solution containing collagenase (45 U/l type B and 185 U/l type P, Boehringer, Mannheim, Germany) and 250 mg/l trypsin inhibitor (Boehringer, Mannheim, Germany) for 15 min at a flow-rate of 12 ml/min. During the last 5 min of this period 250 U/l protease (type XIV, Sigma, St Louis, MO, USA) was also present. All solutions were saturated with 100% $O_2$ and the temperature was maintained at 37°C. Subsequently, the ventricles were cut into pieces which were gently agitated in a small beaker with low $Ca^{2+}$ Tyrode’s solution to obtain single cells. Next, 1 ml of the cell suspension was transferred onto round laminin-pretreated glass cover slips (16-mm diameter) together with 1 ml of culture medium (Hams F-10, Sigma, supplemented with 5% l-glutamine and 1% rabbit serum). The cells were allowed to attach for 2 h after which a medium change with Hams F-10 was performed, thereby removing dead and unattached cells. In this way a homogeneous layer of rod-shaped myocytes at a density of $10^7$ cells per cm² was yielded. Subsequent medium changes were carried out every day. During the entire isolation procedure, starting with the removal of the heart, the following precautions were taken to guarantee sterility: (a) dissection instruments were sterilized and the chest of the rabbit was cleaned with alcohol before opening. (b) the constant-flow perfusion apparatus was placed in a Class II flow hood. (c) before each isolation, the perfusion apparatus was flushed with alcohol followed by deionised water. (d) all other glassware or instruments used to handle cells and solutions were sterilized by autoclaving. (e) all perfusion solutions were filtered (0.2-µm filters, Millipore).

#### 2.2. Solutions

Normal Tyrode’s solution contained (in mM): NaCl 140, KCl 5.4, CaCl₂ 1.8, MgCl₂ 1.0, glucose 5.5, HEPES 5.0, buffered with NaOH at pH 7.4. $Ca^{2+}$-free Tyrode’s solution: NaCl 140, KCl 5.4, MgCl₂ 0.5, KH₁₂PO₄ 1.2, glucose 5.5, HEPES 5.0, buffered with NaOH at pH 7.2. The low-$Ca^{2+}$ Tyrode’s solution was composed of nine parts $Ca^{2+}$-free and one part normal Tyrode’s solution, yielding a final calcium concentration of 180 μM. The pipette (intracellular) solution for whole-cell recording contained (in mM): KCl 140.0, K-ATP 5.0, HEPES 10.0, buffered with KOH at pH 7.2. The bath (extracellular) solution for whole-cell recording consisted of Normal Tyrode’s solution. The pipette (extracellular) solution for single-channel recording in inside-out patches contained (in mM): KCl 140.0, HEPES 10.0, buffered with KOH at pH 7.4. The bath (intracellular) solution for single-channel recording contained (in mM): KCl 140.0, K-ATP 5.0, HEPES 10.0, buffered with KOH at pH 7.2. K-ATP was omitted from the intracellular solution in those experiments where the ATP-sensitive potassium channel was studied.

#### 2.3. Electrophysiological experiments

The cover-slip with cells attached to it was placed in a cell chamber on the temperature-controlled stage of an
inverted microscope (Nikon Diaphot). The coverslip was fixed to the bottom of the cell chamber by the weight of a small stainless steel pin placed on the edge of the coverslip. The cells were continuously superfused with normal Tyrode’s solution (whole-cell experiments) or with a 140 mM K+ solution (single-channel experiments) at a rate of approximately 1 ml/min. The temperature of the bath was maintained at 35±2°C. Currents were recorded in the whole-cell or inside-out patch configuration of the patch–clamp technique [17] with a laboratory-made patch–clamp amplifier. The pipettes were pulled from borosilicate glass by a laboratory-made one-stage puller. The tips of the pipettes were heat-polished. After filling with the appropriate pipette solution, the pipettes had a resistance of 3 to 5 MΩ. Membrane currents and voltage were off-line filtered (low-pass) at 0.5 kHz (single-channel currents) or 2 kHz (whole-cell currents) with a two-pole Butterworth filter, digitized at a sampling interval of 500 μs by an AD converter board (National Instruments), and stored on the hard disk of a computer (Apple Macintosh) for subsequent data analysis.

2.4. Stimulation protocols and data analysis

2.4.1. Whole-cell experiments

Action potentials were elicited at a rate of 1 Hz by current pulses with an amplitude of 1.5 times threshold and 2 ms in duration. Action potential duration was measured at 90% of repolarization (APD90). To determine cell capacitance, 10-ms hyper-and depolarizing pulses of 100 pA were applied during the plateau phase of the action potential, 50 ms after the stimulus pulse. Cell capacitance was calculated by dividing the amplitude of the current pulse by the change of slope in voltage. To measure the quasi steady state current (I_QSS), 500-ms voltage clamp steps were applied from −100 to +60 mV with 10-mV increments from a holding potential of −40 mV. The quasi steady-state current (I_QSS) is defined as the current amplitude at 500 ms after the onset of the voltage step.

2.4.2. Single-channel experiments

The channel open probability (Popen) was calculated as the mean number of open channels (nopen) divided by the total number of channels in the patch membrane (nmax). It was assumed that the total number of channels in the patch membrane was equal to the maximal number of channels simultaneously observed in the open state. The nopen was derived from amplitude histograms constructed from 15 (for IK1 channels) or 10 traces (for K-ATP channels) of 2 s in duration. The area under each peak in the amplitude histogram, representing the fraction of time that 0, 1, 2, ...., n channels are simultaneously in the open state, was calculated from Gaussian functions fitted through these peaks. The height, width and mean of the Gaussian functions were adjusted to the data by the eye. The mean number of open channels was then calculated in the following way:

\[ n_{\text{open}} = \sum_{n=1}^{n_{\text{max}}} n P_n \]

where n is the number of channels simultaneously in the open state, nmax is the total number of channels in the patch membrane, and Pn is the fraction of time that n channels are simultaneously in the open state. Unitary current amplitudes were also derived from amplitude histograms and plotted against voltage to obtain single-channel conductance.

Data are represented as the number of experiments, which is the number of hearts, and the total number of cells (n). Pooled data are presented as means±S.E.M. Significant differences between groups were tested using Student’s t test. The null hypothesis was rejected when the two-tailed P value was <0.05.

3. Results

Freshly isolated rabbit ventricular myocytes were rod-shaped with clear cross striations. During culture the morphology of the myocytes changed. First, there was a rounding of the extreme points, later followed by the formation of multiple processes. Eventually, the cross striated pattern was fading. The time at which these changes in morphology occurred varied among cultures, despite the fact that the isolation and culture procedures were standardized. To get an impression of the electrophysiological changes that occur in ventricular myocytes during culture, action potentials and membrane currents were measured on four successive days. Freshly isolated (day 0) myocytes displayed normal action potential characteristics, i.e. a rapid upstroke followed by a small phase 1 repolarization, a pronounced plateau phase of about 200–300 ms in duration (1 Hz), and a rapid phase three repolarization (Fig. 1a). During culture a number of significant changes in action potential configuration occurred. Firstly, there was a prolongation of the plateau phase, giving rise to the development of early after depolarizations (EAD’s) at days 2 and 3 (Fig. 1B, C). Secondly, there was a slowing down of phase three repolarization. And finally, there was a depolarization of the resting membrane potential which in some cells led to the development of spontaneous activity at day 3 (Fig. 1D). All these changes point into the direction of a decrease in inward rectifier current (I_K1), which is responsible for the fast phase three repolarization and maintenance of the resting membrane potential. To study the changes in I_K1, cells were voltage-clamped at a holding potential of −40 mV and series of 10-mV hyper- and depolarizing steps were applied. Examples of current traces recorded at test potentials between −100 mV and
Fig. 1. Changes in action potential configuration during culture. Example of an action potential recorded from a freshly isolated rabbit ventricular myocyte (A). Examples of action potentials recorded from myocytes maintained in culture for 1 to 3 days (B–D). Note the clear trend for action potential prolongation, giving rise to the development of EAD’s.

+50 mV are shown in Fig. 2A. In freshly isolated myocytes, hyperpolarization induced large inward currents, which were clearly smaller when elicited in myocytes at day 3 of culture. Also outward currents recorded from day 3 myocytes were reduced compared to those in day 0 myocytes. The current at the end of the 500-ms voltage step was measured, normalized to cell capacitance and plotted against the voltage. Fig. 2B shows the resultant steady state current density–voltage relationship at days 0 and 3 of culture. Freshly isolated myocytes showed the typical ‘N’-shaped I–V relation, characteristic for ventricular myocytes with their pronounced inward rectifier current ($I_{K1}$). At day 3 of culture the N-shape had flattened and the reversal potential (the potential at which the I–V relation

Fig. 2. Changes in whole-cell inward rectifier current during culture. (A) Examples of current traces elicited by voltage steps between $-100$ mV and $+50$ mV from a holding potential of $-40$ mV at days 0 and 3 of culture. (B) Average steady-state current density–voltage relation. Amplitudes of membrane currents were measured at the end of a 500-ms voltage steps to various potentials from a holding potential of $-40$ mV at day 0 (filled circles) and day 3 (open circles) of culture. Data at day 0 were from nine experiments, $n=22$. Data at day 3 were from three experiments, $n=9$. 
crosses the x-axis) was shifted in the positive direction, which is in accordance with the occurrence of spontaneous activity at day 3 of culture. Cell capacitance was slightly reduced from 134.3±11.6 pF at day 0 to 126.0±22.9 pF (mean±SEM) at day 3 of culture.

Since we were interested in the underlying cause of the reduction in current we further studied \( I_{K1} \) at the single-channel level. At that level it is possible to discriminate between changes in open probability, single-channel conductance and channel density. Single \( I_{K1} \) channel activity was recorded in inside-out patches with equimolar potassium concentrations (140 mM K\(^+\)) on the intra- and extracellular side. The bathing (intracellular) solution additionally contained 5 mM K-ATP to inhibit ATP-regulated potassium (K.ATP) channels and to prevent rundown of \( I_{K1} \) channels.

Changes in \( I_{K1} \) channel activity were followed in cells from a total of three hearts for three culture days. Fig. 3 shows examples of current traces recorded at −80 mV. Channel activity was characterized by long openings interrupted by short closures, as well at day 0 (Fig. 3A, upper panel) as at day 3 of culture (Fig. 3B, upper panel). The open probabilities (\( P_o \)), determined from the amplitude histograms shown in the lower panels of Fig. 3A, B, were 0.64 and 0.70 for days 0 and 3 respectively. Fig. 3C shows that the mean \( P_o \) at −80 mV was about 0.65 and remained unchanged during culture. Also, the single-channel conductance was determined. Fig. 4A shows current traces with \( I_{K1} \) channel activity recorded at various negative potentials at days 0 and 3 of culture. Single channel conductances were determined from current–voltage relations (not shown). On average, the single-channel conductance was unchanged during culture (Fig. 4B). Thus, neither the open probability nor the single-channel conductance of the \( I_{K1} \) channel was changed during culture.

Striking was the reduction in success rate to find \( I_{K1} \) channel activity in the patch. Whereas at day 0 two-thirds of the patches contained \( I_{K1} \) channel activity, the majority of patches was without \( I_{K1} \) channel activity at day 3. This can clearly be seen from Fig. 5, which shows the distribution of the number of channels per patch. At day 0, about 28% of the patches contain no channels, 52% contain one channel and about 20% contain multiple channels (Fig. 5A). During days 1 and 2, the percentage of patches with one or multiple channels is greatly reduced, and at day 3 more than 80% of the patches is without channels whereas the other 20% of the patches contains only one channel. On average the mean number of channels per patch decreased from 1.2 at day 0 to 0.2 at day 3 (Fig. 5B). From these results it appears that the
necessary to reliably determine the single-channel conductance. Fig. 7 shows the mean values for the three parameters. The open probability was significantly increased during culture. Starting with a relative low value of 0.27 at day 0, \( P_o \) was increased to 0.63 at day 3 after reaching a maximum at day 2 (Fig. 7A). The single-channel current amplitude measured at −50 mV remained constant over time, suggesting no changes in the single-channels conductance (Fig. 7B). The number of channels per patch was clearly increased, although this difference was only significant at day 2. The mean number of channels per patch for days 0 and 2, were 2.3 and 4.7 respectively. From these data it can be concluded that K.ATP channel activity is increased during culture due to an increase in the open probability and channel density.

4. Discussion

This study shows a prominent decrease in inward rectifier current (\( I_{K1} \)) and concomitant changes in action potential configuration in adult rabbit ventricular myocytes in primary culture. Similar changes in \( I_{K1} \) have been described previously [13,14], but it is unknown whether these changes are due to alterations in channel density, open probability or conductive properties of the channel. We studied \( I_{K1} \) at the single-channel level to unravel the mechanism underlying \( I_{K1} \) reduction during primary culture.

4.1. Action potentials

The changes in action potential configuration which occurred during culture can be summarized as follows: (1) Action potential prolongation with early afterdepolarizations (EAD’s) due to postponement of final repolarization. (2) Slowing down of phase 3 repolarization. (3) Depolarization of the resting membrane potential, giving rise to abnormal spontaneous activity in a number of cells. Although these changes are consistent with a decrease in \( I_{K1} \), changes in other currents may also have contributed to the altered action potential configuration. We did not investigate other membrane currents, but the transient outward current [13,14,18], the calcium current [13,14,19] and sodium current [20] have been reported to be also altered during primary culture of adult atrial and ventricular myocytes. The action potential changes observed in this study are partly comparable to those in previous studies. In cat ventricular myocytes a prolongation of the action potential with EAD’s was also prominently present [13]. The depolarization of the resting membrane potential in these cells, however, was less pronounced and the time scale over which changes occurred was more extended. In another study on rabbit ventricular myocytes, the degree of depolarization of the resting membrane potential was similar, but no significant prolongation of the action potential was observed [14]. A reason for the apparent
Fig. 5. Effects of primary culture on inward rectifier channel density. (A) Bar histograms showing the percentage of inside-out patches containing 0, 1, ..., n *I*<sub>K</sub>, channels at various times during culture. (B) Bar histogram showing the mean values for the number of channels per inside-out patch as a function of culture duration. Mean number of channels per patch for days 0, 1, 2, and 3 was 1.16±0.26 (n = 31), 0.83±0.19 (n = 53), 0.27±0.09 (n = 51), 0.17±0.06 (n = 47) respectively. Data were from three different experiments. * P<0.05.

**4.1.2. The inward rectifier channel**

The changes in the steady state current-voltage relation that occurred during culture were consistent with a decrease in *I*<sub>K<sub>1</sub>. There was a flattening of the N-shape of the steady state current-voltage relation combined with a positive shift of the reversal potential. This decrease in *I*<sub>K<sub>1</sub> was studied in more detail at the single-channel level. *I*<sub>K<sub>1</sub> channels in inside-out patches of freshly isolated (day 0) myocytes were characterized by an open probability of 0.64±0.05 and a single-channel conductance of 56.1±2.5 pS. The open probability is in the range of values from previous studies [21,22], the single-channel conductance on the other hand appears to be almost twice as large [21–29]. However, in the majority of studies single-channel conductance was determined at room temperature (24–33 pS at 20–25°C) [21–29]. Taking into account a *Q*<sub>10</sub> of 1.4 for the *I*<sub>K<sub>1</sub> single-channel conductance [30], an increase in temperature from 20°C to 37°C will thus increase the single-channel conductance with a factor 1.8.

Neither the open probability nor the single-channel conductance changed during 3-days culture. There was, however, a prominent decrease in the mean number of channels per patch from 1.16±0.26 at day to 0.17±0.06 at day 3, corresponding to a 85% reduction. Therefore, we can conclude that the decrease in *I*<sub>K<sub>1</sub> during primary culture is due to a decrease in the number of active channels and not to changes in kinetic or conductive properties of the channel.

**4.1.3. The ATP-regulated K<sup>+</sup> channel.**

We also studied the K<sub>ATP</sub> channel, another member of the inwardly rectifying K<sup>+</sup> channel family. K<sub>ATP</sub> channels showed a different response during culture. Although the unitary current amplitude at −50 mV remained un-
changed, suggesting no change in the single-channel conductance, both the mean number of channels per patch and the open probability were significantly changed. $P_o$ was relatively low at day 0 ($0.27 \pm 0.06$) and was significantly increased to $0.64 \pm 0.05$ at day 1 and remained constant during the next two days. The mean number of channels per patch was $2.29 \pm 0.57$ at day 0, increased to $4.43 \pm 0.79$ at day 1 and showed a small decline again at day 3 to $3.25 \pm 0.48$. From these data it can be concluded that K.ATP channel activity increases during culture due to an increase in open probability and channel density.

Since all results point towards a reduction in steady-state $K^+$ current, it might be concluded that the intracellular ATP concentration at day three of culture is still high enough to prevent K.ATP channels from opening. The consequences of the changes in K.ATP activity will only become apparent when cell metabolism is seriously impaired and intracellular ATP is reduced, e.g. by anoxia or the addition of cyanide. Under such circumstances it is to be expected that more outward K.ATP current will be generated in myocytes which are cultured for 3 days than in freshly isolated myocytes, leading to a more pronounced action potential shortening in the former.

### 4.1.4. Background of reduction in $I_{K1}$ channel density

The decrease in $I_{K1}$ channel density may be due to a true reduction in the number of $I_{K1}$ channels, but can also be due to a permanent inactivity of part of the channels. Such a functional inactivation of channels could for example be due to altered concentrations of modulating factors [14]. Since the decrease in channel density was assessed in inside-out patches, providing identical intracellular environments for channels from both freshly isolated and cultured myocytes, an acute effect of modulating factors giving rise to channel inactivation seems unlikely. Long term effects resulting in channel inactivation, however, cannot be ruled out. Assuming that there is a true reduction in the number of $I_{K1}$ channels, several possibilities are conceivable. There may be a decrease in the rate of protein synthesis or an increase in the rate of protein degradation, or both. Alternatively, it is also possible that the transport of channel protein from the ribosome to the membrane is impaired. Studies focussing on changes in the amount of $I_{K1}$ channel protein and mRNA encoding $I_{k1}$ channels, may resolve these questions.

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**Fig. 6.** $K^+$ channel activity at different days of culture. (A) Single ATP-regulated $K^+$ channel currents recorded from inside-out patches of ventricular myocytes at days 0 to 3 of culture. The number of K.ATP channels per patch was 2, 5, 4 and 5 for days 0, 1, 2 and 3 respectively. Channel openings are shown as downward deflections. The dotted line indicates the level at which all channels are closed. (B) Single $I_{K1}$ channel currents recorded from inside-out patches of ventricular myocytes at days 0 to 3 of culture. The number of $I_{K1}$ channels per patch was 2, 1, 1 and 1 for days 0, 1, 2 and 3 respectively. Channel openings are shown as downward deflections. The dotted line indicates the level at which all channels are closed.

**Fig. 7.** Effects of primary culture on K.ATP channel characteristics. (A) The mean open probabilities ($P_o$) for days 0, 1, 2, and 3 were $0.27 \pm 0.06$ ($n=6$), $0.64 \pm 0.05$ ($n=12$), $0.75 \pm 0.05$ ($n=16$), $0.63 \pm 0.06$ ($n=10$) respectively. (B) The mean unitary current amplitudes ($i$) for days 0, 1, 2, and 3 were $4.0 \pm 0.3$ ($n=6$), $4.2 \pm 0.1$ ($n=12$), $4.2 \pm 0.1$ ($n=11$), $4.4 \pm 0.1$ ($n=10$) respectively. (C) The mean number of channels per patch ($n$) for days 0, 1, 2, and 3 was $2.3 \pm 0.6$ ($n=7$), $4.4 \pm 0.8$ ($n=14$), $4.7 \pm 0.7$ ($n=17$), $3.3 \pm 0.5$ ($n=12$) respectively. Data were from two different experiments. * $P<0.05$. 

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We considered the possibility that the culture conditions seriously reduced overall protein synthesis, in which case channel rundown would be a general event during cell culture. The decrease in \( I_{K1} \) channel density would then reflect the consequences of a general degeneration process rather than specific regulation of ion channel gene expression. Although a general reduction in protein synthesis can only be excluded by measuring protein synthetic capacity, the finding that the rate of protein synthesis in cultured adult feline myocytes is maintained at a level very close to that in the intact heart [31] and our observation that K.ATP channel density was increased during culture, makes it plausible that K.ATP channel proteins are still synthesized. This may suggest that the expression of K.ATP channels and \( I_{K1} \) channels may be differently regulated. Similar findings have been made for other sets of tonic currents in adult myocytes maintained in culture. In cat ventricular myocytes \( I_{K1} \) and the transient outward current \( (I_{to}) \) are reduced, while the L-type calcium current \( (I_{Ca,L}) \) is increased [13]. In human myocytes \( I_{to} \) density is reduced, while the ultra-rapid delayed rectifier current density is increased [19,32]. In rabbit ventricular myocytes \( I_{K1}, I_{Ca,L} \) and \( I_{to} \) are all reduced, but on a different time scale [14].

The present study provides no information on the stimuli, or lack of stimuli, inducing the changes in channel expression. A number of environmental factors appear to affect current density under culture conditions. For example, it has been shown that the decrease in \( I_{Ca,L} \) density during primary culture of adult rat [33,34] and rabbit [35] myocytes can be prevented by an increase in the extracellular calcium concentration [33], by continual electric field stimulation [34], or by the addition of L-carnitine, creatine and taurine [35]. Other factors that have also been shown to influence current densities are: serum [36], basic fibroblast growth factor [36,37], the alphal-adrenergic agonist phenylephrine [38], and direct contact with sympathetic neurones [39]. Interestingly, a number of the above-mentioned factors affecting ion channel expression are hypertrophic stimuli affecting e.g. cell size and expression of contractile proteins. This suggests that also with respect to ion channel expression, cell culture may be a useful model for hypertrophy. No studies so far have reported on factors modulating \( I_{K1} \) channel density in culture. The knowledge of factors which up-regulate \( I_{K1} \) channel density may be useful to antagonize the down regulation of \( I_{K1} \) channel density in heart failure and other chronic heart disease.

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