Functional expression of an inactivating potassium channel (Kv4.3) in a mammalian cell line


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Abstract

Objective: The goal of this study was to characterize the electrophysiological properties of the Kv4.3 channels expressed in a mammalian cell line. Methods: Currents were recorded using the whole-cell voltage clamp technique. Results: The threshold for activation of the expressed Kv4.3 current was approximately -30 mV. The dominant time constant for activation was 1.71 ± 0.16 ms (n = 10) at -60 mV. The current inactivated, this process being incomplete, resulting in a sustained level which contributed 15 ± 2% (n = 25) of the total current. The time course of inactivation was fit by a biexponential function, the fast component contributing 74 ± 5% (n = 9) to the overall inactivation. The fast time constant was voltage-dependent [27.6 ± 2.0 ms at +60 mV (n = 10) versus 64.0 ± 3.6 ms at 0 mV (n = 10); P < 0.01], whereas the slow was voltage-independent [142 ± 15 ms at +60 mV (n = 10) versus 129 ± 33 ms at 0 mV (n = 6); P > 0.05]. The voltage-dependence of inactivation exhibited midpoint and slope values of -26.9 ± 1.5 mV and 5.9 ± 0.3 mV (n = 21). Recovery from inactivation was faster at more negative membrane potentials [203 ± 17 ms (n = 13) and 170 ± 19 ms (n = 4), at -90 and -100 mV]. Bupivacaine block of Kv4.3 channels was not stereoselective (K1/2 = 31 μM). Conclusions: The functional profile of Kv4.3 channels expressed in Ltk cells corresponds closely to rat Ito, although differences in recovery do not rule out association with accessory subunits. Nevertheless, the sustained component needs to be considered with respect to native Ito.

Keywords: K-channels; Ion channels; Membrane currents; Antiarrhythmic agents; Arrhythmia

1. Introduction

The transient outward current (Ito) plays an essential role in shaping the early phase of the cardiac action potential [1]. As such, Ito may influence the balance of inward and outward ionic currents flowing during the plateau phase and therefore modulate action potential duration and refractoriness of myocardial tissue. Several K+ channel subunits cloned from cardiac tissue including Kv1.4, Kv4.2 and Kv4.3 generate transient outward currents similar to Ito [2–10]. Many properties of the Kv1.4 currents differ significantly from those of rat ventricular Ito [8]. Moreover, Western blot and immunohistochemical studies have failed to detect Kv1.4 protein in rat ventricular myocytes [11], suggesting that it is not likely that Kv1.4 would be the cloned counterpart of rat cardiac Ito. Kv4.2 cloned from rat heart is abundantly expressed in rat myocytes [12,13]. Moreover, the endo- to epicardial gradient for mRNA expression matches the gradient in Ito density across the left ventricular wall [13–15]. Many properties of Kv4.2 resemble those of Ito recorded in rat and human myocytes [8,9] with the exception of the slower recovery from inactivation. Kv4.2 has been detected in rat myocardium, but is not expressed in significant levels in canine or human ventricular myocytes [6], even though the Ito in these cells is very similar to the Ito recorded in rat myocytes [15–17].

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Recently, a new $K^+$ channel gene, Kv4.3, has been identified. Kv4.3 is expressed in rat brain and at high levels in rat, canine and human hearts [6,18]. Thus, both Kv4.2 and Kv4.3 channels are likely to contribute to the $I_{TX}$ in rat heart, and differential expression of these channels may account for the functional heterogeneity of the $I_{TX}$ in different regions of the rat ventricle [4,6]. The functional properties of Kv4.3 have been reported after expression of Kv4.3 mRNA in Xenopus oocytes [4,6]. The pharmacological properties of Kv4.3 channels, studied after expression in Xenopus oocytes, are very similar to those exhibited by $I_{TX}$, including sensitivity to block by 4-AP, insensitivity to TEA block and lack of redox sensitivity [6]. Although these cells are easily manipulated and are an excellent choice for the initial characterization of channel function, it is also possible that protein processing or membrane environment differs between amphibian oocytes and mammalian cells. For instance differences between the oocyte and mammalian expression systems have been observed in pharmacological studies examining quinidine block of hKv1.5 and hKv1.4 channels [7,8,19,20]. Thus, the purpose of the present study is to characterize the electrophysiological properties of the Kv4.3 channels transiently expressed in a mammalian cell line (Ltk-). We also used this system to determine whether bupivacaine block of Kv4.3 channels display stereoselectivity, which is of importance in the correspondence with native $I_{TX}$ which does not display stereoselective block [21]. Preliminary results of this study have been published in abstract form [22].

### 2. Methods

#### 2.1. Transfection and cell culture

The Kv4.3 clone (1996 bp) [6] was digested from pBluescript with HindIII and XbaI, and subcloned into pBluescript CMV. Previously digested with the same restriction enzymes. Proper insertion was confirmed by the correct size of the fragments of independent restriction digests (HindIII+XbaI, KpnI, SpeI+BamHI). No differences were observed in expression levels or functional properties for two independent positive clones tested. Ltk- cells were cultured in DMEM supplemented with 10% horse serum under a 5% CO$_2$ atmosphere. The cells were transfected using the lipofectamine method (GIBCO-BRL). GFP (green fluorescent protein) [23] was coexpressed to assess the transfection efficiency and to identify expressing cells for voltage clamp analysis. We applied a mixture of 1–5 $\mu$g of Kv4.3/pBK CMV, 2 $\mu$g of GFP/pRC CMV, and 25 $\mu$l of lipofectamine in 0.5 ml serum free DMEM for 6 h, after which the standard medium was restored. The cells were removed from the dish 24 hr later by a brief trypsinization, washed twice in standard media, and stored for use within the next 12 hr. Parallel nontransfected cultures or cells transfected with GFP only (mock transfection) served as controls.

#### 2.2. Solutions

The intracellular pipette filling solution contained (mM): 110 KCl, 10 HEPES, 5 K$_2$BAPTA, 5 K$_2$ATP and 1 MgCl$_2$, adjusted to pH 7.2 with KOH yielding a final intracellular $K^+$ concentration of $\sim$145 mM. The bath solution contained (mM): 130 NaCl, 4 KCl, 1.8 CaCl$_2$, 1 MgCl$_2$, 10 HEPES and 10 glucose, adjusted to pH 7.35 with NaOH. Bupivacaine enantiomers (Astra, Södertälje, Sweden) were added to the external solution from $10^{-2}$ M aqueous solution. All other chemical compounds were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

#### 2.3. Electrical recording

Recordings were made with an Axopatch-200 patch-clamp amplifier (Axon Instruments, Foster City, CA, USA) using the whole-cell configuration of the patch-clamp technique [24]. Cells were allowed to settle to the bottom of a small perfusion chamber (0.5 ml) mounted on the stage of an inverted Nikon diaphot 200 microscope, equipped for epifluorescence with a B2A filter assembly to observe the GFP fluorescence of transfected cells. Currents were recorded at room temperature (21–23$^\circ$C) and sampled at 2–10 kHz after anti-alias filtering at half the sampling frequency. Data acquisition and command potentials were controlled with the use of pClamp software version 6.0.1 (Axon Instruments). Micropipettes were pulled from star-bore borosilicate glass (Radnoti Glass Co., Monrovia, CA, USA) and heat polished. The average pipette resistance was $2.1 \pm 0.5$ MΩ ($n=25$). Junction potentials were zeroed with the pipette in the standard bath solution. Gigaohm seal formation was achieved through the use of suction (10±1 GΩ, $n=15$). After establishing the whole cell configuration, the capacitive transients elicited by symmetrical 10 mV voltage-clamp steps from −80 mV were recorded at 50 kHz for calculation of capacitive surface area and access resistance, which were $20 \pm 2$ pF ($n=18$) and $5.2 \pm 0.2$ MΩ ($n=18$), respectively. Thereafter, capacitance and series resistance compensation were optimized, and 80% compensation of the effective access resistance was usually obtained.

#### 2.4. Pulse protocols and data analysis

The holding potential was maintained at −80 mV unless indicated otherwise. Effects of drug infusion was monitored with test pulses to −60 mV, applied every 30 s until steady-state was obtained. The cycle time for other pulse protocols was 30 s. Steady-state current-voltage relationships (IV) were obtained by averaging the current over a small window (2–5 ms). Between −80 and −40 mV only...
passive linear leak was observed and least squares fits to these data were used for passive leak correction.

Activation curves were fitted with a Boltzmann equation: 
\[ y = \frac{1}{1 + \exp\left(-\frac{E - E_0}{s} \right)} \] 
where \( s \) represents the slope factor, \( E \) the membrane potential and \( E_0 \) the voltage at which 50% of the channels are open. Time course of inactivation and recovery from inactivation were fitted with a sum of exponentials: 
\[ y = A_i \exp\left(-t/t_1\right) + A_e \exp\left(-t/t_2\right) + C \] 
where \( t_1 \) and \( t_2 \) are the time constant and the amplitude of the fastest phase of inactivation, respectively, \( A_i \) and \( A_e \) are the corresponding parameters for the second slow phase of inactivation and \( C \) is the baseline value. The activation kinetics were determined using the approach of the dominant time constant of activation in which a single exponential was fitted to the latter 50% of activation time course [25]. The curve fitting procedure used a nonlinear least-squares (Gauss-Newton) algorithm; results were displayed in linear and semilogarithmic format, together with the difference plot. Goodness of the fit was judged by the \( \chi^2 \) criterion and by inspection for systematic non-random trends in the difference plot. Results are expressed as mean±SEM. Comparisons between mean values in control conditions and mean values in the presence of drug for a single variable were performed by use of Student’s \( t \)-test. A value of \( P<0.05 \) was considered significant.

3. Results

3.1. Expressed Kv4.3 current

Fig. 1 shows original records from a Ltk− cell expressing Kv4.3 currents. Depolarizations positive to −30 mV resulted in a transiently activated (A-type) outward K+ current. The rate of activation increased with depolarization and the time to peak was 6.4±0.6 ms at +60 mV (\( n=13 \)); the dominant time constant of activation at +60 mV averaged 1.71±0.16 ms (\( n=10 \)) (Fig. 1). The average peak outward current at +60 mV was 2.3±0.3 nA (\( n=25 \)) (This number does not include results from those cells in which the expressed current exceeded 5 nA which posed voltage clamp problems). Analysis of the current decay indicates that inactivation was incomplete after 250 ms (Fig. 1). The non-inactivating component of the current remained constant for at least 2 s (data not shown). Its magnitude was proportional to the size of the peak current, contributing to 15±2% of the total outward current (\( n=25 \)) (Fig. 1B). Neither untransfected nor sham-transfected Ltk− cells displayed voltage-gated ionic currents, as has been reported previously (Fig. 1A) [26].

Two exponential components were required to adequately describe the time course of inactivation of the currents during 1000 ms depolarizing steps to +60 mV (Fig. 1C).

Fig. 1. A Current records obtained from non transfected and Kv4.3 transfected L-cells evoked by depolarizing voltage steps from −80 mV to potentials between −30 and +60 mV in 10 mV increments. Cell capacitance, 21 pF. Data filtered at 2 kHz and digitized at 4 kHz. Inset. Activation kinetics of Kv4.3 at +60 mV. B: IV relationship for peak (●) and sustained (○) Kv4.3 currents, the latter measured at the end of the 250 ms depolarizing pulses. C: Inactivation kinetics of Kv4.3 current. Biexponential (a) and monoexponential (b) fits of the time course of fast inactivation of Kv4.3 during depolarizing steps at +60 mV. D: Voltage-dependence of inactivation time constants, \( \tau_1 \) (●) and \( \tau_2 \) (○). Each point represents the mean±SEM of 10 experiments.

*\( P<0.05 \) versus \( \tau_1 \) at +60 mV.
The fast (\(\tau_f\)) and slow (\(\tau_s\)) time constants were 27.6±2.0 ms (\(n=11\)) and 142±15 ms (\(n=11\)), respectively. The fast component of inactivation accounted for 74±5% of the total amount of inactivation (ratio \(A_f/(A_f+A_s)\)). The kinetics of the slow component were voltage-independent: the \(\tau_s\) values remained constant between 0 and +60 mV (range: 125–160 ms; \(P>0.05, n=10\)). In contrast, \(\tau_f\) showed a marked voltage dependence, with significantly larger values (\(P<0.05\)) at potentials negative to +40 mV (Fig. 1D).

3.2. Voltage dependence of Kv4.3 steady-state inactivation and recovery from inactivation

The voltage dependence of Kv4.3 steady-state inactivation was determined using a two-step voltage-clamp protocol. Fig. 2A shows a family of superimposed Kv4.3 currents obtained using this experimental protocol. The magnitude of Kv4.3 currents during the conditioning steps increased with depolarization, while the Kv4.3 current elicited by the test step decreased as the conditioning step was more positive. The voltage dependence of Kv4.3 steady-state inactivation was described by a Boltzmann equation (Fig. 2B), in which the mean \(E_{in}\) value was \(-26.9±1.5\) mV (\(n=21\)) and the slope factor averaged 5.9±0.3 mV (\(n=21\)).

Recovery from inactivation of cardiac rat and human \(I_{Ks}\) is a fast process (\(\tau_{re}\)=50 ms) [21,27,28]. As a result use-dependent accumulation of inactivation at holding potentials negative to −70 mV is either limited or absent. To explore whether Kv4.3 displayed such properties, we analyzed the kinetics of the recovery process of Kv4.3 current using a two-step protocol. Fig. 2C shows records obtained after applying such pulse protocol with the recovery potential at −100 mV. Fig. 2D shows a typical example of the recovery kinetics of Kv4.3 current at −80, −90 and −100 mV. Recovery from inactivation of Kv4.3 current was well defined by a monoeponential process. The time constant (\(\tau_{re}\)) was strongly voltage-dependent, being faster as the membrane potential become more negative. At −90 mV the \(\tau_{re}\) was 203±17 ms (\(n=13\)), compared to 413±48 ms (\(P<0.05; n=4\)) at −80 mV and 170±19 ms at −100 mV (\(P<0.05; n=4\)). Thus, these recovery kinetics display a steep voltage-dependence similar to that of native \(I_{Ks}\), although the time constants are 3–5 fold slower (see discussion).

![Fig. 2. A: Voltage-dependence of Kv4.3 steady-state inactivation. Kv4.3 currents obtained with a double pulse protocol in which a 250 ms conditioning pulse to potentials between −80 and +60 mV was applied from a holding potential of −80 mV and the steady-state inactivation was assessed from the peak outward current during the subsequent step to +40 mV. B: The symbols represent the normalized peak outward current at +40 mV in a typical experiment using the protocol in panel A. The solid line is drawn according to a Boltzmann equation with average values of \(-26.9±1.5\) mV (\(n=21\)) and 5.9±0.3 mV (\(n=21\)) for midpoint and slope factor, respectively. C: Recovery from inactivation of Kv4.3 analyzed with a double pulse protocol in which a 500 ms conditioning prepulse from −80 mV to +50 mV was followed by a fixed 200 ms test pulse to +50 mV after an interstimulus interval of variable duration (5 to 2000 ms) at −80, −90 and −100 mV. Superimposed records are shown for a recovery potential of 200 mV. D: Recovery kinetics expressed as normalized peak amplitude against interpulse interval at −80 (○), −90 (■) and −100 mV (▲). Continuous lines are the best monoeponential fit of the increase of Kv4.3 currents as a function of the interstimulus interval. Time constants for recovery from inactivation (\(\tau_{re}\)) were 413±48 ms (\(n=4\)), 203±17 ms (\(n=13\)) and 170±19 ms (\(n=4\)) at −80, −90 and −100 mV, respectively.](https://academic.oup.com/cardiovascres/article-abstract/41/1/212/316383)
3.3. Inhibition of Kv4.3 by bupivacaine is not stereoselective

It has been shown that bupivacaine inhibition of $I_{10}$ in rat ventricular myocytes is not stereoselective [21]. To test whether this property is shared by Kv4.3, we studied the effects of bupivacaine enantiomers on these channels. Fig. 3A shows original traces elicited during depolarizing pulses from $-80$ mV to $+60$ mV in the absence and in the presence of 10 $\mu$M of S(-)- and R(+)-bupivacaine, respectively. S(-)- and R(+)-bupivacaine decreased the peak current in a similar fashion [23±5% (n=4) and 18±7% (n=4), respectively; $P>0.05$]. However, their most prominent effect was on the time course of inactivation: the time constant for inactivation decreased from 30.8±2.0 ms to 10.0±1.8 ms (n=4) in the presence of 10 $\mu$M S(-)-bupivacaine and from 24.6±4.1 ms to 14.2±0.7 ms (n=4) in the presence of 10 $\mu$M R(+)-bupivacaine. This accelerated decline of current was suggestive of an open channel block mechanism in which case the reduction of peak current would be a non-equilibrium measure of block. Therefore, block of the Kv4.3 channels by bupivacaine enantiomers was also measured as the reduction in the amount of charge crossing the membrane (estimated from the integral of the current for each test potential) during the application of 500 ms depolarizing pulses from $-80$ mV to $+50$ mV. As it is illustrated in Fig. 3 both enantiomers produced similar levels of Kv4.3 inhibition: R(+)-bupivacaine at 10 and 30 $\mu$M reduced the integrated current by 29±9% (n=4) and 42±7% (n=4), respectively. S(-)-bupivacaine at 10 and 30 $\mu$M reduced the charge by 24±2% (n=4) and 40±9% (n=4), respectively. Thus, both enantiomers reduced the current-time integral to the same extent at both concentrations ($P>0.05$). Assuming a bimolecular reaction and equilibrium conditions we estimated, from these experimental data, $K_D$ values of 31.5±7.2 $\mu$M (n=4) and 31.6±1.5 $\mu$M (n=4) for R(+)- and S(-)-bupivacaine, respectively ($P>0.05$). Using the rate of fast exponential decline of the current induced by the bupivacaine enantiomers as an estimate for the rate of block $\lambda=k[D]+l$, the following estimates were obtained for the apparent association (k) and dissociation (l) rate constants: k=(5.4±1.2) $\times$ 10$^3$ M$^{-1}$s$^{-1}$ and l=157±37 s$^{-1}$ (n=4) for R(+)-bupivacaine and k=(4.7±0.8) $\times$ 10$^3$ M$^{-1}$s$^{-1}$ and l=153±32 s$^{-1}$ for S(-)-bupivacaine ($P>0.05$). Thus, bupivacaine block of Kv4.3 channels is not stereoselective, as is the case for its effects on the native rat $I_{10}$ [21].

4. Discussion

Our results indicate that the biophysical properties of Kv4.3 channels expressed in Ltk$^-$ cells are comparable to
those observed when expressed in *Xenopus* oocytes, including the presence of a non-inactivating component. However, in neither system does Kv4.3 recapitulate the native $I_{\text{TO}}$ properties in full detail. The lack of stereoselectivity observed for bupivacaine and the overall similarity in their effect on the expressed Kv4.3 current with their effect on $I_{\text{TO}}$ in rat myocytes further supports the link between Kv4.3 and the native $I_{\text{TO}}$.

4.1. Comparison between Kv4.3 expressed in Ltk$^-$ cells and native $I_{\text{TO}}$

The time course for inactivation of $I_{\text{TO}}$ has been described either as monoeponential or biexponential [1]. The inactivation of Kv4.3 current expressed in Ltk$^-$ cells was better fit to a biexponential process, at least for depolarizations positive to 0 mV. The fast time constant of inactivation of Kv4.3 current was similar to the time constant of the single exponential fitted observed in human atrium, rat ventricle, canine ventricle and Kv4.2 channels expressed in Ltk$^-$ (Table 1). It is possible that the single-exponential description of the inactivation resulted from native $I_{\text{TO}}$ records which were too short to reveal the slower phase. At potentials positive to 0 mV, voltage dependence of $I_{\text{TO}}$ inactivation kinetics is very weak; only the fast component of inactivation of Kv4.3 current was voltage-dependent, consistent with results obtained for Kv4.1 channels [29].

The presence of a sustained component was a consistent finding and indicates that inactivation in Kv4.3 is incomplete. It is unlikely that this represents an endogenous current since (1) the size of this component was proportional (~15%) to the peak current, (2) no such currents were observed in control cells, and (3) this component was equally sensitive to bupivacaine block. The presence of such sustained component complicates direct comparison with cardiac $I_{\text{TO}}$. Indeed, due to the presence of other currents, the native $I_{\text{TO}}$ is frequently defined as the transient component, in which case the sustained component is assumed to represent a different current. If Kv4.3 contributes to native $I_{\text{TO}}$, then either (1) it undergoes additional (possibly cardiac-specific) modification or assembles with functional-altering subunits to induce full inactivation, as has been demonstrated in brain Kv4.3 channels [4] or (2) the sustained component of Kv4.3 in effect contributes to the outward current during the remainder of the plateau phase of the cardiac action potential.

4.2. Voltage dependence of inactivation and recovery from inactivation

Inactivation of Kv4.3 channels expressed both in Ltk$^-$ cells and in *Xenopus* oocytes displayed an apparent voltage-dependence. This does not necessarily imply that inactivation is intrinsically voltage-dependent, since most N-type inactivation derives its voltage-dependence primarily from that of channel opening [30]. The presence of two kinetic components is consistent with a more complex inactivation process. Indeed, a recent molecular analysis of the inactivation mechanism of mKv4.1 indicated a concerted action of both N- and C-terminal domains [29]. The midpoint of steady-state inactivation of the current was significantly more positive (~27 mV) for Kv4.3 expressed in Ltk$^-$ cells, than that observed when Kv4.3 was expressed in *Xenopus* oocytes (~59 mV) [6] (Fig. 2, Table 1). The $E_{\text{h}}$ value obtained in Ltk$^-$ cells is closer to that obtained for $I_{\text{TO}}$ in most cardiac preparations, especially in human cells (range ~14 to ~45 mV), with the more positive values obtained when divalent cations were used to block calcium currents [28].

The time course of reactivation is a major determinant of the functional role of $I_{\text{TO}}$ in the action potential duration and is responsible for some of its frequency-dependent properties. In rat, canine and human cardiac myocytes, $I_{\text{TO}}$ recovers quickly from inactivation (Table 1) and the time constant that describes this process is strongly voltage-dependent [15]. The $\tau_{\text{rec}}$ values for Kv4.3 when expressed in *Xenopus* oocytes and Ltk$^-$ cells were voltage-dependent and similar in magnitude with values of 202 ms [6] and 203 ms at ~90 mV, respectively. These values are somewhat slower compared to recovery kinetics of Kv4.2 channels expressed in Ltk$^-$ cells [8]. Interestingly the recovery rates for Kv4.2 and Kv4.3 are 3–6 times slower than in rat and human cardiac myocytes. The reason for

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<th>$\tau_{\text{rec}}$ (ms)</th>
<th>$\tau_{\text{in}}$ (ms)</th>
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<td>~14, ~23</td>
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$E_{\text{h}}$: midpoint of inactivation; $\tau_{\text{rec}}$: time constant at or around +50 mV; $\tau_{\text{in}}$: time constant for recovery from inactivation (~80 mV); *value of ~45 mV in the absence of Cd$^{2+}$; $^3$value in the presence of 300 μM Cd$^{2+}$; $^3$time constant obtained at a holding potential of ~90 mV.
these slower recovery in cloned channels remains to be elucidated. This does not appear to be due to the expression system, because similar rates of recovery are observed in different expression systems. One possibility is that some cofactor or subunit that modulates inactivation is missing in the expression systems studied [4]. Another possibility would be that native cardiac channels are heterotetramers which consist of different α-subunits [7,18].

4.3. Block by bupivacaine enantiomers is comparable for Kv4.3 and native ITO

Inhibition of rat ventricular ITO by bupivacaine is not stereoselective [21], a property shared by Kv4.3 channels. The accelerated decline of current in the presence of drug was approximately 3 times faster than the fast component of current inactivation in drug-free channels, this parameter should be a reasonable estimator of the time course of block. Furthermore, a process with a time constant of 10–15 ms should achieve steady-state in 35–50 ms in which case the integration over 250 ms should be sufficient. The Kp values calculated for Kv4.3 in the present study (30 μM) are similar to those obtained for inhibition of native rat ITO (25 μM) [21]. These results are consistent with the effects of mutagenesis of S6 residues on stereoselective bupivacaine block in hKv1.5 [31]: hydrophobic substitutions of threonine 505 (in hKv1.5) eliminated stereoselectivity. Both Kv2.1 and Kv4.3 carry a valine at the equivalent S6 position, and block of both channels is not stereoselective [31], suggesting a common determinant for stereoselective binding of bupivacaine to Kv1.5, Kv2.1 and Kv4.3 channels.

5. Conclusions

The functional profile of Kv4.3 channels expressed in Ltk− cells closely corresponds to the ITO recorded in native cells. However, our results do not rule out association of Kv4.3 with accessory subunits, or some – possibly cardiac-specific – postranslational modification. The presence of a sustained component alerts to a potential direct role of ITO in the control of the duration of the cardiac action potential, which might have therapeutic implications.

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