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Different effects of gadolinium on I_{KR}, I_{KS} and I_{K1} in guinea-pig isolated ventricular myocytes

C. Pascarel, [1]K. Hongo, O. Cazorla, [1]E. White & [2]J-Y. Le Guennec

Laboratoire de Physiologie des Cellules Cardiaques et Vasculaires, UMR 6542 CNRS, Parc de Grandmont, Faculté des Sciences, 37200 Tours, France and [1]Department of Physiology, University of Leeds, Leeds LS2 9JT U.K.

1 Using the whole cell configuration of the patch clamp technique, we studied the potential blocking effects of gadolinium (1 μM to 1 mM) on potassium currents: I_{KR}, I_{KS} and I_{K1}. The study was performed on guinea-pig isolated ventricular myocytes.

2 The background current, I_{K1} was insensitive to Gd^{3+}. Thus, we found that no obvious screening of surface charges was visible with concentrations of Gd^{3+} up to 100 μM.

3 By use of three different protocols: tail currents fitting, analysis of envelope of tails and electrophysiological dissection, we found that I_{KR} was the only component of I_K that was sensitive to Gd^{3+}. The sensitivity was apparently different depending on the protocol used.

4 Comparison of the results obtained with the different protocols revealed that the rapid component of I_{KR} is more sensitive to Gd^{3+} than the slow one.

5 Of the different protocols used to distinguish between I_{KR} and I_{KS}, the electrophysiological dissection seems to be the more accurate.

Keywords: Lanthanides; gadolinium; potassium currents; I_{KR}; I_{KS}; I_{K1}; stretch

Introduction

The delayed rectifier potassium current of guinea-pig cardiac cells consists of two components: I_{KR} and I_{KS} (Sanguinetti & Jurkiewicz, 1990a). I_{KR} is rapidly activated compared to I_{KS}. However, the deactivation of I_{KR} can be fitted by two exponentials at room temperature: a fast and a slow one, while I_{KS} deactivation can just be fitted by a slow one (Chinn, 1992). These two components can be distinguished by use of different electrophysiological methods. Following the work by Noble and Tsien (1969) and of Sanguinetti & Jurkiewicz (1990a,b), the ratio of tail to pulse currents activated by a series of depolarizations of increasing duration ('envelope of tails' test) has often been used to show the dual activation of I_{KR} and I_{KS} and to show selective block of components of I_K (Balser *et al*., 1990; Sanguinetti & Jurkiewicz, 1990a,b). Another way of distinguishing between these two components is by the analysis of the decay of the tail current. This method is useful for the rapid phase of the current which, at room temperature, represents I_{KR}, but not for the slow phase which is a composite of I_{KR} and I_{KS} (Chinn, 1992). The last method used to distinguish I_{KR} and I_{KS} is based on their different voltage sensitivity. Sanguinetti & Jurkiewicz (1990a) showed that the activation threshold of I_{KR} is more negative than that of I_{KS}: -40 mV *vs* -20 mV. Moreover I_{KR} is maximally activated at 0 mV, whereas I_{KS} is maximally activated at $+60$ mV. Using this difference in voltage-dependence, Carmeliet (1992) was able to differentiate both components of I_K. All these protocols can be completed by specific pharmacological blockade (Heath & Terrar, 1996).

The trivalent cation gadolinium (Gd^{3+}) is the most commonly used blocker of stretch-activated channels (SACs, Yank & Sachs, 1989) and has been shown to block stretch-induced arrhythmias in heart muscle (Hansen *et al*., 1991). In addition to this effect, Gd^{3+} (at 10 μM) has also been shown to block completely the high threshold, L-type calcium current (I_{Ca}) in single cardiac myocytes (Lacampagne *et al*., 1994). Interestingly Hansen *et al*. (1991) showed that 10 μM Gd^{3+} induced a prolongation of the cardiac action potential duration. This observation is inconsistent with the findings of Lacampagne *et al*. (1994) and may be explained by a blockade of the repolarizing potassium current. We observed the same increase in action potential duration with Gd^{3+} only when I_{Ca} had been previously blocked by nifedipine (Hongo *et al*., 1995). Sanguinetti and Jurkiewitz (1990b) found that of the two components of the delayed rectifier potassium current, only one is sensitive to La^{3+}: I_{KR}. In bullfrog atrial cells, Nathan *et al*. (1988) observed that I_K and I_{K1} were insensitive to Gd^{3+}. Since Gd^{3+} is more widely used as a stretch-activated channel blocker than La^{3+}, we undertook a study on the effect of this lanthanide on potassium currents of guinea-pig ventricular myocytes: I_{K1}, I_{KS} and I_{KR} using different protocols.

Methods

Cell isolation

Guinea-pigs were killed by cervical dislocation following stunning and hearts removed. Single ventricular myocytes were isolated by use of collagenase and pronase digestion as described elsewhere (Le Guennec *et al*., 1993; Lacampagne *et al*., 1994). Isolated cells were placed in a 1.5 ml perpex chamber on the stage of an inverted microscope (Diaphot, Nikon, Japan). The chamber was continuously perfused at a rate of 1–2 ml min^{-1} with a 'standard' Tyrode solution (containing in mM): NaCl 140, KCl 5.4, $MgCl_2$ 1, $CaCl_2$ 1.8, glucose 11, NaH_2PO_4 0.33 & HEPES 10; pH adjusted to 7.3 with NaOH.

Electrophysiological studies

Whole cell voltage clamp experiments were conducted with a patch-clamp amplifier (Biologic RK 400, Grenoble, France).

[2] Author for correspondence.

Cells were internally perfused with pipette solution (which contained, in mM): K-aspartate 110, KCl 15, NaCl 5, ATP-Mg 5, HEPES 11 and EGTA 10; pH adjusted to 7.1 with KOH.

The holding potential was set to −40 mV to inactivate the sodium current and 5 μM nifedipine was added to the Tyrode solution to block I_{Ca}.

Test solutions were applied to the cell by the positioning of a microcapillary tube, carrying the desired solution, adjacent to the cell (Lacampagne *et al*., 1994). $GdCl_3$ (Aldrich, France) was used in the concentration range of 1 μM to 1 mM. Experiments were performed at room temperature (23–26°C). The osmolarity of all the solutions was carefully controlled (290±5 mosmol) to prevent activation of swelling conductances.

The effects of Gd^{3+} on I_K were studied by use of different protocols: (1) I_K was elicited by voltage clamp steps from a holding potential of −40 mV to +60 mV for 2.25 s at a frequency of 0.1 Hz (Kiyosue *et al*., 1993). Current at the end of the pulse and upon repolarization (tail current) were measured before and after exposure to Gd^{3+}. Tail currents were fitted by use of 2 exponentials to distinguish between I_{KR} and I_{KS} (Chinn, 1992). To construct current-voltage relationships, cells were step depolarized for 2.25 s from −40 mV to test potentials between −80 and +60 mV at 10 mV increments, with an interpulse duration of 10 s. Exponential fittings with one or two exponentials were performed on tail currents. (2) An envelope of tail currents was constructed by clamping cells at a holding potential of −40 mV and stepping to +60 mV for 150–4500 ms. (3) Both components of I_K tail current were separated by their differential voltage-dependence by use of a protocol from Carmeliet (1992), in which cells were clamped at −40 mV, depolarized by a prepulse to +60 mV for 3 s, repolarized to 0 mV for 6 s (during which period the tail current that deactivated was primarily I_{KS}) and subsequently repolarized to −40 mV, allowing I_{KR} to deactivate.

Experiments were conducted and current acquired by means of pClamp software (v5.51) installed on a 386 SX 33 computer. Currents were filtered at 3 kHz by a 8 pole-Butterworth filtre and acquired at a sampling frequency of 0.5 kHz.

Statistical analysis

Results are expressed as mean±s.e.mean (n = number of cells). Statistical analysis was performed by paired t test. Statistical significance was tested at 5% of confidence limits.

Results

As shown on Figure 1, during a current/voltage (I/V) curve procedure (see Methods), the averaged current measured between 50 and 100 ms was not affected by the application of 50 μM Gd^{3+} when the depolarization did not exceed 0 mV. Under 0 mV, this current represents mainly I_{K1}. For depolarizations above 0 mV, a small drop of the current was observed indicating that a component of the current is Gd^{3+}-sensitive. Figure 2 clearly shows that this component is the delayed rectifier potassium current. From this figure, we observed that currents elicited by depolarizations between −10 and 60 mV for 2.25 s are blocked by Gd^{3+} in a dose-dependent manner: 100 μM Gd^{3+} being more efficient than 10 μM. This current has all the characteristics of the delayed rectifier potassium current, I_K. When we looked carefully at the Gd^{3+}-sensitive and the Gd^{3+}-insensitive current, it seems that the kinetic of the former is much slower. To test for specific

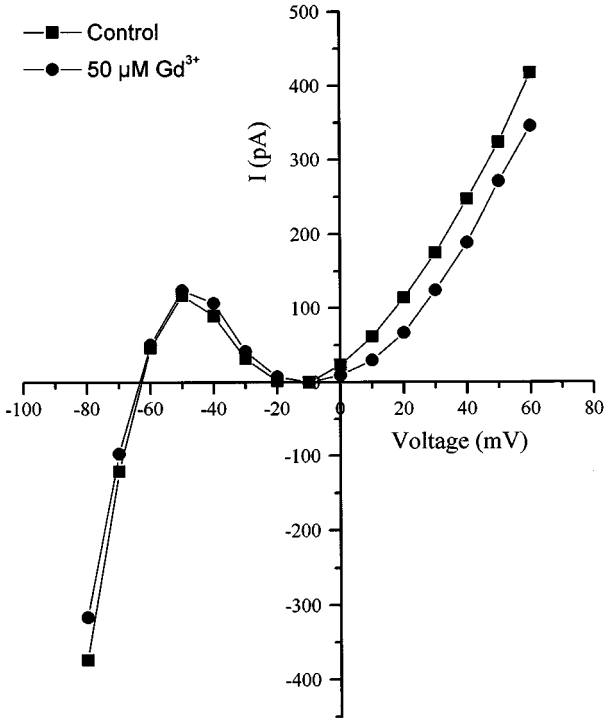

Figure 1 Current-voltage relationship in a single cardiac myocyte in control and during the application of 50 μM Gd^{3+}. The current was measured as the averaged current between times 50 and 100 ms during the depolarization to represent mainly the background potassium current I_{K1}.

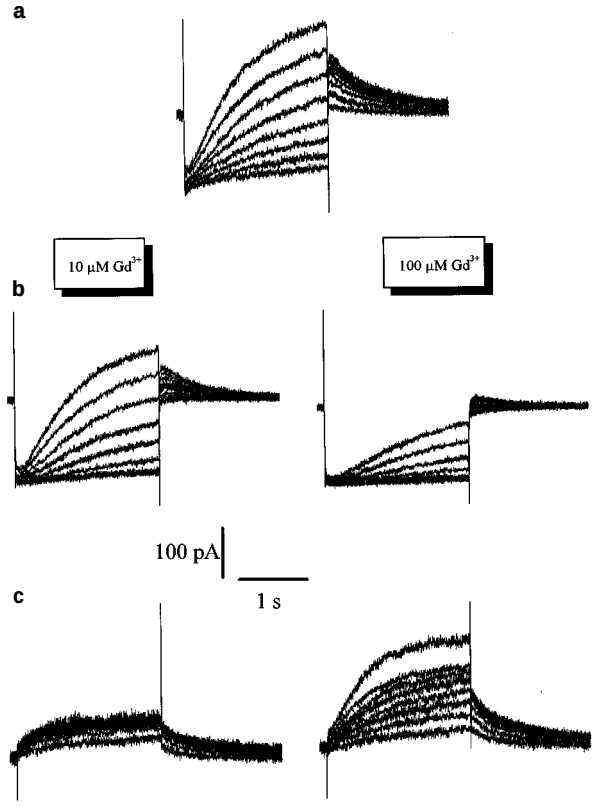

Figure 2 Membrane currents in a whole cell voltage-clamped myocyte evoked in response to 10 mV step depolarizations from −40 mV to potentials between −10 and +60 mV. Currents shown in control (a) and in presence of 10 μM (b, left) or 100 μM (b, right) Gd^{3+}. In (c), the Gd^{3+}-sensitive fraction of the current, obtained as the difference between the current in control and during application of the lanthanide, is shown.

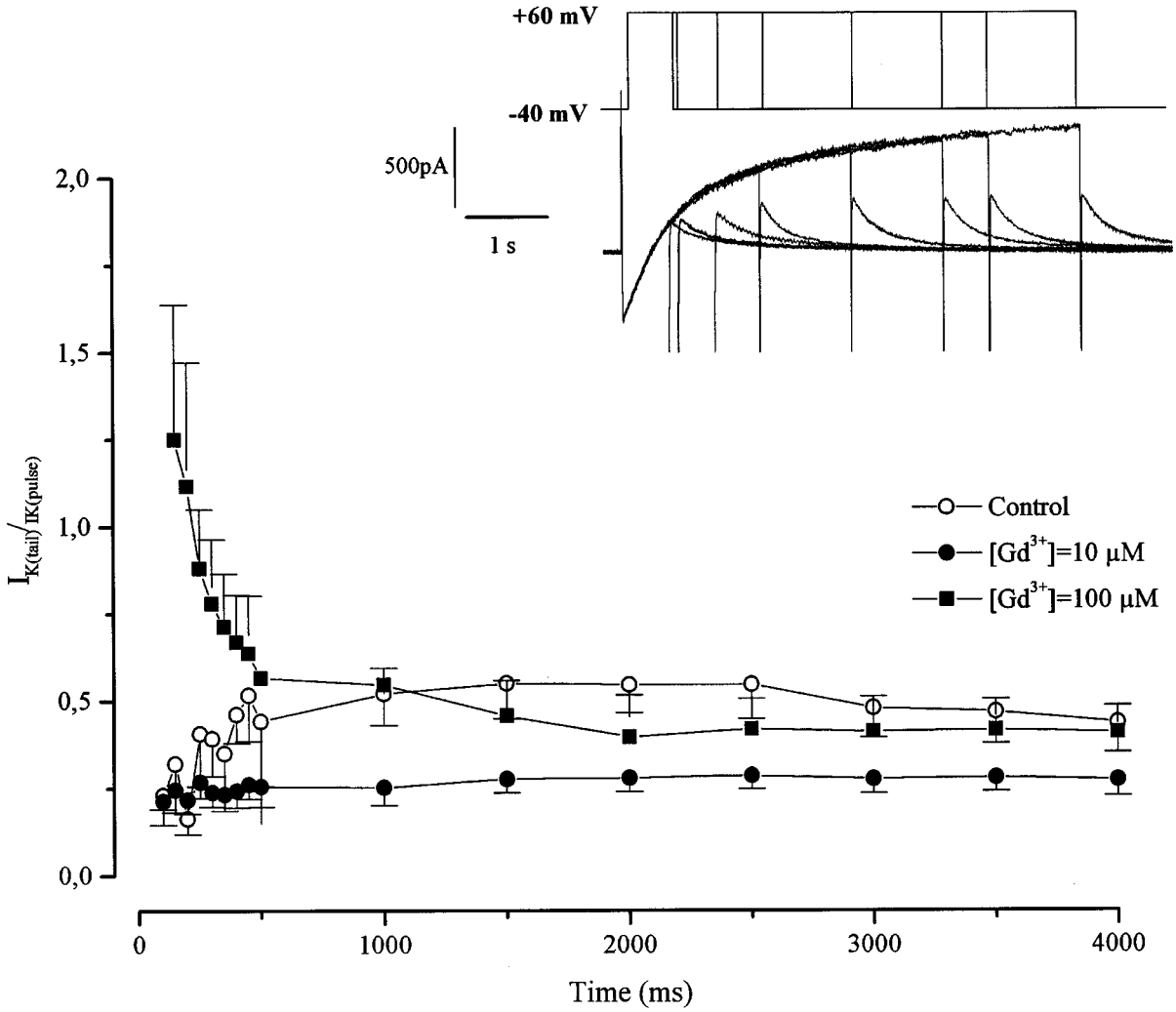

Figure 3 Envelope of tails analysis. Mean ratio of tail current to pulse current in control conditions ($n=11$ cells), during application of 1 μM ($n=3$ cells) or 10 μM Gd^{3+} ($n=6$ cells). The inset shows a typical record obtained with this protocol. Each point represents the mean and vertical lines show s.e.mean.

Table 1 Effects of different Gd^{3+} concentrations on tail current kinetics

$[Gd^{3+}]$	τ_1 (ms)	Amp_1 (pA)	τ_2 (ms)	Amp_2 (pA)	*Number of cells*
0	237±20	19±5	1599±210	58±5	32
10 μM	149±32	5±5	868±222	39±10	7
50 μM	138±22	0±3	1016±94	59±8	9
100 μM	59±9	−5±1	537±101	34±22	6
200 μM	169±20	2±1	1014±158	25±3	4
1 mM	111±16	−3±1	963±103	35±6	6

Tail currents were elicited by repolarizing cells to −40 mV after a 2.25 s depolarization to +60 mV. Tail currents were fitted with two exponentials having time constants and amplitudes given in the table.

blockade of one component of I_K by Gd^{3+}, we used different methods. I_K was first activated by a series of depolarizations, to +60 mV, of increasing duration, and both the tail and the time-dependent pulse currents measured. The ratio of tail current to pulse current indicates (Figure 3), as has already been shown by Sanguinetti and Jurkiewicz (1990a), that at least two currents are present in control conditions since the ratio was not constant. In the presence of 1 and 10 μM Gd^{3+} the ratio became constant, indicating that one component of I_K was blocked by Gd^{3+}. To differentiate between these two components, we also used exponential fitting analysis of the tail current when repolarizing cells from +60 mV to −40 mV (Chinn, 1992). Table 1 shows, in accordance with Chinn (1992), that in control conditions 2 exponentials can be found: a fast one representing I_{KR} and a slow one representing a mixture of I_{KR} and I_{KS}. When increasing concentrations of Gd^{3+} were applied to the cells, the amplitude of the first exponential decreased, being null at 50 μM Gd^{3+}. At the same time, the amplitude of the slow exponential was not significantly affected by Gd^{3+} up to a concentration of 200 μM. To differentiate I_{KR} and I_{KS} electrophysiologically, we used a double pulse protocol as described by Carmeliet (1992). Figure 4 shows that 50 μM Gd^{3+} blocked almost completely the tail current on repolarizing to −40 mV from 0 mV, without a measurable effect on the tail current on repolarizing to 0 mV from +60 mV. Thus, it is clear that the Gd^{3+}-sensitive current is I_{KR} and that I_{KS} is not sensitive to Gd^{3+}. The downward shift observed during the repolarization to 0 mV is consequent to I_{KR} blockade since it is a component of the 'background' current at this voltage. A dose-response curve was obtained to characterize the blockade (Figure 5). A

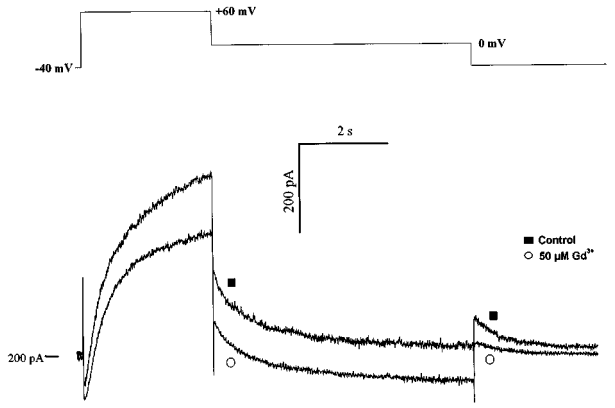

Figure 4 Effect, in a single cardiac myocyte, of 50 μM Gd^{3+} on the tail current deactivating at 0 mV and -40 mV. The protocol used is given on the upper part of the figure and described in the Methods section.

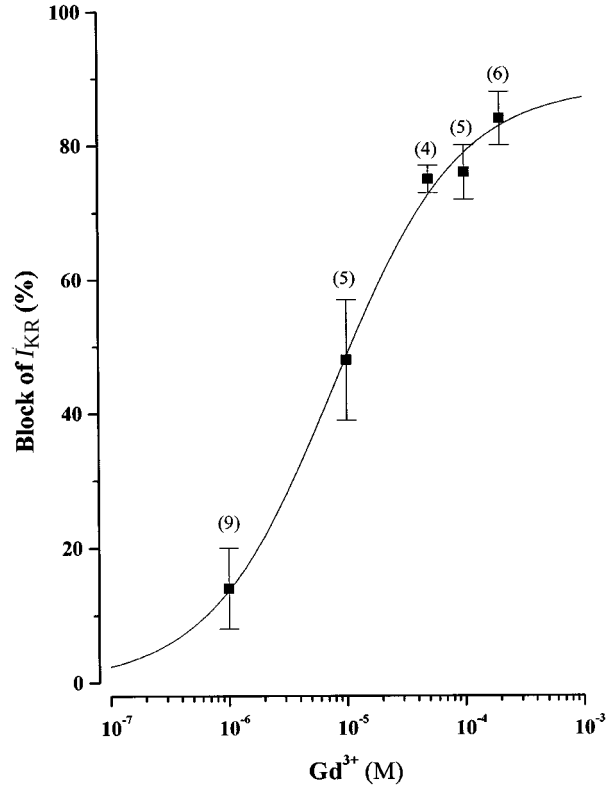

Figure 5 Dose-response curve for the effect of Gd^{3+} upon I_K tail currents deactivating at -40 mV following a 6 s prepulse to 0 mV. Each point represents the mean of the numbers of cells at each dose given in parentheses; vertical lines show s.e.mean. The maximum effect of Gd^{3+} was a 88% blockade, the EC_{50} was 7.9 μM and the Hill coefficient 0.82.

maximal blockade of 88% with an EC_{50} of 7.9 μM and a Hill coefficient of 0.82 has been found by use of a logistic fit (equivalent to a Hill plot, Barlow & Blake, 1989).

Discussion

The main findings from this study were that, whatever the protocol used, we obtained a specific component of I_K, I_{KR}, that was sensitive to Gd^{3+} while I_{KS} and I_{K1} were not affected by Gd^{3+}. This result is comparable to the specific blockade of I_{KR} by La^{3+} described by Sanguinetti and Jurkiewicz (1990b). A big difference between their results and our findings concerns the screening of negative surface charges. Sanguinetti and Jurkiewicz (1990b) observed that, for concentrations of $La^{3+} \geqslant 10$ μM, a measurable screening occurred. In our study, we observed that even at a concentration of 50 μM, Gd^{3+} had no such an effect; as observed in Figure 1, no shift of the reversal potential of the current was obtained. This shift appeared at 100 μM Gd^{3+}. A former study on the L-type Ca^{2+} current (Lacampagne *et al*., 1994) (in guinea-pig ventricular myocytes at 22°C) showed no screening of surface charges with 10 μM Gd^{3+}. Erlinder and Arhem (1994) did not find any screening with 10 μM Gd^{3+} on myelinated axon of *Xenopus laevis*. The differences between these findings and those of Sanguinetti and Jurkiewicz (1990b) might be related to the lanthanide used i.e. La^{3+} versus Gd^{3+}. Differences in experimental temperature might also account for these effects, as the study by Sanguinetti and Jurkiewicz (1990b), in guinea-pig ventricular myocytes was performed at 35°C and a screening of surface charges of about 10 mV was obtained with 10 μM La^{3+}. However, Nathan *et al*. (1988) did not find any screening with 100 μM La^{3+} while working on bullfrog atrial cells at 22°C.

When we compared carefully the results given by the 3 methods used in this study, envelope of tail test, exponential fitting of tail current and electrophysiological dissection, some interesting information could be obtained. The envelope of tail test gave a complete blockade at 1 μM, since the curve was flat at this concentration. If just a partial block had occurred, a decrease of the initial ratio might have been observed with no flattening. The fitting of tail currents indicates a complete blockade at a concentration of 50 μM (Chinn, 1992). The electrophysiological dissection (Carmeliet, 1992) indicates that the maximal blockade was obtained at Gd^{3+} concentrations of around 100 μM. In fact, the complete flattening of the ratio of tail to pulse currents curve with 1 μM Gd^{3+} indicates that one component of I_K was completely blocked or that the difference between the kinetics of the 2 components disappeared. Since deactivating current at -40 mV is sensitive to Gd^{3+} concentrations up to 100 μM while the deactivating current at 0 mV is insensitive, we can conclude that I_{KR} is not completely blocked by 1 μM Gd^{3+}. Thus it is likely that the constant ratio of tail to pulse currents amplitude is due to the fact that the fast component of I_{KR} is more sensitive to Gd^{3+} than is the slow one. This fast component is probably completely blocked by 1 μM Gd^{3+} while the slow one needs up to 100 μM Gd^{3+} to be completely blocked. The slow component of the tail current observed during the repolarization to -40 mV (Figure 4) probably represents some I_{KS} deactivating current responsible for the 12% of the current not sensitive to Gd^{3+}.

This result is interesting since it is the first time that a differential affinity for a blocker has been found, allowing us to distinguish between the fast and the slow component of I_{KR} at room temperature. This result demonstrates that the envelope of tail test must be use with great care: a constant ratio of tail to pulse current amplitude does not mean that just one component of I_K is present. For the same kind of reason, the fitting of the tail currents when repolarizing from positive voltages to -40 mV does not give good indications of the specificity of a potassium channel blocker. The best protocol to study the pharmacological properties of I_K is probably the electrophysiological dissection method (Carmeliet, 1992), the other protocols being able to give additional information.

In conclusion, Gd^{3+} blocks specifically I_{KR} and not I_{KS} or I_{K1}. The fast component of I_{KR} is more sensitive than the slow one.

It is interesting to note that the concentration of Gd^{3+} necessary to block I_{KR} ($EC_{50} = 7.9$ μM) is in the same range as those typically used to block stretch-induced arrhythmias (1–10 μM, Hansen *et al*., 1991). Given that at this dose Gd^{3+} also blocks L-type Ca^{2+} channels (Lacampagne *et al*., 1994), it is clear that the effects of Gd^{3+} cannot be assumed to be solely mediated by blockade of SACs.

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