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The stretch-activated ion channel blocker gadolinium also blocks L-type calcium channels in isolated ventricular myocytes of the guinea-pig

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Abstract

We show that gadolinium (Gd^{3+}) is a potent calcium channel blocker in guinea-pig isolated ventricular myocytes. A dose-dependent inhibition of I_{CaL} was found with an EC_{50} of 1.4 μM and a complete inhibition at 10 μM Gd^{3+}. When compared with Cd^{2+}, it appeared that the blockade of I_{CaL} is a complex phenomenon probably involving more than one site of interaction (a Hill coefficient of 1.6 was found for Gd^{3+} vs. 1.0 for Cd^{2+}). It is concluded that Gd^{3+} ions completely block I_{CaL} at concentrations used to block stretch-activated channels (SAC), rendering its use as a specific SAC inhibitor problematic.

Key words: Gadolinium; Cadmium; Calcium current; Stretch-activated channel

1. Introduction

Gadolinium is considered to be the most potent and specific stretch-activated channel (SAC) blocker [1]. Stretch-induced increase of resting intracellular calcium was found in different preparations such as smooth muscle cells [2], cultured chick cardiac cells [3] or endothelial cells [4]. SAC seem to be involved in this phenomenon since it is blocked by 10–20 μM Gd^{3+}. As a lanthanide cationic trivalent ion, Gd^{3+} may also interact with calcium channels. To our knowledge, there are only three electrophysiological studies on the effects of Gd^{3+} on the calcium currents (I_{Ca}) and the results are contradictory. 10 μM Gd^{3+} was found to selectively inhibit an I_{Ca} which shared some common properties with the N-type I_{Ca} in neuronal cells while L- and T-type I_{Ca} were not affected [5]. Opposing this, it was found that 5 μM Gd^{3+} inhibited completely L- and T-type I_{Ca} in pituitary cells [6]. A partial inhibition of L-type I_{Ca} by 10 and 50 μM Gd^{3+} in cultured rat cardiac ventricular myocytes was also observed [7]. These discrepancies may be due to the different cell type. Studies in this laboratory involve the effects of stretch upon single isolated ventricular myocytes [8,9] and so it was important for us to determine whether Gd^{3+} may influence other channels. Thus, we studied the effects of Gd^{3+} on L-type I_{Ca} of isolated guinea-pig cardiac ventricular cells. A blockade of the current has been found and compared to the inhibition induced by cadmium which is a reference inorganic calcium channel blocker [10].

2. Materials and methods

Single ventricular myocytes were isolated as described elsewhere [11]. Isolated cells were placed in a 1 ml perpex chamber on the stage of an inverted microscope (CK 2, Olympus, Japan). The chamber was continuously perfused at a rate of 1 ml · min^{-1} with a 'standard' Tyrode solution (in mM): 140 NaCl, 5.4 KCl, 1 $MgCl_2$, 1.8 $CaCl_2$, 11 glucose, 0.33 NaH_2PO_4, 10 Hepes; pH adjusted to 7.3 with NaOH.

Whole-cell voltage clamp experiments were conducted using a patch-clamp amplifier (Biologic RK 300, Grenoble, France). Cells were internally perfused with the intracellular solution (in mM): 125 CsCl, 5 ATP-Mg, 11 Hepes, 10 EGTA (ethylene glycol bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid); pH ad-

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justed to 7.1 with CsOH. To eliminate ion currents other than I_{Ca}, cells were superfused (18 $\mu l \cdot min^{-1}$) with an extracellular solution (TEA-Cs solution), where Na^+ ions were substituted by tetraethylammonium (TEA) and K^+ ions replaced by Cs^+ ions (in mM): 140 TEA-Cl, 6 CsCl, 1 $MgCl_2$, 1.8 $CaCl_2$, 11 glucose, 10 Hepes; pH adjusted to 7.3 with TEA-OH. Test solutions were applied to the cell by microcapillaries. Less than 10 s were needed to completely change the superfusing solution around a cell. $GdCl_3$ (Aldrich, France) and $CdCl_2$ (Sigma, France) were prepared as 2 mM stock solutions in distilled water and diluted in the TEA-Cs solution, at the final concentrations as mentioned in the text.

I_{Ca} was elicited with 300 ms depolarizing pulses to 0 mV from a holding potential of −80 mV at a stimulation frequency of 0.1 Hz. I_{Ca} was measured as the difference between peak inward current and the current at the end of the depolarizing pulse. The temperature at which experiments were performed (26–27°C) and the calcium concentration used in this study (1.8 mM) led us to reasonably assume that this I_{Ca} is mainly constituted by I_{CaL} [12]. This is confirmed by the lack of shoulder at negative voltages on the I–V curve shown on Fig. 2B. The protocol to build the I–V curves was the following: at a holding potential of −80 mV, 25 depolarizing pulses of 500 ms from −60 to 60 mV, with a 5 mV interval, were applied to the cell with an interpulse duration of 6 s. For each cell, the capacitance was determined and currents were expressed in current density. The capacitance was 125 ± 7 pF (mean ± S.E., $n = 26$ cells). Under steady-state, I_{CaL} had a current density of −7.3 ± 0.7 pA/pF.

Estimates of EC_{50}, Hill coefficients and lines on graph were obtained by fitting individual data with an unweighted non-linear least-square algorithm to the following equation:

$$I_{Ca}/I_{Ca_{max}} = 100 + 100/\left(\left(1 + \left([I]/EC_{50}\right)^{p}\right)\right)$$

where I represents the calcium channel blocker concentration, p the Hill coefficient and EC_{50} the concentration of blocker inducing an half inhibition of I_{Ca}.

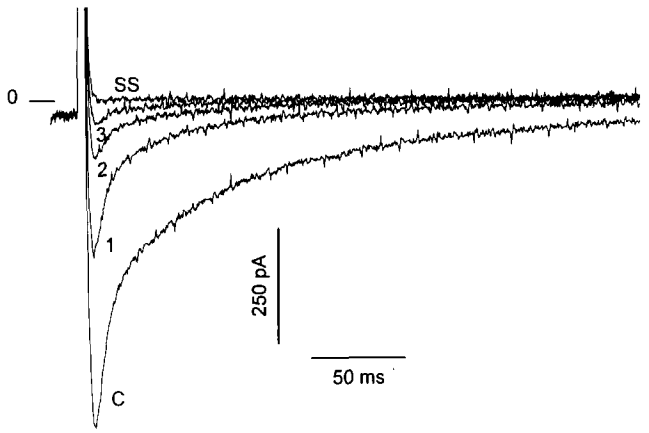

Fig. 1. Effects of 10 μM Gd^{3+} on I_{CaL}. (c) represents the control current (in TEA-Cs solution without Gd^{3+}). (1), (2) and (3) are the stimulation numbers after application of Gd^{3+}. (ss) represents steady-state current (6th depolarization after application of 10 μM Gd^{3+}). Complete inhibition was found in 6 out of 7 cells. For 1 cell, there was 95% inhibition.

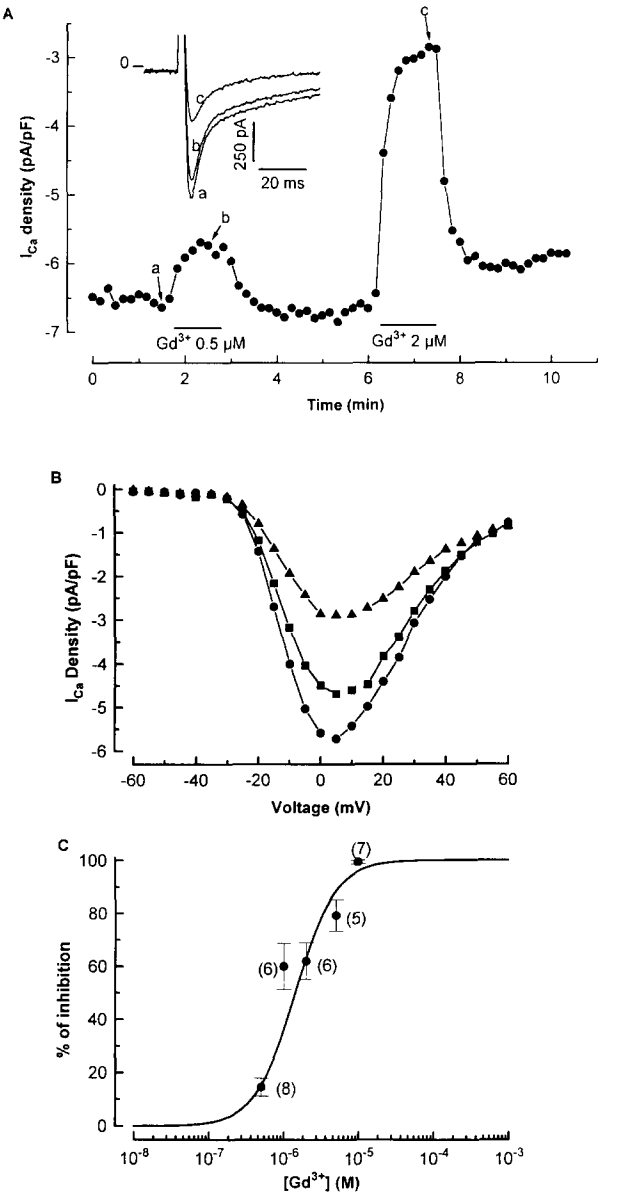

Fig. 2. Dose-dependence of the inhibitory effect of Gd^{3+} on I_{CaL}. (A) Evolution with time of the amplitude of I_{CaL} density in control conditions and during application of two concentrations of Gd^{3+}: 0.5 and 2 μM. The inset shows steady-state currents at the different concentrations: a: control current, b: 0.5 μM Gd^{3+}, c: 2 μM Gd^{3+}. (B) I–V curves of I_{CaL} density in control (circle) and with 0.5 (square) and 2 μM (triangle) Gd^{3+}. (C) Dose–response curve of the inhibitory effect of Gd^{3+} on I_{CaL}. A Hill coefficient p of 1.6 and an EC_{50} of 1.4 μM were determined from the fitting. Numbers between brackets indicate the number of cells studied at each concentration.

Statistical comparisons were analysed using an unpaired non-parametric test (Mann-Whitney U). Results are expressed as mean ± S.E., and $P < 0.05$ was considered significant.

3. Results

Effects of Gd^{3+} on I_{CaL}

We first tested the effects of 10 μM Gd^{3+} on I_{CaL}, and as can be seen on Fig. 1, the current is completely blocked after a few depolarizations (here the 6th). This effect was dependent upon the concentration of Gd^{3+} (Fig. 2A). The effect of Gd^{3+} was completely reversible except when a Gd^{3+} concentration of 10 μM was applied for more than 3 min. Fig. 2B illustrates that there was no significant shift of the I–V curves during application of 0.5 and 2 μM Gd^{3+}. To quantify more precisely this concentration dependent inhibition of I_{CaL} by Gd^{3+}, a dose–response curve is shown on Fig. 2C.

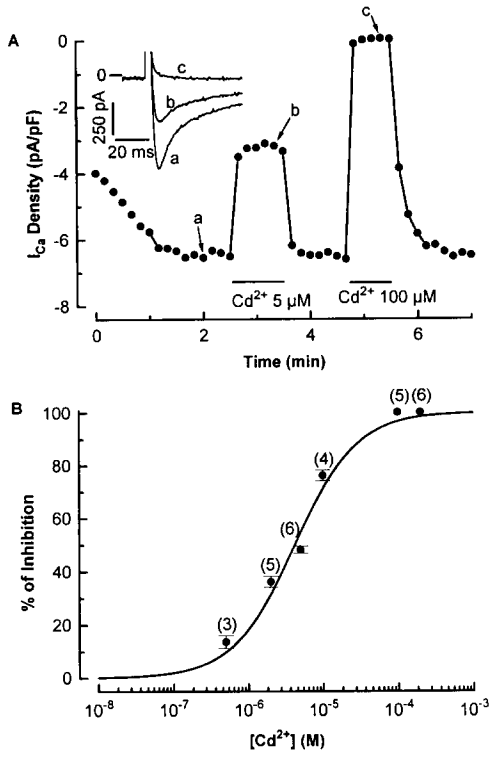

Fig. 3. Dose-dependence of the inhibitory effect of Cd^{2+} on I_{CaL}. (A) Temporal evolution of the amplitude of I_{CaL} density in control conditions and during application of two concentrations of Cd^{2+}: 5 and 100 μM. The inset shows steady-state currents obtained in control condition (a), during application of 5 μM Cd^{2+} (b) and 100 μM Cd^{2+} (c). (B) Dose–response curve of the inhibitory effect of Cd^{2+} on I_{CaL}. A Hill coefficient of 1.0 and an EC_{50} of 4.1 μM were determined. Numbers between brackets indicate the number of cells studied at each concentration.

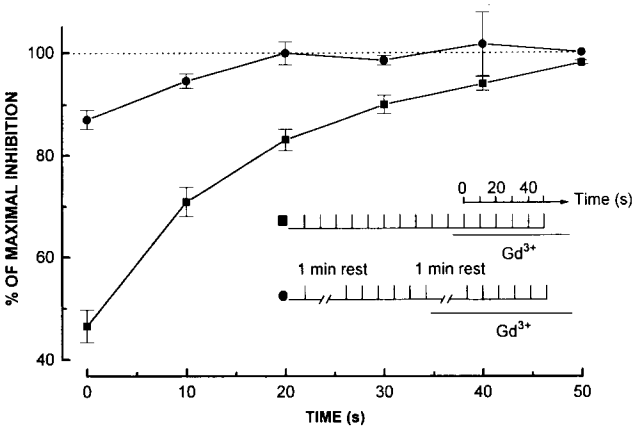

Fig. 4. Testing the use-dependence of the effect of Gd^{3+} on I_{CaL}. Since the kinetics of block followed the same pattern whatever the concentration of Gd^{3+} used (1, 2, 5 or 10 μM) and since no statistical differences were found between results obtained at the different concentrations, the results were pooled. Squares represent the evolution of the effect of Gd^{3+} without interrupting the stimulus protocol ($n = 21$ pooled results, upper part of the inset). The effect was normalized to the maximal inhibitory effect in each experiment. Circles represents the evolution of the effect of Gd^{3+} upon resumption of the stimulus protocol after 1 min rest in the presence of Gd^{3+} (5 cells pooled from 3 cells in 2 μM and 2 cells in 5 μM Gd^{3+}, lower part of the inset). The difference between I_{CaL} in presence and in absence of Gd^{3+}, to remove any possible effect of rest on I_{CaL}, was normalized to the maximal inhibitory effect in each experiment.

Comparison with the blocking effect of Cd^{2+}

Inhibition of I_{CaL} by Cd^{2+} was examined for comparison with the responses to Gd^{3+}. After breaking the patch, a run-up of I_{CaL} was seen sometimes [13] and it was the case of the cell shown in Fig. 3A. When the steady state of the control currrent was reached, application of Cd^{2+} induced an immediate partial (5 μM) or complete (100 μM) blockade of I_{CaL}. The dose–response curve for Cd^{2+} is shown on Fig. 3B.

As shown on Figs. 1 and 2A, the blockade of I_{CaL} by Gd^{3+} is not instantaneaous. This is not due to the speed of solution changing around the cell since, as shown on Fig. 3A, under the same conditions, the inhibitory effect of Cd^{2+} was faster than the resolution of the experimental protocol (< 10 s). We therefore assumed that, as already suggested [6], Gd^{3+} may need a depolarization to exert its effects. To test this assumption, knowing that maximal blockade at each dose was reached in about 1 min (50 ± 20 s), we performed the experiments shown in Fig. 4. The results show little or no obvious need of depolarization for Gd^{3+} to block I_{CaL}.

4. Discussion

The main finding of this study is that Gd^{3+} is a very potent blocker of the cardiac L-type calcium channel.

This contradicts the results obtained by Sadoshima et al. [7]. At least two explanations can be given for this difference: (1) different species (rat vs. guinea-pig). (2) the presence of Na^+ ions in the bathing solution and the voltage-clamp protocol used to study I_{Ca} (pulses from −60 to 0 mV) by Sadoshima et al. [7] makes likely that there was a contamination of I_{Ca} by I_{Na}. Its inhibitory effect upon I_{CaL} is comparable in some aspects to the one observed on pituitary cells [6]. The main difference seems to be localized in the need or not of a depolarization. Biagy & Enyeart [6] found that the blocking effect was much stronger with longer depolarizations. We have not performed exactly the same experiments but we found that a 1 min rest from stimulation allowed a blockade of I_{CaL} which is, if not complete, at least comparable to that recorded 40–50 s after the cell was superfused by a solution-containing Gd^{3+} during a maintained train of stimuli. Docherty [5], working on a neuroblastoma cell line, did not find any blocking effect of Gd^{3+}, at the same concentration range, on L-type calcium channels but rather a specific inhibition of N-type calcium current. This may be explained by differences in calcium channels in heart and neurone [14,15].

When comparing the dose–response curves of Cd^{2+} and Gd^{3+}, a difference was found in the Hill coefficient (1.0 for Cd^{2+} and 1.6 for Gd^{3+}). For Gd^{3+}, the result agrees with the two interaction sites found for the inhibition of contraction of guinea-pig papillary muscle by lanthanum [16]. This observation, together with the difference in kinetics of I_{CaL} indicate that the mechanism of blockade by Gd^{3+} is more complex than the 1:1 interaction found for Cd^{2+}. A screening of surface charges does not appear to be a good explanation for the inhibition of I_{CaL} by Gd^{3+} for at least three reasons: (1) the concentrations needed to block I_{CaL} are much lower in comparison with the external calcium concentration. (2) There was no significant shift of the I–V curves (see Fig. 2B). (3) During the development of the inhibition, no shift of the time to peak of the current was seen (see Fig. 1).

Gd^{3+} is a more potent I_{CaL} blocker than is Cd^{2+} (EC_{50} of 1.4 μM vs. 4.1 μM for Cd^{2+}). However, it has some properties which may render it less useful than Cd^{2+}. The blockade is not instantaneous. The recovery is much longer and can be incomplete when high doses (10 μM) are applied for a long time (5–7 min) (data not shown).

In the literature, it was reported that stretch may induce an increase in intracellular calcium concentration in different preparations [2–4]. To demonstrate the role played by SAC in this phenomenon, experiments showing its blockade by Gd^{3+} have to be completed by other experiments excluding the involvement of I_{CaL} [2–4].

5. Acknowledgements

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