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Short communication

Alkalinisation does not modify the effect of verapamil on myocardial Ca^{2+} current

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Abstract

The effect of verapamil on L-type Ca^{2+} current (I_{Ca}) was compared at external pH 7.4 and 8.5 in rat ventricular myocytes. Alkalinisation increases the fraction of uncharged molecules of verapamil (pK 8.75) and thereby facilitates membrane permeation of the drug. Verapamil (1 μM) reduced the amplitude of I_{Ca} ($I_{Ca(peak)}$) by 36 ± 4% at pH 7.4 and by 40 ± 6% at pH 8.5, whereas alkalinisation from pH 7.4 to 8.5, without drug, increased $I_{Ca(peak)}$ by 12 ± 3%. It is suggested that the efficiency of verapamil is not influenced by the amount of protonation or membrane permeation.

Keywords: Ca^{2+} current, L-type; Verapamil; Binding site; Ventricular myocyte, rat; Patch-clamp technique

1. Introduction

L-type Ca^{2+} channels mediate the increase in intracellular Ca^{2+} level in cardiac myocytes leading to the initiation of myocardial contraction. Phenylalkylamines like verapamil affect the function of Ca^{2+} channels and have been established in the therapy of cardiovascular disorders (Spedding and Paoletti, 1992). Identification of the receptor site for phenylalkylamines could offer the chance of modelling new Ca^{2+} antagonists (Catterall and Striessnig, 1992). Photoaffinity-labeling studies have revealed that phenylalkylamines exert their effects by binding to receptor sites placed on the α_1 subunit of the Ca^{2+} channel protein (Catterall and Striessnig, 1992). Electrophysiological investigations suggest that the binding site is located at the intracellular side of the cell membrane assuming a similar mode of action for phenylalkylamines as found for local anaesthetics (Hescheler et al., 1982). However, other studies do not support this hypothesis (Ohya et al., 1987; Wegener and Nawrath, 1995).

It is well established that local anaesthetics affect Na^+ channels by binding to intracellular receptor sites. As local anaesthetics are weak bases, they exist in a charged and uncharged form at pH 7.4. It is supposed that the uncharged form permeates cell membranes and the charged form binds to intracellular sites of the channel molecules. The hypothesis was supported by experiments at different external pH values leading to different amounts of uncharged drug molecules: extracellular acidification decreased, whereas alkalinisation increased the potency of the drugs acting on Na^+ currents (Hille, 1977). Here we report the action of verapamil on L-type Ca^{2+} currents (I_{Ca}) when external pH was changed from 7.4 to 8.5. Thereby, the fraction of unprotonated verapamil molecules is assumed to increase about 10-fold (according to a pK value of 8.75; Hasegawa et al., 1984). Our results demonstrate that the action of verapamil on I_{Ca} is not different at pH 7.4 and 8.5. This finding calls in question the hypothesis that phenylalkylamines have to penetrate the cell membrane to act on I_{Ca}.

2. Material and methods

Ventricular myocytes from rat hearts were isolated as described (Wegener and Nawrath, 1995). Electrophysiological experiments were performed at 36 ± 1°C on myocytes using the whole-cell configuration of the patch clamp technique (Hamill et al., 1981). Myocytes were superfused with bath solution buffered with Hepes/NaOH to pH 7.4 or with Tris/HCl to pH 8.5

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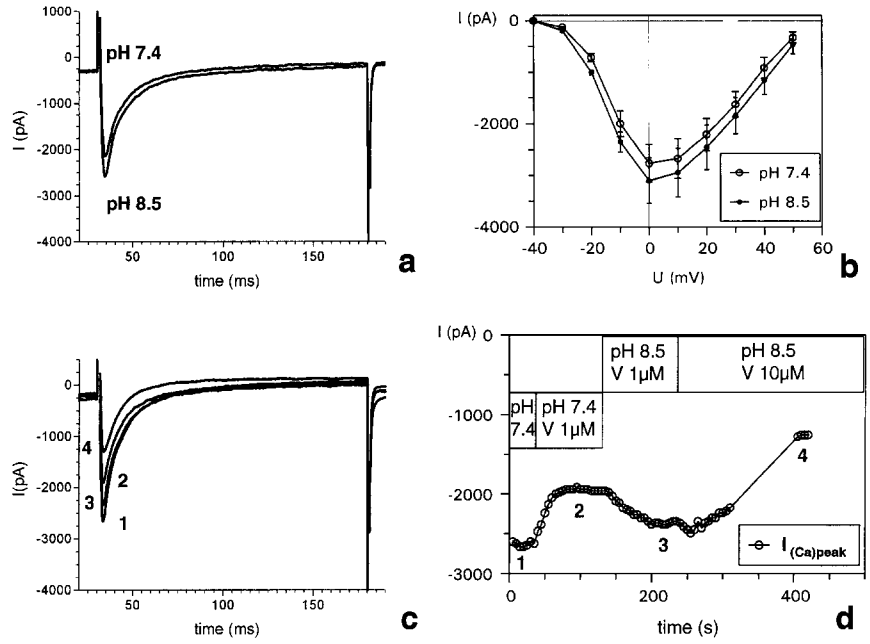

Fig. 1. Effect of change in external pH on I_{Ca} with and without application of verapamil (V). (a) Original recording of I_{Ca} activated by a voltage step from −40 mV to 0 mV. Fast sodium currents were inactivated by a prepulse from the holding potential of −80 mV to −40 mV. Changing extracellular pH from 7.4 to 8.5 slightly increased I_{Ca}. (b) Current-voltage relation of $I_{Ca(peak)}$ at pH 7.4 and 8.5. At 0 mV $I_{Ca(peak)}$ increased from -2763 ± 363 pA (pH 7.4) to -3097 ± 551 pA (pH 8.5; $n = 3$ each). (c) Original recording of I_{Ca} activated as described in (a) at pH 7.4 (1). Verapamil (1 μM) reduced I_{Ca} at pH 7.4 (2), whereas a change of the superfusing solution containing verapamil (1 μM) at pH 8.5 increased I_{Ca} (3). Application of verapamil (10 μM) at pH 8.5 further depressed I_{Ca} (4). (d) Time course of $I_{Ca(peak)}$ of the experiment in (c), to which the numbers depicted correspond.

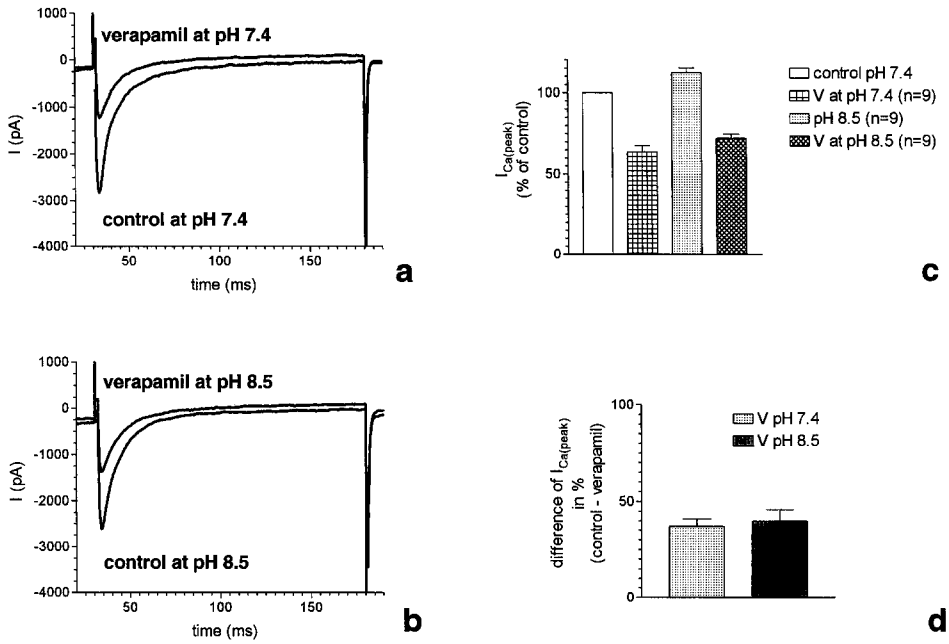

Fig. 2. Effect of verapamil (V) on I_{Ca} at external pH of 7.4 and 8.5. Original recording of I_{Ca} activated as described in Fig. 1a at pH 7.4 (a) and pH 8.5 (b). Application of verapamil (1 μM) reduced I_{Ca} under both conditions. (c) $I_{Ca(peak)}$ at different pH with and without verapamil (1 μM) with respect to $I_{Ca(peak)}$ at pH 7.4 (100%). A rise in pH from 7.4 to 8.5 increased $I_{Ca(peak)}$ to $112 \pm 3\%$ ($n = 9$). Application of 1 μM verapamil reduced $I_{Ca(peak)}$ to $64 \pm 4\%$ ($n = 9$) at pH 7.4 and to $72 \pm 3\%$ ($n = 9$) at pH 8.5. (d) Depression of $I_{Ca(peak)}$ as difference of control and verapamil at both pH 7.4 and 8.5. $I_{Ca(peak)}$ was reduced by $36 \pm 4\%$ ($n = 9$) at pH 7.4 and by $40 \pm 6\%$ ($n = 9$) at pH 8.5. A statistical analysis using Student's t-test for unpaired data revealed no significant difference between both sets of values ($P = 0.34$).

(in mM: NaCl 137, CsCl 5.4, $MgCl_2$ 0.5, $CaCl_2$ 1.8, glucose 5, buffer 10). Verapamil was added to the superfusate. The pipette solution contained in mM: CsCl 125, $MgCl_2$ 6, $CaCl_2$ 0.15, Na_2ATP 5, Na_2GTP 0.1, EGTA 5, Hepes 10; pH was adjusted to 7.4 with CsOH. During the experiments, myocytes were voltage-clamped at a holding potential of −80 mV. To inactivate fast sodium currents, a prepulse to −40 mV was set for 30 ms before activating Ca^{2+} currents. I_{Ca} was activated by depolarising voltage pulses up to 0 mV for 150 ms at 0.2 Hz. The voltage dependence of I_{Ca} was examined using voltage pulses up to +50 mV in 10 mV increments at 1 Hz. Amplitude of I_{Ca} ($I_{Ca(peak)}$) was measured as the difference between peak inward and steady-state current at the end of the voltage pulse. Current-voltage relations were obtained by plotting the current values as function of the voltages examined. Data were presented as original recordings filtered at 1 kHz or expressed as means ± S.E.M. The significance of differences was evaluated by Student's *t*-test for unpaired data.

All salts and solvents used were at least p.a. grade and purchased from Sigma (St. Louis, MO, USA). Verapamil was a gift from Knoll (Ludwigshafen, Germany).

3. Results

Fig. 1a demonstrates original recordings of I_{Ca} at external pH (pH_o) of 7.4 and 8.5. The change of pH_o from 7.4 to 8.5 slightly enhanced I_{Ca} as described (Krafte and Kass, 1988). Current-voltage relations of $I_{Ca(peak)}$ are shown in Fig. 1b at pH_o 7.4 and 8.5. The rise in pH_o increased $I_{Ca(peak)}$ at 0 mV from −2763 ± 363 pA to −3097 ± 551 pA ($n = 4$). No change in the voltage dependence of I_{Ca} was observed.

Fig. 1c shows original recordings of I_{Ca} under control conditions and after application of verapamil in sequence at pH_o 7.4 and pH 8.5. Verapamil (1 μM) significantly depressed the magnitude of I_{Ca} at pH_o 7.4. Increasing the pH of the superfusing solution from pH 7.4 to 8.5 enlarged I_{Ca}, similar to control experiments without drug. Application of verapamil (10 μM) further decreased I_{Ca} indicating that verapamil at 1 μM had not reached a maximal effect on I_{Ca}. Fig. 1d demonstrates the time course of $I_{Ca(peak)}$ during the course of the experiment.

Fig. 2 summarizes the results obtained with verapamil at pH_o 7.4 and 8.5. Original recordings of I_{Ca} are shown under control conditions and after application of verapamil (1 μM) at pH_o 7.4 and 8.5. Verapamil depressed the magnitude of I_{Ca} under both conditions (Fig. 2a and b). $I_{Ca(peak)}$ was reduced by verapamil (1 μM) to 64 ± 4% of control ($n = 9$) at pH_o 7.4 and to 72 ± 3% of control ($n = 9$) at pH_o 8.5 (Fig. 2c). A rise of pH_o from 7.4 to 8.5 without drug increased $I_{Ca(peak)}$ to 112 ± 3% of control ($n = 9$). Fig. 2d shows the depression of $I_{Ca(peak)}$ by verapamil as difference (control − verapamil) at pH_o 7.4 and 8.5; $I_{Ca(peak)}$ was reduced by 36 ± 4% ($n = 9$) at pH_o 7.4 and 40 ± 6% ($n = 9$) at pH_o 8.5. A statistical analysis using Student's *t*-test for unpaired data revealed no significant difference between both sets of values ($P = 0.34$).

4. Discussion

L-type Ca^{2+} channels are the primary target of phenylalkylamines which inhibit Ca^{2+} channel function by binding to the channel protein (Catterall and Striessnig, 1992). It is commonly thought that the binding site for phenylalkylamines is located at an intracellular site of the Ca^{2+} channel molecule. This view mainly originated from the finding that the phenylalkylamine gallopamil suppressed cardiac action potential plateau both when applied extra- and intracellularly, whereas its permanently charged derivative was only effective when injected intracellularly (Hescheler et al., 1982). As phenylalkylamines are weak bases, it was suggested that they permeate the cell membrane in their uncharged form to exhibit their effect on the Ca^{2+} channel molecule from the inside. Another approach, to test for the side of action, has been achieved by changes in external pH resulting in different amounts of charged drug molecules. However, measurements of force of contraction, as influenced by phenylalkylamines at low or high pH, have yielded contradictory results. At pH 10, reversal of the inhibition by gallopamil in smooth muscle tone during washout of the drug was retarded (Triggle, 1980) supporting an intracellular pathway of action for the drugs. In contrast, under acidic conditions, the Ca^{2+}-antagonistic effects of verapamil were increased about 7 to 9-fold in cat papillary muscle (Briscoe and Smith, 1982) and guinea pig papillary muscle under conditions resembling ischaemia (Robertson and Lumley, 1989). In another study, both acidosis and alkalosis enhanced the efficiency of verapamil in rat hearts (Achike and Dai, 1991).

In the present study, the Ca^{2+} antagonistic action of verapamil was determined more directly by measuring I_{Ca} at pH 7.4 and 8.5. At pH 7.4, verapamil is supposed to exist in its uncharged form to about 4% according to the pK value of about 8.75 (Hasegawa et al., 1984). A rise in pH from 7.4 to 8.5 increases the fraction of uncharged molecules almost 10-fold, from about 4% to about 36%. If membrane permeation is required for the action of the drug, the efficiency in blocking I_{Ca} should be enhanced at pH 8.5. In a similar study, the efficiency of veratridine on Na^+ tail currents was enlarged at pH 8.3 (Honerjäger et al., 1992). However, in

our study, the efficiency of verapamil in blocking I_{Ca} was not different at pH 7.4 and 8.5. This is in line with a previous report which showed that an increase of extracellular pH failed to enhance the action of verapamil on I_{si}-mediated action potentials in guinea pig papillary muscle (Kohlhardt, 1983). Therefore, the results do not support the view that phenylalkylamines have to penetrate the cell membrane to exhibit their effect on I_{Ca}. It is suggested that phenylalkylamines may act from the outside on I_{Ca} by binding to an extracellular part of the channel molecule. This view is also favoured by the finding that several phenylalkylamines did not affect the magnitude of I_{Ca} when applied intracellularly (Wegener and Nawrath, 1995).

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