Short communication

Sodium Conductance in Calcium Channels of Single Smooth Muscle Cells of Guinea-Pig Taenia Caeci

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During the last few years, the Ca^{2+}-channels in isolated smooth muscle cells have been intensively studied (Walsh and Singer 1981; Bury et al. 1982; Klöckner and Isenberg 1985; Droogmans and Callewaert 1986). It was shown that under physiological conditions Ca^{2+}-channels are not measurably permeable for Na-ions (Klöckner and Isenberg 1985). On the other hand, inward currents carried by Na^+ through Ca^{2+}-channels have been detected in a variety of excitable tissues after removing external Ca^{2+} by calcium chelators, such as EDTA and EGTA (neurons: Kostyuk et al. (1983); cardiac muscle: Hess and Tsien (1984) and Matsuda (1986); smooth muscle: Isenberg and Klöckner (1985), Jmari et al. (1987), Katzka and Morad (1989). This procedure could be used to study some of the properties of Ca^{2+}-channels (Kostyuk et al. 1983).

In investigating Na^+-conductivity in single smooth muscle cells of guinea-pig taenia caeci, we obtained data concerning the inactivation of Ca^{2+}-channels and the role of Ca^{2+} in this process. Preliminary results of these studies have already been published (Bonev and Boev 1989).

Single smooth muscle cells were isolated from guinea-pig taenia caeci. Whole cell currents were studied by the patch-clamp technique (Hamill et al. 1981) using a List-electronic (EPC-7) patch-clamp amplifier. Patch pipettes with resistances ranging between 1.5—2 MΩ were made of Pyrex glass (Jencons H 15/10). The solutions used were of the following composition: (in mmol/l). (*A*) External solution: NaCl 135, KCl 4.74, KH_2PO_4 1.2, $CaCl_2$ 2.5; glucose 11.5. In Ca^{2+}-free solution $CaCl_2$ was absent and 1 mmol/l EGTA was added. The solution was buffered with Na.HEPES (5 mmol/l) to pH 7.4. (*B*) Internal (pipette) solution: (in mmol/l) CsCl 110, $CaCl_2$ 1, EGTA 11, $MgCl_2$ 2, N-methyl--α-glucamine-40, Na-pyruvate-5, Na_2-succinate — 3, oxalacetic acid — 5, Na_2-ATP — 2 and cyclic AMP — 5×10^{-6} mol/l. The solution was buffered with

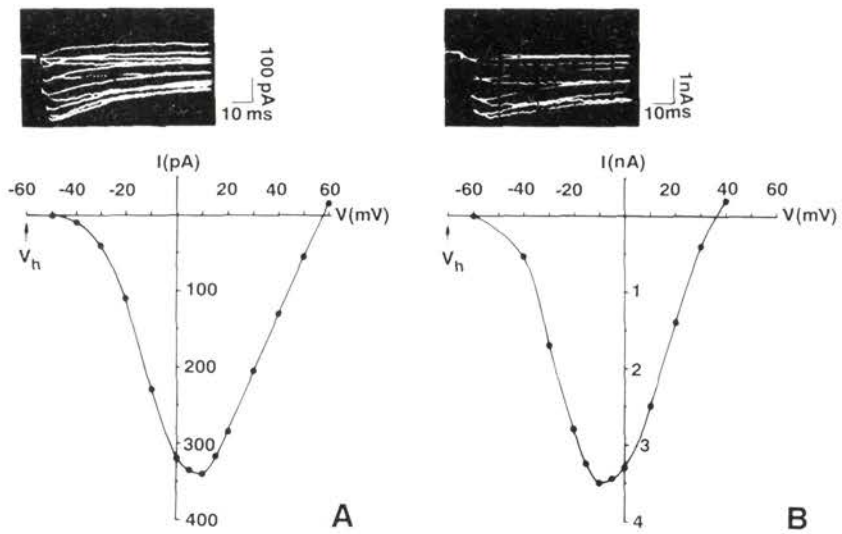

Fig. 1. Membrane currents evoked by 100 ms depolarization steps (*top*), and the dependences of the peak currents on membrane potential (*bottom*) in the same cell. (*A*) Ca^{2+}-containing solution from holding potential $V_h = -60$ mV and potential steps -40, -30, -20, -10, 0, 10, 20, 30, 40, 50, 60 mV. (*B*) Ca^{2+}-free solution, containing 1 mmol/l EGTA from $V_h = -70$ mV and potential steps -50, -40, -30, -20, -10, 0, 10, 20, 30, 40 mV.

HEPES (10 mmol/l) to pH 7.2. All experiments were carried out at room temperature (22—25 °C).

Depolarizing clamp steps evoked Ca^{2+}-inward current (I_{Ca}) (Fig. 1*A*, upper part). Activation of I_{Ca} occurred at a membrane potential of about -40 mV. I_{Ca} increased to a maximal value at $+10$ mV and then decreased for more positive voltage steps (Fig. 1*A*, lower part). The mean maximal amplitude of I_{Ca} was 379 ∓ 35 pA per cell ($n = 24$). The data obtained are in good agreement with the results reported previously for single smooth muscle cells of guinea-pig taenia caeci (Ganitkevich et al. 1985, 1986, 1988; Yamamoto et al. 1989). I_{Ca} was blocked by calcium antagonist nifedipine (10^{-7} mol/l) and remained unchanged after the replacement of the external Na^+ by N-methyl-α-glucamine. This indicated that Ca^{2+}-channels were not permeable for Na^+ if Ca-ions were present in external solution.

In Ca^{2+}-free solution, containing 1 mmol/l EGTA, a Na^+-dependent inward current appeared (I_{Ca}^{Na}) (Fig. 1*B*, upper part). As seen from the current--voltage relationship (Fig. 1*B*, lower part) the maximal amplitude of the current

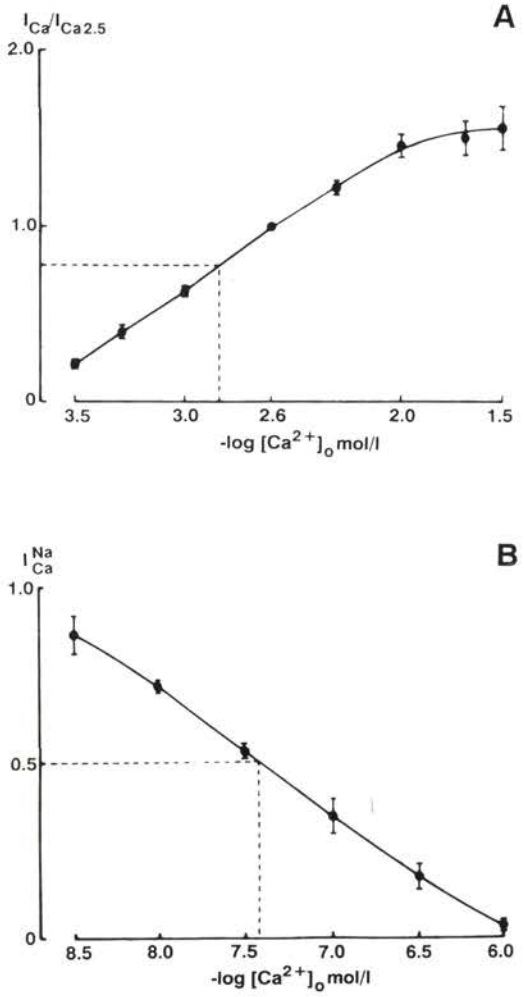

Fig. 2. Dependence of peak I_{Ca} (*A*) and I^{Na}_{Ca} (*B*) on the external Ca^{2+}-concentration. The peak currents obtained in 2.5 mmol/l external Ca^{2+} (I_{Ca}) and in Ca^{2+}--free, EGTA (1 mmol/l) containing solution (I^{Na}_{Ca}) were taken for unity. The clamp potential was chosen to evoke the largest inward current for each single external Ca^{2+} concentration. The vertical bars represent ±SEM (6 cells each).

was about 10-fold greater than that of I_{Ca} of the same cell; the mean maximal amplitude of I^{Na}_{Ca} was 6.19 ∓ 0.66 nA ($n = 24$). This current decreased with the decreasing extracellular Na^+, and disappeared at low Na^+-concentrations close to that in the pipette solution. The reversal potential was about 35 to 40 mV and

was near to reversal of pure Na^+-current according to the Nernst equation by 15 mmol/l Na^+ in the pipette solution. The inward current induced in Ca^{2+}-free, Mg^{2+}-free EGTA-containing solution was tetrodotoxin-resistant and was blocked by nifedipine (3×10^{-8} mol/l). The Ca^{2+}-agonist BAY-k 8644 (10^{-6} mol/l) increased both I_{Ca} and I_{Ca}^{Na}. These observations indicate that in Ca^{2+}-free, EGTA-containing solution Na^+-conductance is likely due to Na^+-entry through Ca^{2+}-channels in the smooth muscle cell membrane of guinea-pig taenia caeci. As seen from Fig. 1*A* and 1*B*, the voltage dependence of peak I_{Ca}^{Na} was shifted by about -20 mV compared to the peak I_{Ca} of the same cell. A similar shift may be explained by a change of the surface charge of cell membrane caused by removal of divalent ions from the external solution.

It is known that both the Ca^{2+}- and the Na^+-currents depend on the extracellular Ca^{2+}-concentration (Kostyuk et al. 1983; Hess and Tsien 1984; Matsuda 1986; Jmari et al. 1987). Fig. 2*A* shows the dependence of the peak amplitude of I_{Ca} on the Ca^{2+}-concentration in the external solution. I_{Ca} increased almost proportionally to the Ca^{2+}-concentration in the range 3×10^{-4} —10^{-2} mol/l; saturation occurred at higher concentrations. This dependence fits well to a Langmuir curve with a dissociation constant for Ca^{2+} (K_{dCa}) of 1.5×10^{-3} mol/l. Unlike I_{Ca}, I_{Ca}^{Na} was suppressed by addition of Ca^{2+} into the external solution, and decreased almost linearly if the Ca^{2+}-concentration was raised from 3×10^{-9} to 10^{-6} mol/l (up to 10^{-4} mol/l), until undetectable. K_{dCa} estimated under these conditions was 4×10^{-8} mol/l, i.e. approx. 5 orders smaller than K_{dCa} for Ca-ions being the charge carrier through Ca^{2+}-channels. Two different values of dissociation constants for Ca^{2+} have been reported previously for neuronal membranes (Kostyuk et al. 1983), cardiomyocytes (Hess and Tsien 1984) and uterine multicellular preparations (Jmari et al. 1987). This can be explained by the model proposed by Hess and Tsien (1984), which has postulated the existence of two Ca^{2+}-binding sites within the channel, controlling ionic selectivity and movement through Ca^{2+}-channels in cardiomyocyte membrane.

The value of K_{dCa} estimated in our experiments with Na^+ as the charge carrier (4×10^{-8} mol/l) is comparable to that reported for the uterine multicellular preparation (10^{-7} mol/l) (Jmari et al. 1987). However, it is approx. an order smaller than those reported for neurons (2×10^{-7} mol/l; Kostyuk et al. 1983), and single smooth muscle cells isolated from the urinary bladder (6×10^{-7} mol/l; Isenberg and Klöckner 1985). The value is approx. two orders smaller than those reported for cardiomyocytes (1.3×10^{-6} mol/l and 1.2×10^{-6} mol/l; Hess and Tsien 1984; Matsuda 1986). Ca^{2+}-channels in the smooth muscle cell membranes of taenia caeci seem to have higher affinity for Ca^{2+} than do the channels in other excitable cells.

The voltage dependences of I_{Ca} and I_{Ca}^{Na} inactivation were investigated using

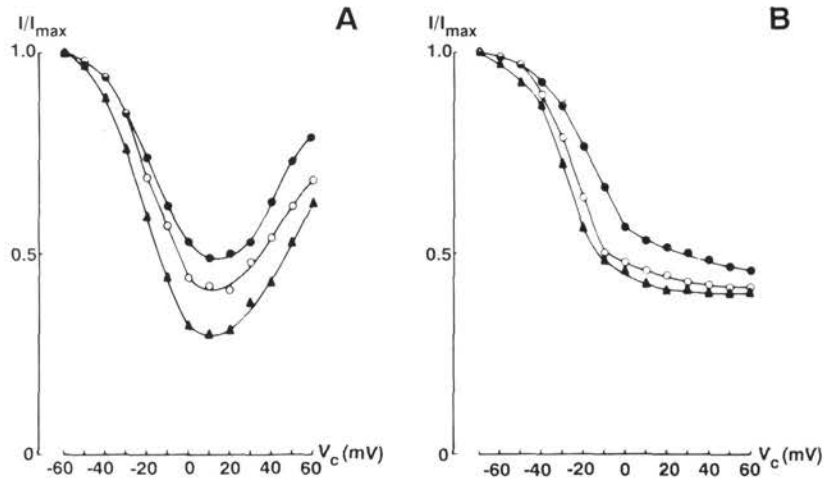

Fig. 3. Voltage dependences of I_{Ca} (*A*) and I_{Ca}^{Na} (*B*) inactivation in the same cell, obtained by a two-pulse protocol. V_c — condition potential with durations: 100 ms (●) 200 ms (○) and 500 ms (▲). Duration of the second pulse — 100 ms.

a two-pulse voltage-clamp protocol. The inactivation induced by the first depolarization pulse was measured as the reduction of the peak current induced by the second depolarizing test pulse. The test pulse was chosen to elicit maximal current. Fig. 3*A* illustrates the voltage dependence of I_{Ca} inactivation. Inactivation appeared at the lowest levels of conditioning depolarization, which could not evoke I_{Ca}. The latter developed simultaneously with the increasing prepulse amplitude, reached a maximal value at the peak potential of I_{Ca}, and then decreased (Ganitkevich et al. 1986, 1987). The U-shaped relationship indicates that both the voltage and the Ca^{2+}-mediated mechanism are involved in I_{Ca} inactivation. I_{Ca}^{Na} inactivation was enhanced upon increasing the prepulse amplitude; on contrast to I_{Ca}, however, it did not decrease at higher potentials of conditioning depolarization (Fig. 3*B*). I_{Ca} and I_{Ca}^{Na} differed also in their respective dependences of inactivation on prepulse duration. I_{Ca} inactivation was enhanced in direct proportion to prepulse duration (Fig. 3*A*). Unlike I_{Ca}, I_{Ca}^{Na} inactivation was not significantly changed at prepulse duration exceeding 100 ms (Fig. 3*B*). All these findings allow the suggestion that Na-ions do not alter the inactivation of Ca^{2+}-channels through which they flow, i.e. Na^+ cannot substitute Ca^{2+} in inactivation processes.

In conclusion, in a Ca^{2+}-free, Mg^{2+}-free, EGTA-containing solution Na-ions can pass through Ca-channels in smooth muscle cells of guinea-pig taenia caeci; the Na^+-current inactivation is determined only by a voltage-dependent mechanism.

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