Inactivation of Calcium Channels in Single Vascular and Visceral Smooth Muscle Cells of the Guinea-Pig

V. Ya. GANITKEVICH, M. F. SHUBA and S. V. SMIRNOV

Department of Neuromuscular Physiology, A. A. Bogomoletz Institute of Physiology, Academy of Sciences of the Ukrainian S.S.R., Bogomoletz str. 4, Kiev-24, 252601, USSR

Abstract. Inactivation of currents carried through calcium channels by calcium (I_{Ca}), barium (I_{Ba}) and monovalent cations ($I_{n.s.}$) was studied in single smooth muscle cell (SMC) of the guinea-pig coronary artery and taenia caeci by the whole-cell patch-clamp method. The rate of I_{Ca} inactivation in the coronary artery SMC was correlated with I_{Ca} amplitude, and acceleration was observed with the increasing I_{Ca} peak amplitude. The availability curve of I_{Ca} in double-pulse experiments was found to be U-shaped, however, no complete restoration of I_{Ca} availability was observed. Inactivation of I_{Ba} was considerably slowlier than that of I_{Ca}. These findings may indicate that inactivation of calcium channels in the membrane of coronary artery SMC is, at least partially, a Ca-dependent process. However, some facts observed contradict the validity of this hypothesis for coronary artery SMC in contrast to taenia caeci: 1) elevation of external Ca^{2+} concentration did not affect the time course of I_{Ca} inactivation; 2) inactivation of $I_{n.s.}$, i.e. without calcium entry into the cell, was faster than that of I_{Ca}. It was concluded that the characteristics of Ca channel inactivation were changed by the removal of divalent cations from extracellular solution. Differences and similarities in Ca channel inactivation between coronary artery and taenia caeci SMC are discussed.

Key words: Calcium channels — Smooth muscle — Coronary artery

Introduction

Inactivation of Ca channels in smooth muscle cells (SMC), as well as in other tissues, seems to be a complex phenomenon affected by both the calcium entry and the membrane potential (Ganitkevich et al. 1986, 1987; Jmari et al. 1986; Amedee et al. 1987; Ohya et al. 1986, 1988). With barium ions as the current carrier, Ca channel inactivation has been found to be slowlier than that of calcium current (Ohya et al. 1986; Jmari et al. 1986; Ganitkevich et al. 1987). It has been suggested that I_{Ba} is inactivated in a purely voltage-dependent manner (Jmari et al. 1986).

Complete removal of divalent cations from extracellular solution allows monovalent cations to carry the currents through Ca channels in many tissues (Kostyuk and Krishtal 1977; Hadley and Hume 1987; Almers and McCleskey 1984; Hess and Tsien 1984) including smooth muscles (Isenberg and Klöckner 1985; Ohya et al. 1986; Jmari et al. 1987).

However, changes in the extracellular concentration of divalent cations are known to affect the surface membrane potential in SMC (Ganitkevich et al. 1988). So, it may be expected that upon introducing the appropriate corrections for the surface potential change, similar inactivation will be observed for I_{Ba} and $I_{n.s.}$ if both processes represent the operation of the potential-sensitive Ca channel inactivation gate. The present work was performed in order to test the above possibility.

Materials and Methods

Experiments were carried out on single SMC freshly dissociated from the taenia caeci and the coronary artery of the guinea-pig.

Preparation of isolated cells

The procedure used for the taenia caeci has been described earlier (Ganitkevich et al. 1986; 1987). For the preparation of isolated cells from the circumflex coronary artery (length approx. 5 mm), the vessel was carefully removed from the heart of the guinea-pig (the animals weighing 500 to 1000 g) and placed in a Ca^{2+}-free solution for 1 hour. Thereafter, it was transferred to 1 ml of Ca^{2+}-free solution which contained 0.1 % collagenase (Serva) and 1 % elastase (Reanal), and incubated during 30 min at 35 °C. Then the solution was gently pipetted for about 1 min and the artery, still as a tube, was placed in 1 ml of a fresh enzyme solution heated to 35 °C; the fresh solution was also Ca^{2+}-free and contained additionally 0.1 % soyabean trypsin inhibitor (Reanal). The vessel was incubated for additional 25—50 min at 35 °C. After the incubation the artery was transferred to 0.5 ml of Ca^{2+}- and enzyme-free solution and the solution was pipetted until the appearance of isolated cells. The cell suspension thus obtained was placed into Petri dishes and diluted with potassium-free external solution.

Solutions

The Ca^{2+}-free solution used for cell preparation was of the following composition (in mmol/l): NaCl 130; KCl 5; $MgCl_2$ 5; HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid, Sigma) 10; glucose 6; pH was adjusted to 7.2 with NaOH.

To suppress K^+ conductance, K^+-free external and internal solution were used. The composition of the external solution (in mmol/l) was: NaCl 110; CsCl 20; $CaCl_2$ 2.5; HEPES 10; TEACL 5; glucose 6; pH was adjusted to 7.2 with NaOH. The intracellular solution of the following composition was used (in mmol/l): CsCl 130; $MgCl_2$ 2.25; HEPES 5; Na_2ATP 5; cAMP 0.1; EGTA (ethyleneglycol-*bis*-β-amino-ethylether-N,N'-tetraacetic acid, Sigma) 0.42; 5 mmol/l TEA^+ ions (as hydroxide) was added to the solution and the pH was adjusted to 7.2 with NaOH. The intracellular

solution with elevated EGTA concentration contained 10 mmol/l of EGTA instead of 0.42 mmol/l.

Extracellular solutions containing Ba^{2+} or EGTA were prepared by addition of the appropriate amounts of $BaCl_2$ or EGTA (as acid) to Ca^{2+}-free external solution.

Recording of membrane currents and the external solution change

Membrane currents were recorded as described previously (Ganitkevich et al. 1986, 1987). A 500 MΩ feed-back resistor (Victoreen, Florida) was used in current-voltage convertor. All currents were recorded at a bandwith of 2.5 kHz. Experiments were performed at room temperature.

If not indicated otherwise, holding potential was −60 mV (in Ca^{2+} and Ba^{2+} containing external solutions) and −80 (in EGTa- containing external solutions).

The inactivation of Ca channel currents (in the text indicated as the fraction of noninactivated current) was evaluated as the ratio of current amplitude at the end of a depolarizing pulse to the peak current at the same membrane potential. To evaluate shifts of current-voltage (I V) relationships along the voltage axis due to a change of the surface membrane potential upon changing the external concentration of divalent cations, the parameter $V_{1/2}$ (the potential on the descending branch of the I V curve at which the current through Ca channels was half the peak one) was used (for details see Ganitkevich et al. 1988).

All values are given as mean ± s.d. with the number of cells tested in parentheses.

Results

Inactivation of coronary artery I_{Ca} at different membrane potentials

Fig. 1 shows a family of calcium currents elicited by step depolarization of the cell membrane. Upon stepwise increasing the depolarization form −30 mV to 20 mV, I_{Ca} peak amplitude increased and its decay was accelerated as can be seen from a decrease of the I_{Ca} decay half-time (arrows in Fig. 1). Further stepwise increase of depolarization resulted in a decrease of I_{Ca} peak amplitude and slowing down of its decay. It should be noted that though I_{Ca} decay was slowed down at positive potentials, it inactivated considerably faster than I_{Ca} of comparable amplitudes at negative potentials (compare I_{Ca} at −20 mV and at 60 mV, or I_{Ca} at −10 mV and at 50 mV, Fig. 1). Thus, a maximal speed of I_{Ca} inactivation was observed at potentials corresponding to a peak of I_{Ca}, i.e. from 10 mV to 20 mV.

In most cells studied a "flat" current was observed at 80 or 90 mV. We believe that this is the indication of the purity of I_{Ca}, since a time-dependent outward current which clearly disturbed the time course of I_{Ca} decay was observed in some cells. This current could be visualized after addition of Co^{2+} ions (5 mmol/l) to block I_{Ca}. So, in most cells the time course of I_{Ca} decay presumably resembled the time course of Ca conductance inactivation.

Fig. 2*A* shows the availability curve of I_{Ca} obtained with the two-pulse

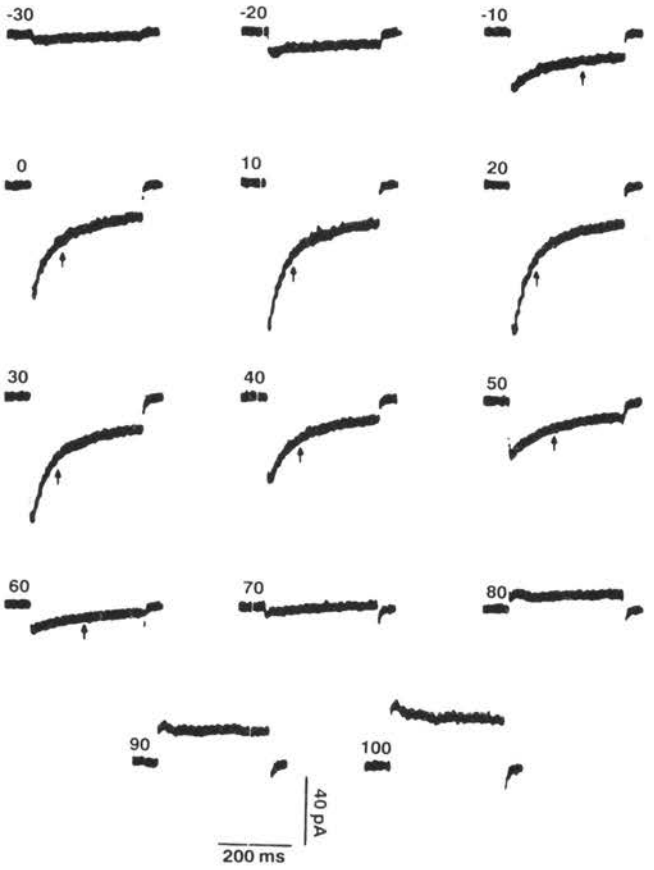

Fig. 1. Calcium currents in coronary artery SMC. Membrane potentials are indicated near each current trace in mV. The half-amplitudes of each I_{Ca} are indicated by the arrows.

protocol. As can be seen, I_{Ca} availability started decreasing at potentials close to I_{Ca} activation and was minimal at potentials corresponding to maximal inward I_{Ca}. Further increase of conditioning depolarization raised the I_{Ca} availability; however, it failed to restore completely although at 10 mV calcium entry into the cell seems negligible (Fig. 2).

Also, it should be noted that in agreement with the results shown in Fig. 1, inactivation induced by conditioning depolarization to -20 mV was much less than that induced by prepulse to 60 mV (Fig. 2*A*), although I_{Ca} at these potentials were of comparable amplitude (Fig. 2*B*). In general, in all 10 cells studied with the two-pulse protocol the availability curve was asymmetrical, i.e. its

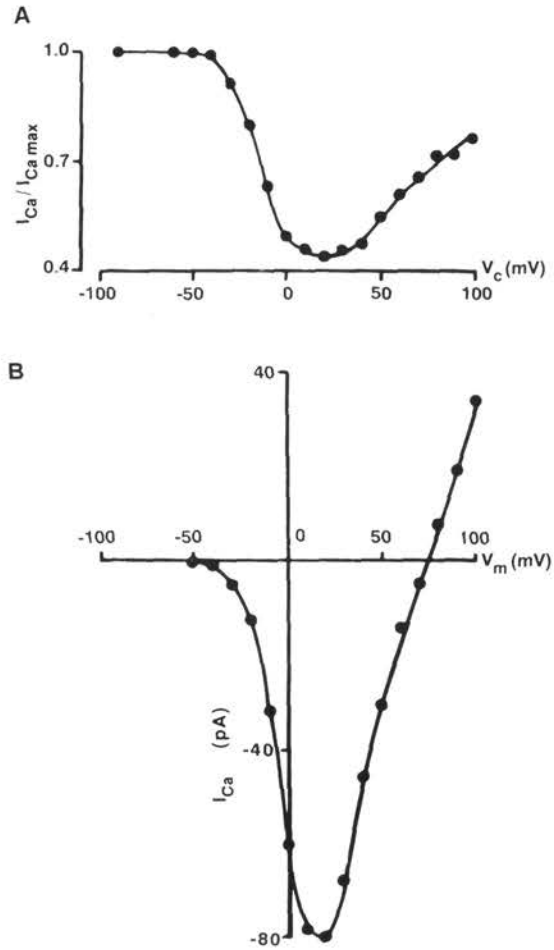

Fig. 2. Coronary artery. *A*: Availability of I_{Ca} defined using the two-pulse protocol. $I_{Ca}/I_{Ca_{max}}$, ratio of peak I_{Ca} amplitudes at test potential to 20 mV with and without conditioning prepulse (V_c). Prepulse duration 300 ms, interpulse interval 50 ms. *B*: I—V relationship of I_{Ca} peak. V_m, membrane potential. The same cell as in *A*.

descending branch was steeper than the ascending one, and the restoration of I_{Ca} availability was not complete.

It should be noted that in two-pulse experiments I_{Ca} elicited by test pulse may be affected by I_{Ca} tail current flowing during the interpulse interval. This may result in an underestimation of the true availability of I_{Ca}, especially at large positive potentials. However, tail current effect cannot be the reason for the

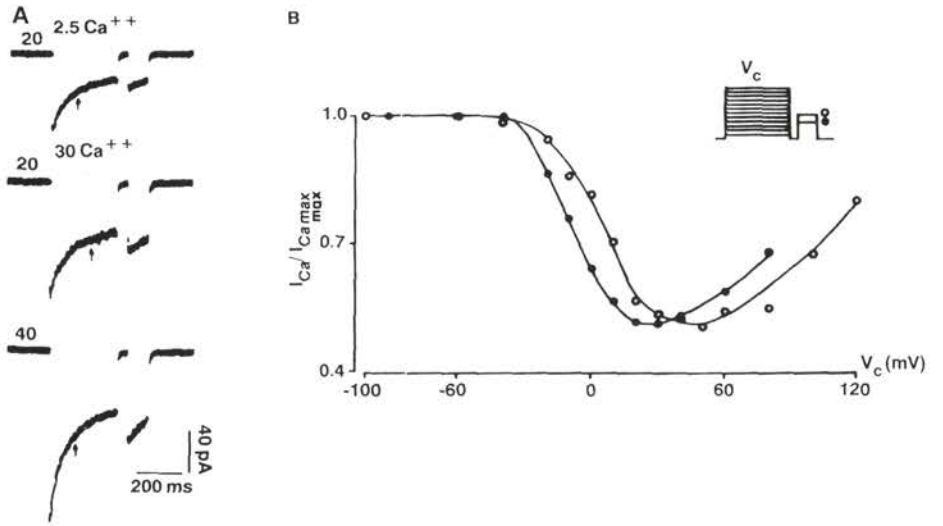

Fig. 3. Effect of elevated $[Ca^{2+}]_o$ on I_{Ca} in coronary artery SMC. *A*: I_{Ca} during prepulse and testpulse depolarizations to the potentials indicated (mV) in 2.5 and 30 mmol/l $[Ca^{2+}]_o$. The half-amplitudes of each I_{Ca} are indicated by the arrows. *B*: Availability of I_{Ca} in 2.5 mmol/l (filled circles) and 30 mmol/l (open circles) $[Ca^{2+}]_o$ defined using the two-pulse protocol (inset). $I_{Ca}/I_{Ca_{max}}$ was defined as described in the legend to Fig. 2. Test potentials were to 20 mV in 2.5 mmol/l and to 40 mV in 30 mmol/l $[Ca^{2+}]_o$. Prepulse duration was 300 ms, interpulse interval 50 ms. V_c, conditioning potential.

incomplete restoration of I_{Ca} availability at positive potentials, since at 90 mV I_{Ca} inactivation was enhanced with the increasing prepulse duration (Fig. 10*A*) whereas the tail current presumably, cannot be enhanced by prepulse prolongation. So, at large positive potentials I_{Ca} inactivation developed in a manner apparently independent of calcium entry into the cell.

Coronary artery I_{Ca} inactivation in the presence of elevated $[Ca^{2+}]_o$.

When the external calcium concentration ($[Ca^{2+}]_o$) was increased from 2.5 to 30 mmol/l the I—V relationship for I_{Ca} was shifted by about 20 mV toward positive potentials ($V_{1/2}$ for I_{Ca} was -5.5 ± 0.6 mV in 2.5 mmol/l $[Ca^{2+}]_o$ ($n = 39$) and 13.9 ± 1.0 mV in 30 mmol/l ($n = 9$)). The peak I_{Ca} amplitude increased by a factor 1.75 ± 0.08 ($n = 11$) when $[Ca^{2+}]_o$ was raised from 2.5 to 30 mmol/l.

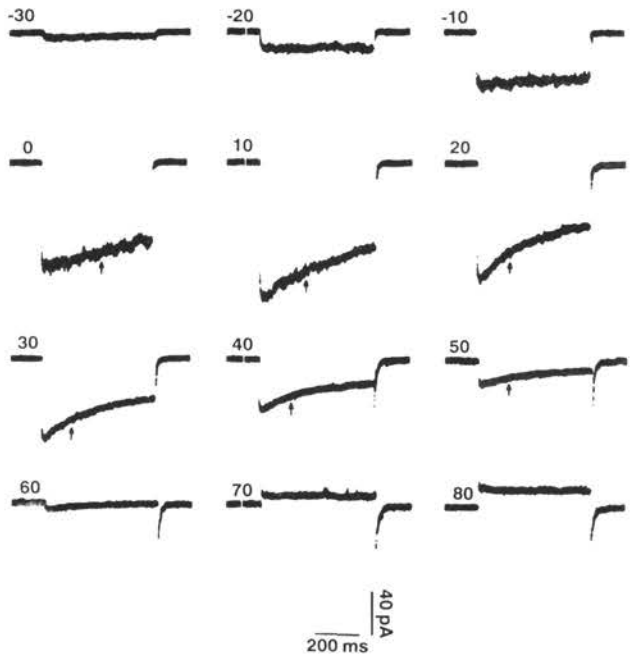

Fig. 4. Barium currents in coronary artery SMC. Membrane potentials are indicated near each current trace in mV. Pulse duration 500 ms. The half-time of each current change is indicated by the arrows.

I_{Ca} current traces obtained with the two-pulse protocol in the presence of 2.5 mmol/l and in 30 mmol/l $[Ca^{2+}]_o$ are shown in Fig. 3*A*. Peak I_{Ca} amplitudes were reached at 20 mV and 40 mV respectively. A comparison of the currents at 20 mV shows that I_{Ca} decay at 30 mmol/l $[Ca^{2+}]_o$ was slowlier than that at 2.5 mmol/l despite a larger amplitude of the former. Moreover, I_{Ca} half-time of decay at 20 mV and 2.5 mmol/l $[Ca^{2+}]_o$, and at 40 mV and 30 mmol/l $[Ca^{2+}]_o$ were similar (Fig. 3*A*). So, when accounting for the change in surface membrane potential, $[Ca^{2+}]_o$ elevation and increase I_{Ca} amplitude had no effects on the time course of I_{Ca} inactivation. These results may suggest that the membrane potential affected the development of I_{Ca} inactivation even in the potential range where inactivation is expected to be mediated to a great extent by the calcium entry into the cell (Eckert and Chad 1984).

This is also confirmed by comparing the availability curves of I_{Ca} in the presence of 2.5 and 30 mmol/l of $[Ca^{2+}]_o$ (Fig. 3). The only significant effect of $[Ca^{2+}]_o$ elevation was a shift of the availability curve toward positive potentials in agreement with the shift of the I—V relationship for I_{Ca}. The minimum of I_{Ca}

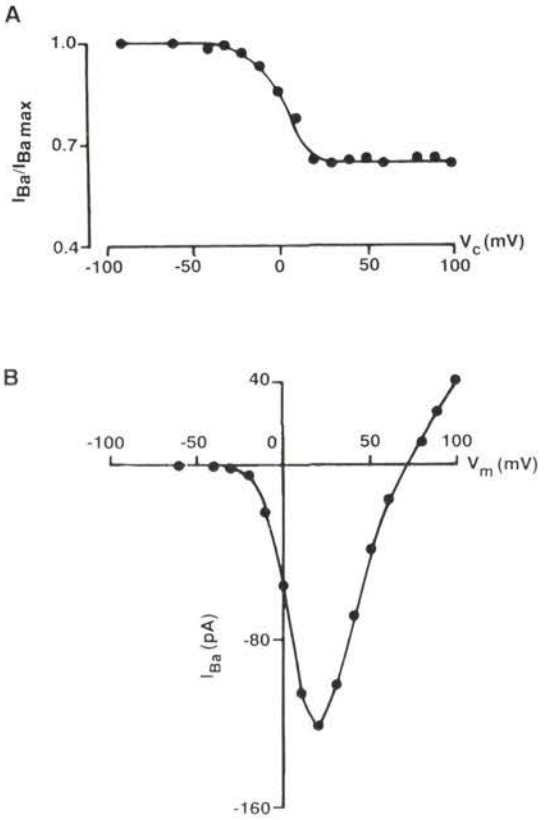

Fig. 5. Coronary artery SMC. *A*: Availability of I_{Ba} defined in two-pulse experiments. I_{Ba}, $I_{Ba_{max}}$, see legend to Fig. 2. Test potential was to 20 mV. Conditioning pulse duration 300 ms, interpulse interval 50 ms. V_c, conditioning potential. *B*: I V relationship of I_{Ba} peak from the same cell as in *A*. V_m, membrane potential.

availability was not decreased at 30 mmol/l $[Ca^{2+}]_o$ in comparison with that at 2.5 mmol/l $[Ca^{2+}]_o$. Also, even if the minimum of I_{Ca} availability represented some "saturation" level, the descending branch of the availability curve would be expected to be steeper at 30 mmol/l $[Ca^{2+}]_o$, if inactivation were controlled by calcium entry which was enhanced under these conditions. Fig. 3*B* shows that this was not the case.

Inactivation of coronary artery I_{Ba}

In these experiments external solution containing 10 mmol/l Ba^{2+} was used for the following reason. At this $[Ba^{2+}]_o$ the shift of I—V relationship for I_{Ba} was small in respect to that of I_{Ca} at 2.5 mmol/l $[Ca^{2+}]_o$: $V_{1/2}$ for I_{Ba} at 10 mmol/l $[Ba^{2+}]_o$ was -5.2 ± 2.2 mV ($n = 8$) in comparison with -5.5 for I_{Ca} (see above). Therefore, I_{Ca} and I_{Ba} were compared at the same potential.

When 2.5 mmol/l $(Ca^{2+}]_o$ was replaced by 10 mmol/l $[Ba^{2+}]_o$ the peak amplitude of I_{Ba} was higher by a factor 2.4 ± 0.1 ($n = 13$) than that of I_{Ca}, whereas its inactivation was slowlier than that of I_{Ca} (see Fig. 9*A*). The average fractions of noninactivated current at the end of the 300 ms depolarizing step to 20 mV were 0.34 ± 0.02 ($n = 18$) for I_{Ca} and 0.55 ± 0.04 ($n = 7$) for I_{Ba}.

I_{Ba} current traces recorded at different levels of step membrane depolarization are shown in Fig. 4. Again, the "flat" current appears at 80 mV, suggested to the evidence the purity of I_{Ba}. To characterize the rate of I_{Ba} decay the half-time of current change during the depolarizing pulse was used since the decay of I_{Ba} was considerably slowlier than that of I_{Ca}.

At -30 mV or -20 mV the inactivation of I_{Ba} was very slow (Fig. 4). Upon increasing depolarization the inactivation of I_{Ba} was accelerated, and within the potential range between 20 mV and 50 mV its rate was apparently independent of the membrane potential (Fig. 4).

The I_{Ba} availability curve was "saturated" in the potential range between 20 mV and 100 mV (Fig. 5). The inactivation of I_{Ba} was enhanced by prepulse prolongation, but within this potential range its rate was apparently independent of the membrane voltage (see Fig. 10*B*). This rules out the possibility that "saturation" of I_{Ca} availability was due to some noninactivating component of I_{Ba}. Similar curves were obtained for I_{Ba} in SMC myometrium (Jmari et al. 1986) and heart cells (Tsien and Marban 1982).

A comparison of I_{Ca} and I_{Ba} availabilities at 80 mV in the same cell shows that the values were similar. This presumably indicates that the availability curves of I_{Ca} and I_{Ba} meet at large positive potentials, as it was reported for heart cells (Tsien and Marban 1982), and supports the assumption that I_{Ba} inactivation is a potential-dependent process.

So, considering all above facts, one may expect that the inactivation of current carried through Ca channels by monovalent cations ($I_{n.s.}$) is similar to the inactivation of I_{Ba}, if the identity of the permeable cation does not affect the potential-dependent inactivation gate of Ca channels. To test this point we performed experiments in divalent-cation-free external solution.

Inactivation of coronary artery $I_{n.s.}$

When the nominally Ca^{2+}-free solution, containing additionally 2 to 5 mmol/l

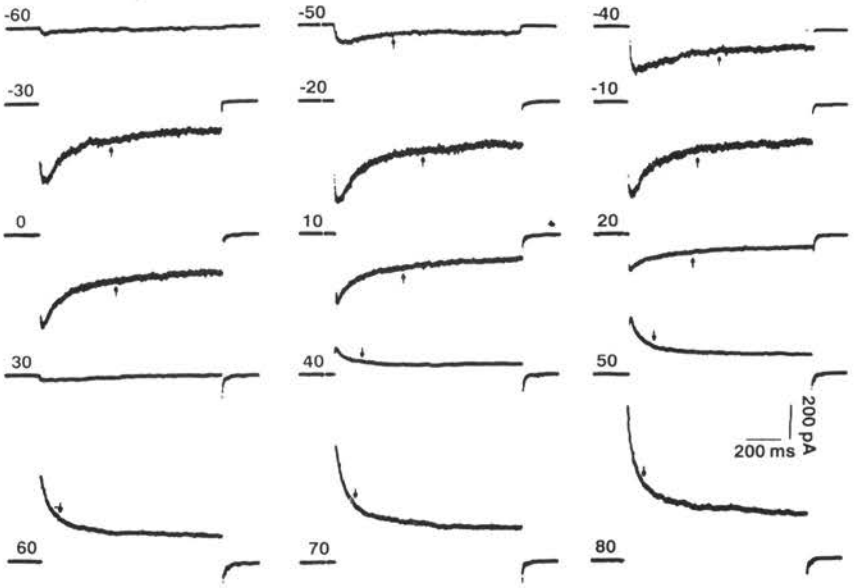

Fig. 6. Coronary artery SMC. $I_{n.s.}$ during potential steps to the potentials indicated (mV). Pulse duration 1 s. The half-amplitudes of each $I_{n.s.}$ are indicated by the arrows.

EGTA, was applied to coronary artery cell step depolarization from the holding potential −80 mV to different levels of membrane potential resulted in the appearence of $I_{n.s.}$ (Fig. 6). We assumed that this current was carried through Ca channels by monovalent cations since it was greatly suppressed by addition of nitrendipine (5 μmol/l) or Co^{2+} ions (2 mmol/l, but without removal of EGTA).

The peak of inward $I_{n.s.}$ was greater by a factor 10.4 ± 0.8 (n = 18) than that of I_{Ca} at 2.5 mmol/l $[Ca^{2+}]_o$ ions and occurred at potentials between −20 mV and −10 mV. $I_{n.s.}$ was activated in the potential range between −50 mV and −60 mV, i.e. at potentials approx. 30 mV more negative than those at which I_{Ca} is activated ($[Ca^{2+}]_o$ = 2.5 mmol/l). The value of $V_{1/2}$ for $I_{n.s.}$ was −34 ± 1.3 mV (n = 12). Upon increasing depolarization $I_{n.s.}$ reversed and an outward current was recorded. The reversal potential of $I_{n.s.}$ was 28.9 ± 1.3 mV (n = 14). This may indicate that in divalent cations-free solution the selectivity of Ca channels to monovalent cations is moderate since the reversal potential of $I_{n.s.}$ differed significantly from the equilibrium potentials of the cations used (Na^+ and Cs^+). Assuming an effective perfusion of cells with Na^+ and Cs^+ ions, and Ca channel permeability only to these ions, the ratio of permeabilities for Na^+ and Cs^+ can be calculated from the value of $I_{n.s.}$ reversal potential and the concentrations of

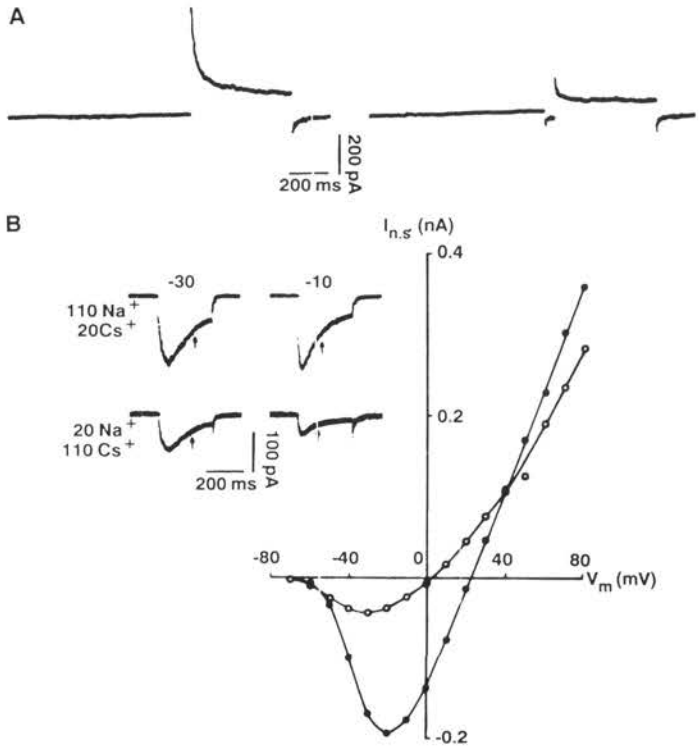

Fig. 7. *A*: $I_{n.s.}$ recorded with (*right trace*) and without (*left trace*) conditioning prepulse to 35 mV. Test-pulse potential to 80 mV. *B*: I—V relationship of $I_{n.s.}$ in 110 mmol/l Na^+ nad 20 mmol l Cs^+ (filled circles) and in 20 mmol/l Na^+ and 110 mmol/l Cs^+ (open circles) in the external solution. Pulse duration 300 ms. Current traces of $I_{n.s.}$ at -30 mV and at -10 mV in each solution are shown (*left medium and bottom*). Note the different current calibrations (200 pA *upper trace* and 100 pA *medium* and *lower trace*). The half amplitudes of each $I_{n.s.}$ are indicated by the arrows. External solution in *B* contained 3 mmol/l EGTA. *A* and *B* from two different cells of a coronary artery.

Na^+ and Cs^+ ions in the extra- and the intracellular solution. With the above assumptions a ratio $P_{Na}:P_{Cs} = 1:0.2$ was obtained. This is similar to reports by other authors (Kostyuk et al. 1983; Almers and McCleskey 1984; Hess et al. 1986).

Fig. 6 shows $I_{n.s.}$ current traces at different levels of step membrane depolarization of 1 s duration. It can be seen from the figure that after reaching its peak $I_{n.s.}$ decayed. The inactivation of $I_{n.s.}$ in coronary artery SMC was obvious at all potentials tested (from -70 mV to 100 mV). However, it was not complete at the end of the 1 s depolarizing pulses to any potentials. The rate of

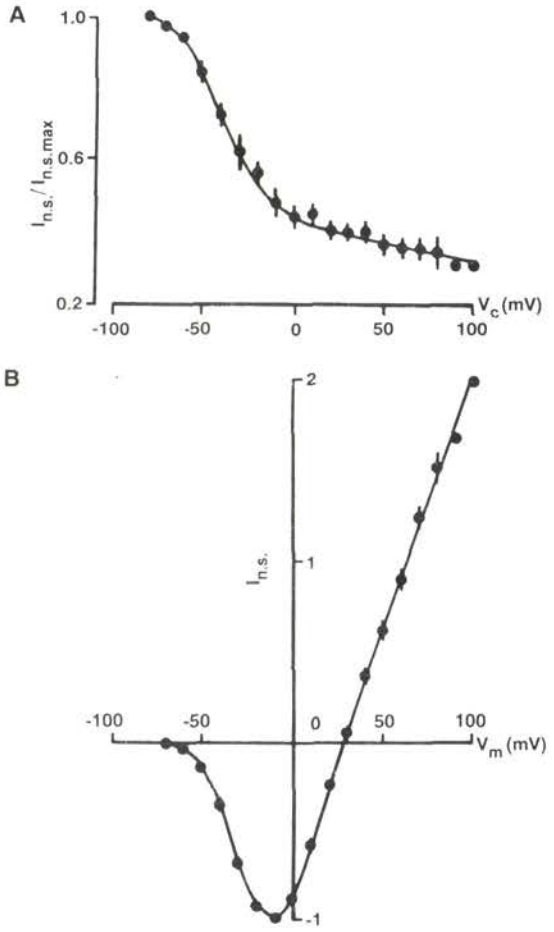

Fig. 8. *A*: availability of $I_{n.s.}$ defined using the two-pulse protocol. $I_{n.s.}/I_{n.s.max}$, see legend to Fig. 2. Test-pulse potential to −10 mV. Conditioning pulse duration 500 ms, interpulse interval 50 ms. *B*: normalized I—V relationship of $I_{n.s.}$. The values in *A* and *B* represent mean ± S.D. for 9 coronary artery cells, except for points at 90 and 100 mV which were obtained from one cell each. V_c and V_m, conditioning and membrane potential.

inactivation, characterized by the half-time of $I_{n.s.}$ decay, showed a weak dependence on voltage in the membrane potential range between −50 mV and 20 mV (Fig. 6). However, after $I_{n.s.}$ reversal its decay was markedly accelerated. For outward $I_{n.s.}$ the rate of current decay also showed a weak dependence on the membrane voltage.

In order to test whether the $I_{n.s.}$ inactivation was due to accumulation-depletion phenomena the following experiments were performed. The concentration of external Na^+ ions was lowered from 110 mmol/l to 20 mmol/l whereas the concentration of external Cs^+ ions was elevated from 20 to 110 mmol/l. Under these conditions the reversal potential of $I_{n.s.}$ decreased to 3.5 ± 1.5 mV ($n = 2$). The $I_{n.s.}$ amplitude was markedly decreased at -30 mV or -10 mV (Fig. 7*B*) whereas its time course was not slowed down as might be expected if $I_{n.s.}$ inactivation were current dependent.

Another evidence against current dependence of $I_{n.s.}$ inactivation is shown in Fig. 7*A*. A prepulse to 35 mV (close to the reversal potential of $I_{n.s.}$ in this cell) produced a significant inactivation of the test current whereas current flowing during the prepulse was negligible.

The possibility that the time course of $I_{n.s.}$ inactivation was considerably disturbed by other conductances can be excluded since the values of reversal potentials for $I_{n.s.}$ peak and for $I_{n.s.}$ at the end of the depolarizing pulse were very close (Fig. 6, depolarization to 30 mV; Fig. 7*A*, depolarization to 35 mV).

Inactivation of $I_{n.s.}$ was studied with the two-pulse protocol. Fig. 8*A* shows the availability curve obtained from 9 cells. Also, the normalized I—V relationship of $I_{n.s.}$ is shown (Fig. 8*B*). The availability curve of $I_{n.s.}$ considerably differs from those I_{Ca} and I_{Ba} (Figs. 2 and 5). Upon increasing the conditioning depolarization up to 100 mV the $I_{n.s.}$ availability monotonically decreased. This finding is in agreement with the results of other studies in which $I_{n.s.}$ availability was studied (Hadley and Hume 1987; Jmari et al. 1987).

The results presented herein indicate that $I_{n.s.}$ inactivation is potential-dependent, at least in that it is induced by membrane depolarisation. However, the properties of $I_{n.s.}$ seem to be quite different from those of I_{Ba}. Therefore, I_{Ca}, I_{Ba} and $I_{n.s.}$ in coronary artery SMC were directly compared to each other.

Comparison of I_{Ca}, I_{Ba} and $I_{n.s.}$ inactivation in coronary artery SMC

When 2.5 mmol/l Ca^{2+} in the external solution was replaced by 10 mmol/l Ba^{2+} the inactivation of Ca channels was slowed down (Fig. 9*A*). To evaluate the inactivation induced by 500 ms pulse the ratio I_2/I_1 (where I_1 is the peak amplitude of current induced by the first pulse and I_2 is that induced by the second pulse) can be used. This ratio was 0.54 for I_{Ca} and 0.74 for I_{Ba} (Fig. 9*A*).

If the currents carried by calcium and monovalent cations were compared at the peaks of their respective I—V relationships (i.e. at 20 mV for I_{Ca} and at —10 mV for $I_{n.s.}$) the decay of the currents did not differ very much (Fig. 9*B*). The ratios I_2/I_1 were 0.57 for $I_{n.s.}$ and 0.56 for I_{Ca}. In general, at the end of a 300 ms pulse the average fractions of noninactivating current were 0.38 ± 0.03

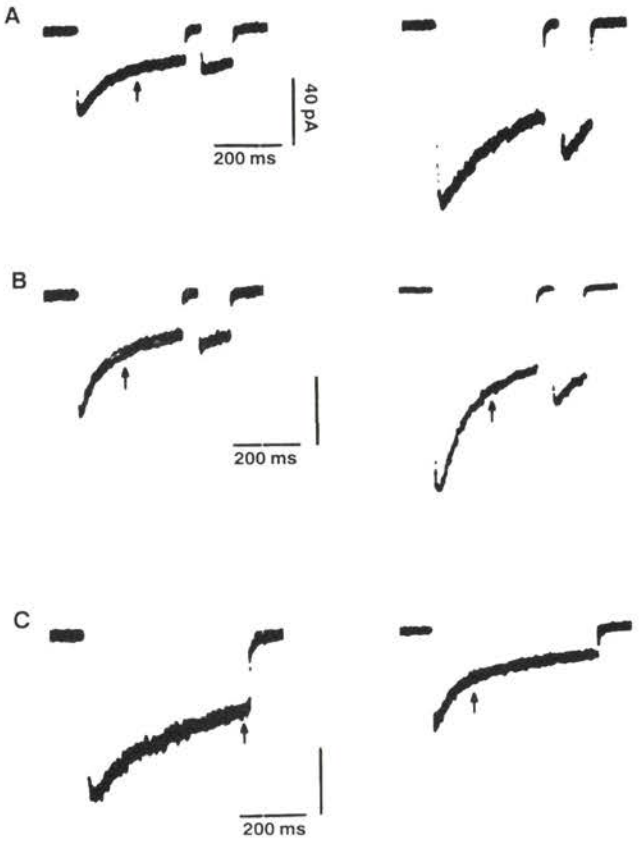

Fig. 9. Coronary artery SMC. *A*: I_{Ca} (*left trace*) and I_{Ba} (*right trace*) recorded during prepulse and testpulse steps to 20 mV. *B*: I_{Ca} (*left trace*) and $I_{n.s.}$ (*right trace*) during prepulse and test pulse steps to 20 mV for I_{Ca}, and to −10 mV for $I_{n.s.}$. Note the different calibrations for I_{Ca} (40 pA) and $I_{n.s.}$ (400 pA). *C*) I_{Ba} (*left trace*) and $I_{n.s.}$ (*right trace*) during step depolarization from holding potential −80 mV to 0 mV. Current calibration for I_{Ba} 40 pA, for $I_{n.s.}$ 400 pA. *A*, *B* and *C* were obtained from three different cells. The half amplitudes of each current are indicated by the arrows.

($n = 22$) for $I_{n.s.}$ at −10 mV and 0.34 ± 0.02 ($n = 18$) for I_{Ca} at 20 mV. Moreover, a comparison of I_{Ba} and $I_{n.s.}$ at 0 mV in the same cell shows that the inactivation of $I_{n.s.}$ proceeds significantly faster than that of I_{Ba} (Fig. 9*C*).

However, the above comparisons did not consider changes of the surface membrane potential. Such a correction should be introduced, especially for I_{Ba} and $I_{n.s.}$, since inactivation of both currents seems to be dependent on the membrane potential. The best way to do this is to measure the shift of activation

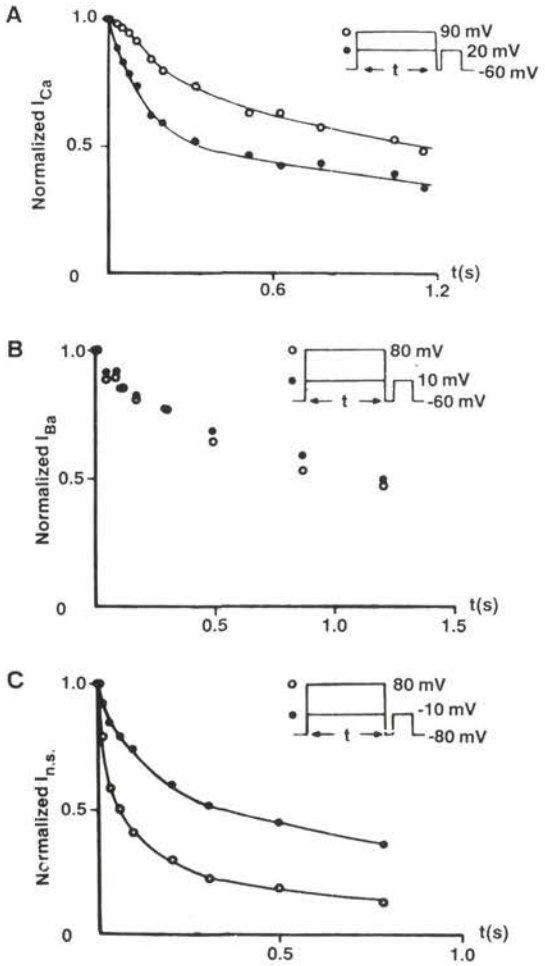

Fig. 10. Development of I_{Ca} (*A*), I_{Ba} (*B*) and $I_{n.s.}$ (*C*) inactivation in coronary artery SMC. Experimental protocols are shown in the insets. The currents were normalized in respect to the peak of a current elicited by a testpulse without conditioning prepulse. *A*, *B* and *C* from three different cells.

curve of $I_{n.s.}$ with respect to I_{Ba}, and to suppose that the inactivation gate of Ca channel has the same sensitivity to surface potential changes as does the activation gate. The activation curve of $I_{n.s.}$ can be calculated from I—V relation for $I_{n.s.}$ (Fig. 8*B*) (the values of parameters in Boltzman equation were $V_{0.5} = -25$ mV and $k = -8.5$ mV, cf. Klöckner and Isenberg 1985b). However, we suggested that the activation curve cannot be calculated correctly from I_{Ba}

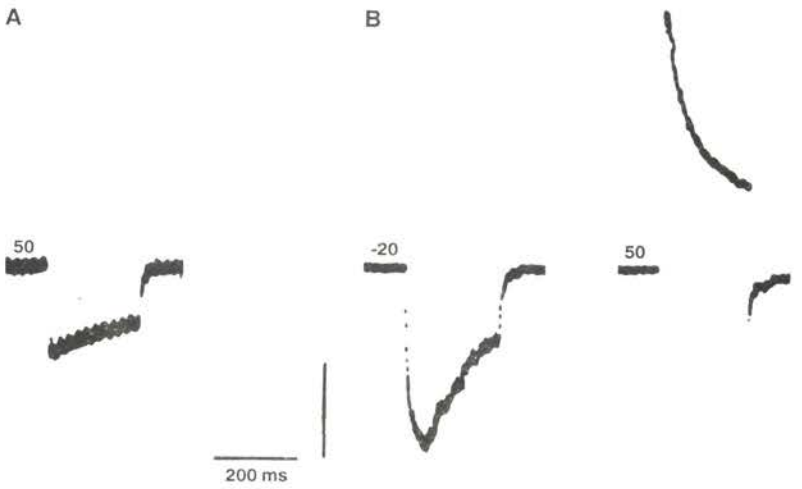

Fig. 11. I_{Ca} (*A*) and $I_{n.s.}$ (*B*) during 200 ms step depolarizations from holding potential −80 mV to potentials indicated (mV) near each current traces. Note the different calibrations for *A* (40 pA) and *B* (200 pA). pH of the external solution in *A* was 8.0. *A* and *B* from the same coronary artery SMC.

(of I_{Ca}) I—V relationship due to the insufficient accuracy in estimating I_{Ba} (or I_{Ca}) amplitude at potentials close to its reversal.

To overcome the above difficulties experiments were performed where the development of current inactivation was measured at two potentials 70 mV (or 90 mV) apart using the twopulse protocol.

In agreement with the shape of the availability curve of I_{Ca} (Fig. 2), I_{Ca} inactivation developed slowlier at 90 mV than at 20 mV (Fig. 10*A*). The patterns of I_{Ba} inactivation at 80 mV and at 10 mV were nearly similar suggesting that the current rate was weakly dependent on the membrane voltage in this potential range (compare with Fig. 4). In contrast, $I_{n.s.}$ inactivated significantly faster at 80 mV than at −10 mV (compare with Fig. 8). Fig. 10 illustrates quite different effects of comparable membrane potential changes on the rates of Ca channel inactivation for currents carried by calcium, barium and monovalent cations.

Moreover, from the opposite effects of membrane voltage on I_{Ca} and $I_{n.s.}$ inactivation (Fig. 10*A*, *C*) and from our finding that at peaks $I_{n.s.}$ and I_{Ca} decay did not show marked differences (Fig. 9*B*), it can be expected that at large positive potentials I_{Ca} inactivation is slowlier than that of $I_{n.s.}$.

Fig. 11 presents current traces of I_{Ca} (*A*) and $I_{n.s.}$ (*B*) recorded from one cell. At 50 mV, I_{Ca} (which inactivated slowlier than at peak potential) decayed during the depolarization to 0.62 of its peak value (Fig. 11*A*). The fraction of the

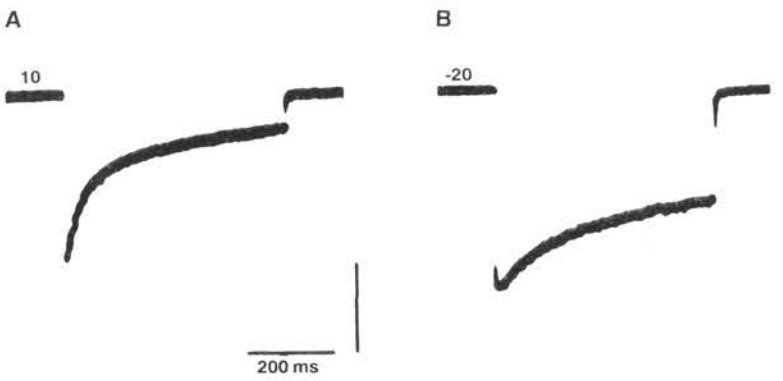

Fig. 12. I_{Ca} (*A*) and $I_{n.s.}$ (*B*) elicited by step depolarizations from holding potential −80 mV to the membrane potential indicated near each current traces (mV); taenia caeci SMC. Note the different current calibrations for I_{Ca} (100 pA) and $I_{n.s.}$ (1 nA).

noninactivating $I_{n.s.}$ at the end of the depolarization was 0.37 (Fig. 11*B*, right trace). At 50 mV, $I_{n.s.}$ clearly inactivated faster than did I_{Ca}. This, however, can be also due to the shift of potential-dependent parameters of Ca channels in negative direction. There are some difficulties in evaluating this shift (see above); for different preparations values of approx. 30 to 40 mV have been reported (Almers and McCleskey 1984; Hess et al. 1986; Carbone and Lux 1987) but even 70 mV (Kostyuk et al. 1983). Upon introducing 70 mV correction, a comparison of I_{Ca} at 50 mV with $I_{n.s.}$ at −20 mV shows that $I_{n.s.}$ still inactivates faster than does I_{Ca} (Fig. 11*A* and *B*, *left trace*). The fraction of noninactivated current at the end of depolarization to −20 mV was 0.4 for $I_{n.s.}$. This suggests that there is some range of membrane potentials within which I_{Ca} inactivates slowlier than does $I_{n.s.}$, i.e. than do Ca channels without any calcium entry.

In general, the results of the comparison of I_{Ca} and $I_{n.s.}$ inactivation in coronary artery SMC were opposite to those reported for other preparations, where $I_{n.s.}$ inactivation was markedly slowlier than that of I_{Ca} (Hadley and Hume 1987; Hess and Tsien 1984; Isenberg and Klöckner 1985; Ohya et al. 1986; Klöckner and Isenberg 1987). In an attempt to understand the above discrepancy we elevated the intracellular EGTA concentration up to 10 mmol/l, but the results were similar to that obtained with 0.42 mmol/l EGTA. So, we applied the same intra- and extracellular solutions to isolated SMC from taenia caeci of the guinea-pig. In taenia caeci SMC, inactivation of Ca channels was found to be influenced by calcium entry into the cell (Ganitkevich et al. 1987).

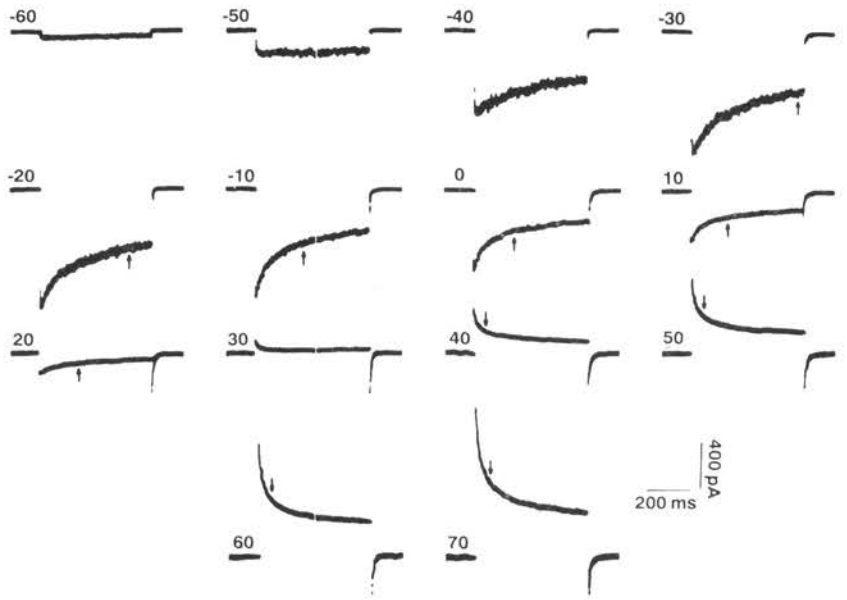

Fig. 13. $I_{n.s.}$ during different potential steps of 500 ms duration; taenia caeci SMC. Membrane potentials are indicated (mV) near each current trace. The half amplitudes of each $I_{n.s.}$ are indicated by the arrows.

Inactivation of taenia caeci $I_{n.s.}$

Upon removing external calcium and adding 2 mmol/l EGTA, $I_{n.s.}$ can be recorded instead of I_{Ca} in taenia caeci SMC. The ratio of $I_{n.s.}$ to I_{Ca} peak amplitudes was similar to that found for coronary artery SMC (approx. 10, Fig. 12). The I—V relationship of $I_{n.s.}$ was shifted by about 30 mV toward negative potentials in comparison with that of I_{Ca}. The reversal potential of $I_{n.s.}$ in taenia caeci SMC was also similar to that measured in coronary artery SMC (approx. 30 mV, Fig. 13).

However, two significant differences in the properties of $I_{n.s.}$ were found for taenia caeci SMC in comparison to coronary artery SMC: 1) comparing I_{Ca} and $I_{n.s.}$ peaks in taenia caeci, $I_{n.s.}$ inactivation was always significantly slower than that of I_{Ca} (Fig. 12, compare with Fig. 9*B* for coronary artery SMC); 2) $I_{n.s.}$ inactivation in taenia caeci was very slow close to potentials of its activation, i.e. from −60 mV to −50 mV (Fig. 13). With the increasing step depolarization it was accelerated. For outward $I_{n.s.}$ the half-time of its decay shows a weak

dependence on membrane voltage (Fig. 13), similarly as in coronary artery SMC. The availability curve of $I_{n.s.}$ in taenia caeci showed a similar monotonic dependence on the conditioning potential as it was shown for coronary artery SMC (see Fig. 8).

Discussion

Calcium-dependence of I_{Ca} inactivation in coronary artery SMC

The results presented in this paper provide evidence for calcium dependence of I_{Ca} inactivation in coronary artery SMC. The criteria usually employed to check this possibility are next: 1) the time course of I_{Ca} inactivation was slowed down at large positive potentials together with a decrease of I_{Ca} peak amplitude; 2) the availability curve of I_{Ca} (but not of I_{Ba}) was U-shaped; 3) inactivation of I_{Ba} was much retarded in comparison with that of I_{Ca}. This has also been demonstrated in taenia caeci SMC (Ganitkevich et al. 1987) as well as in other tissues (Ashcroft and Stanfield 1982; Mentrard et al. 1984; Lee et al. 1985).

On the other hand, two our findings strongly contradict the simplest hypothesis concerning calcium-dependent inactivation (Eckert and Chad 1984): 1) $[Ca^{2+}]_o$ elevation has no effect on the time course of I_{Ca} inactivation in coronary artery SMC. Similar results were reported for heart cells (Lee et al. 1985; Campbell et al. 1988). Neither the enhancement of calcium entry into heart cells by isoprenaline or by cyclic AMP was accompanied by changes in the U-shaped availability curve of I_{Ca}. The peak amplitude of I_{Ca} was increased several times whereas its time course was not changed (Mentrard et al. 1984; Isenberg and Belardinelli 1984; Fischmeister and Hartzell 1986; Kameyama et al. 1986; and Argibay et al. 1988). Moreover, at the same depolarization, the inactivation of I_{Ca} was slowed down despite the increase of I_{Ca} peak amplitude in the presence of elevated $[Ca^{2+}]_o$. This strongly suggests that, at least at potentials close to 20 mV (Fig. 3*A*), I_{Ca} inactivation is controlled mainly by the membrane potential, it was shifted toward positive potentials in the presence of elevated $[Ca^{2+}]_o$ due to the surface potential changes. However, at the same potential inactivation of Ca channels was slowed down with Ba^{2+} ions as the current carrier. 2) Inactivation of $I_{n.s.}$ appears to be faster than that of I_{Ca} in the same potential range, i.e. without calcium entry Ca channels inactivate faster than with calcium entry into the cell.

The above findings make us to suggest the possibility of interaction of calcium ions with the potential-sensitive gate of Ca channel. Some evidence for this possibility may also be derived from the characteristics of $I_{n.s.}$.

Properties of $I_{n.s.}$ in coronary artery SMC

The ability of Ca channels to allow monovalent cations to pass when divalent cations have been removed from the external solution has been demonstrated in a variety of tissues (Kostyuk and Krishtal 1977; Almers and McCleskey 1984; Hess and Tsien 1984; Hadley and Hume 1987). However, there have been only few reports dealing with the characteristics of $I_{n.s.}$ in SMC. The results reported so far have partially been contradictory. Jmari et al. (1987) found $I_{n.s.}$ peak amplitudes in myometrial cells smaller than those of I_{Ca}; Ohya et al. (1986) reported higher peaks of $I_{n.s.}$ than I_{Ca} in ileal SMC (by a factor of 3), whereas Isenberg and Klöckner (1985) found an $I_{n.s.}$ peak to I_{Ca} peak ratio of approx. 8 in urinary bladder SMC. In our opinion, the above differences can be due to relatively low holding potential in some of these studies (approx. −60 mV). In our experiments $I_{n.s.}$ was greatly reduced (to 55 %, $n = 2$) at the holding potential of −60 mV, and $I_{n.s.}$ peak to I_{Ca} peak ratio was approx. 5.

In most works mentioned above shifts of the I—V relationships of $I_{n.s.}$ toward negative potentials in respect to those of I_{Ca} have been reported.

A much slower inactivation of $I_{n.s.}$ than I_{Ca} has been reported for ileal (Ohya et al. 1986), urinary bladder (Isenberg and Klöckner 1985) and myometrial SMC (Jmari et al. 1987). This is in agreement with our results obtained with taenia caeci (Fig. 12), and opposite to those with coronary artery SMC (Fig. 9*B*).

Inactivation of $I_{n.s.}$ in heart cells has been shown to be a potential-dependent process (Hadley and Hume 1987). Also in our experiments there were no observations to suggest a current-dependence of $I_{n.s.}$ inactivation; i.e., it was not due to accumulation or depletion of current carriers. We could conclude that it is mainly a potential-mediated process.

At present we cannot offer any explanation for the enhanced $I_{n.s.}$ inactivation occurring upon $I_{n.s.}$ reversal. Possibly, it is linked with the asymmetry of intra- and extracellular media; however, additional experiments are necessary to check this possibility. The latter is also true for the mechanisms involved in $I_{n.s.}$ inactivation.

What is calcium-dependent inactivation?

It was shown that calcium- dependent inactivation of Ca channels involves the operation of intracellular enzymes (Chad and Eckert 1986). There is no doubt that the membrane potential also affects the inactivation and subsequent recovery of Ca channels in a variety of tissues, i.e. that there is a joint regulation of Ca channels by calcium ions and membrane potential (Lee et al. 1985; Ohya et

al. 1986; Jmari et al. 1986; Ganitkevich et al. 1987). One hypothesis of a joint regulation has been offered by Lee et al. (1985).

Usually, it is assumed that I_{Ba} inactivation is potential- dependent process (Lee et al. 1985; Amadee et al. 1987) whereas additional inactivation of I_{Ca} represents a calcium-dependent component of Ca channel inactivation (Jmari et. al. 1986). There is nothing to contradict this assumption in coronary artery SMC (the present study) or in taenia caeci (Ganitkevich et al. 1987). The only unusual finding was that within a defined range of membrane potentials the rate of I_{Ba} inactivation was virtually independent of voltage. However, this can be explained also by assuming a purely potential-dependent mechanism of I_{Ba} inactivation.

We could find no indication for the current dependence of I_{Ba} inactivation as reported for heart cells (Markwardt and Nilius 1988).

Inactivation of $I_{n.s.}$ has also been suggested to be a potential-dependent process whereas additional inactivation in heart cells with calcium ions as the current carrier has been suggested to be a calcium-mediated mechanism (Hadley and Hume 1987).

All the three currents (I_{Ca}, I_{Ba}, $I_{n.s.}$) were studied in the present work, and they could be compared as for their inactivation properties. Considerable differences could be stated to exist between those characteristics (Fig. 10). To explain these results two possibilities have be considered: 1) Inactivation of $I_{n.s.}$ represents a pure potential-dependent process which is "normal" for Ca channels. The operation of this inactivation gate is affected by barium as well as calcium ions when they carry a current through Ca channels; 2) The potential-dependent inactivation gate of Ca channels is affected by removal of divalent cations from the external solution. In this case, the I_{Ba} inactivation probably represents "normal" potential-dependent inactivation whereas $I_{n.s.}$ inactivation is due to the operation of the modified potential-dependent inactivation gate of Ca channel.

Our results do not allow to distinguish between the above possibilities. Nevertheless, we tried to understand at least the interaction of divalent cations with the potential-sensitive inactivation gate of Ca channel.

Alteration of kinetic properties of calcium-permeable single channels by permeant cations was demonstrated by Chesnoy-Marshais (1985) in Aplysia neurones.

Comparison of Ca channel properties in coronary artery and taenia caeci SMC

In general, there are some similarities as well as differences in the properties of inactivation of Ca channels in coronary artery (described in this work) and taenia caeci SMC (mainly Ganitkevich et al. 1987; this paper).

The similarities include: 1) slowing down of I_{Ca} decay at potentials more positive than the potential of I_{Ca} peak; 2) U-shaped availability curves of I_{Ca} in both tissues; 3) slowed down inactivation of currents through Ca channels upon substitution of Ba^{2+} for external Ca^{2+} ions.

The differences were the following: 1) in coronary artery SMC, elevation of $[Ca^{2+}]_o$ had no effect on the time course of I_{Ca} inactivation, if the effect of the surface potential changes was accounted for, whereas in taenia caeci SMC I_{Ca} inactivation was accelerated in this condition (Ganitkevich et al. 1987); 2) comparison of I_{Ca} and $I_{n.s.}$ decays at the maxima of their I– V relationships showed a slowing down of $I_{n.s.}$ decay in comparison with that of I_{Ca} in taenia caeci with no significant change in coronary artery SMC (Figs. 12 and 9*B*); 3) the rate of $I_{n.s.}$ inactivation in coronary artery SMC at potentials close to its activation (Figs. 6 and 13) in coronary artery shows different potential dependence in comparison with that in taenia caeci.

Since the mechanisms involved in $I_{n.s.}$ inactivation remain unknown, we can only speculate about the reasons for the different inactivation properties of $I_{n.s.}$ in coronary artery and taenia caeci SMC.

The above differences may be due to different types of Ca channels present in the membranes of coronary artery and taenia caeci SMC, as suggested by Shuba (1981) for smooth muscle. However, we have no evidence for the presence of T-type Ca channels in both tissues. This type of Ca channels was found in neurons (Carbone and Lux 1984; 1987; Fedulova et al. 1985) and it was difficult to make any conclusions in this respect based on whole-cell I_{Ca} in coronary artery or taenia caeci SMC. In both tissues, inactivation of whole-cell I_{Ca} as well as its other properties (Ganitkevich et al. 1987; 1988; 1989), match better with the properties of L-type Ca channels.

Another possibility is that the differences in Ca channel properties between coronary artery and taenia caeci are due to some intracellular factors affecting the properties of Ca channel inactivation.

With our cell preparation method and in the presence of K^+ instead of Cs^+ ions in the internal solution spontaneous transient outward currents (STOC) were recorded in coronary artery SMC (Ganitkevich and Shuba 1988). STOC were similar to those described in other SMC (Klöckner and Isenberg 1985a; Benham and Bolton 1986; Ohya et al. 1987), and there is good evidence for their occurrence being due to cyclical release of calcium in the cell. STOC were abolished in calcium-free external solutions in visceral but not in vascular SMC (Benham and Bolton 1986), as well as in coronary artery SMC (Ganitkevich and Shuba 1988). However, in taenia caeci SMC, no STOC were generally seen (our unpublished observation). The appearance of STOC may indicate an elevated concentration of calcium ions close to the internal side of the membrane of vascular SMC. Also, there is additional evidence suggesting that large amounts

of calcium are stored and can cyclically be released within vascular SMC (Bond et al. 1984).

In our experiments elevation of intracellular concentration of EGTA up to 10 mmol/l had no clear effect on the properties of I_{Ca} or $I_{n.s.}$ in any of the tissues studied. However, it cannot be excluded that EGTA failed to buffer intracellular Ca^{2+} ions close to the inner mouth of Ca channels (Bechem and Pott (1985).

So, the question is if there is some link between different calcium contents in coronary artery and taenia caeci SMC and the observed differences in Ca channel inactivation properties in these tissues.

We have failed to answer this question at present. It is possible some answer might be derived from measurements of intracellular Ca^{2+} concentrations.

References

Almers W., McCleskey E. W. (1984): Non-selective conductance in calcium channels of frog muscle: calcium selectivity in a single- file pore. J. Physiol. (London) **353,** 585—608

Amedee T., Mironneau C., Mironneau J. (1987): The calcium channel current of pregnant rat single myometrial cells in short-term primary culture. J. Physiol. (London) **392,** 253—272

Argibay J. A., Fischmeister R., Hartzell H. C. (1988): Inactivation, reactivation and pacing dependence of calcium current in frog cardiocytes: correlation with current density. J. Physiol. (London) **401,** 201—226

Ashcroft F. M., Stanfield P. R. (1982): Calcium inactivation in skeletal muscle fibres of the stick insect, Carausius morosus. J. Physiol. (London) **330,** 349—372

Bechem M., Pott L. (1985): Removal of Ca current inactivation in dialysed guinea-pig atrial cardioballs by Ca chelators. Pflügers Arch. **404,** 10—20

Benham C. D., Bolton T. B. (1986): Spontaneous transient outward currents in single visceral and vascular smooth muscle cells of the rabbit. J. Physiol. (London) **381,** 385—406

Bond M., Kitazawa T., Somlyo A. P., Somlyo A. V. (1984): Release and recycling of calcium by the sarcoplasmic reticulum in guinea-pig portal vein smooth muscle. J. Physiol. (London) **355,** 677—695

Campbell D. L., Giles W. R., Hume J. R., Shibata E. F. (1988): Inactivation of calcium current in bull-frog atrial myocytes. J. Physiol. (London) **403,** 287—315

Carbone E., Lux H. D. (1984): A low voltage-activated, fully inactivating Ca channel in vertebrate sensory neurones. Nature **310,** 501—502

Carbone E., Lux H. D. (1987): Kinetics and selectivity of a low-voltage-activated calcium current in chick and rat sensory neurones. J. Physiol. (London) **386,** 547—570

Chad J. E., Eckert R. (1986): An enzymatic mechanism for calcium current inactivation in dialyzed Helix neurones. J. Physiol. (London) **378,** 31—52

Chesnoy-Marchais D. (1985): Kinetic properties and selectivity of calcium-permeable single channels in Aplysia neurones. J. Physiol. (London) **367,** 457—488

Eckert R., Chad J. D. (1984): Inactivation of Ca channels. Prog. Biophys. Mol. Biol. **44,** 215—267

Fedulova S. A., Kostyuk P. G., Veselovski N. S. (1985): Two types of calcium channels in the somatic membrane of new-born rat dorsal root ganglion neurones. J. Physiol. (London) **359,** 431—446

Fischmeister R., Hartzell H. C. (1986): Mechanism of action of acetylcholine on calcium current in single cells from frog ventricle. J. Physiol. (London) **376,** 183—202

Ganitkevich V. Ya., Shuba M. F. (1988): Spontaneous outward currents in the membrane of single isolated smooth muscle cell of coronary artery. Biol. Membrany **5,** 1312 1320 (in Russian)

Ganitkevich V. Ya., Shuba M. F., Smirnov S. V. (1986): Potential-dependent calcium inward current in a single isolated smooth muscle cell of the guinea-pig taenia caeci. J. Physiol. (London) **380,** 1 16

Ganitkevich V. Ya., Shuba M. F., Smirnov S. V. (1987): Calcium-dependent inactivation of potential-dependent calcium inward current in an isolated guinea-pig smooth muscle cell. J. Physiol. (London) **392,** 431 449

Ganitkevich V. Ya., Shuba M. F., Smirnov S. V. (1988): Saturation of calcium channels in single isolated smooth muscle cells of guinea- pig taenia caeci. J. Physiol. (London) **399,** 419 436

Ganitkevich V. Ya., Smirnov S. V., Shuba M. F. (1989): Characteristics of potential-dependent calcium inward current in the membrane of isolated smooth muscle cell of coronary artery. Biol. Membrany **6,** 51 58 (in Russian)

Hadley R. W., Hume J. R. (1987): An intrinsic potential-dependent inactivation mechanism associated with calcium channels in guinea- pig myocytes. J. Physiol. (London) **389,** 205 222

Hess P., Tsien R. W. (1984): Mechanism of ion permeation through calcium channels. Nature **309,** 453 456

Hess P., Lansman J. B., Tsien R. V. (1986): Calcium channel selectivity for divalent and monovalent cations. Voltage and concentration dependence of single channel current in ventricular heart cells. J. Gen. Physiol. **88,** 293 320

Isenberg G., Belardinelli L. (1984): Ionic basis for the antagonism between adenosine and isoproterenol in isolated mammalian ventricular myocytes. Circ. Res. **55,** 309 325

Isenberg G., Klöckner U. (1985): Calcium currents of isolated guinea-pig smooth muscle cells: inactivation, conductance and selectivity is controlled by micromolar amounts of $[Ca]_o$. J. Physiol. (London) **358,** 60P

Jmari K., Mironneau C., Mironneau J. (1986): Inactivation of calcium channel current in rat uterine smooth muscle: evidence for calcium- and voltage-metiated mechanisms. J. Physiol. (London) **380,** 111 126

Jmari K., Mironneau C., Mironneau J. (1987): Selectivity of calcium channels in rat uterine smooth muscle: interactions between sodium, calcium and barium ions. J. Physiol. (London) **384,** 247 261

Kameyama M., Heschler J., Mieskes G., Trautwein W. (1986): The protein-specific phosphatase 1 antagonizes the β-adrenergic increase of the cardiac Ca current. Pflügers Arch. **407,** 461 463

Klöckner U., Isenberg G. (1985a): Calcium-activated potassium currents as an indicator for intracellular Ca-transients (single smooth muscle cells from trachea and urinary bladder). Pflügers Arch. **405,** R61

Klöckner U., Isenberg G. (1985b): Calcium currents of ceasium loaded isolated smooth muscle cells (urinary bladder of the quinea- pig). Pflügers Arch. **405,** 340 348

Klöckner U., Isenberg G. (1987): Calmoduline antagonists depress calcium and potassium currents in ventricular and vascular myocytes. Amer. J. Physiol. **253,** H1601 1611

Kostyuk P. G., Krishtal O. A. (1977): Effect of calcium and calcium- chelating agents on the inward and outward currents in the membrane of mollusc neurones. J. Physiol. (London) **270,** 569 580

Kostyuk P. G., Mironov S. L., Shuba Ya. M. (1983): Two ionselecting filters in the calcium channel of the somatic membrane of mollusc neurons. J. Membrane Biol. **76,** 83 93

Lee K. S., Marban E., Tsien R. W. (1985): Inactivation of calcium channels in mammalian heart cells: joint dependence on the membrane potential and intracellular calcium. J. Physiol. (London) **364,** 395 411

Markwardt F., Nilius B. (1988): Modulation of calcium channel currents in guinea-pig single ventricular cells by the dihydropyridine BAY K 8644. J. Physiol. (London) **399,** 559—575

Mentrard D., Vassort G., Fischmeister R. (1984): Calcium mediated inactivation of the calcium conductance in caesium-loaded frog heart cells. J. Gen. Physiol. **83,** 105—131

Ohya Y., Terada K., Kitamura K., Kuriyama H. (1986): Membrane currents recorded from a fragment of rabbit intestinal smooth muscle cell. Amer. J. Physiol. **251,** C335—346

Ohya Y., Kitamura K., Kuriyama H. (1987): Cellular calcium regulates outward currents in rabbit intestinal smooth muscle cell. Amer. J. Physiol. **252,** C401—410

Ohya Y., Kitamura K., Kuriyama H. (1988): Regulation of calcium current by intracellular calcium in smooth muscle cells of rabbit portal vein. Circ. Res. **62,** 375—383

Shuba M. F. (1981): The transport mechanisms by which contraction activating extracellular Ca^{2+} ions enter smooth muscle cells. In: Advances in Physiological Sciences, Vol. 5, Molecular and Cellular Aspects of Muscle Function (Eds. Varga E., Kövér A., Kovacs T. and Kovacs L.) pp. 83—94. Pergamon Press—Akadémiai Kiadó, Budapest

Tsien R. W., Marban E. (1982): Digitalis and slow inward current in heart muscle: Evidence for regulatory effects of intracellular calcium on calcium channels. In: Advances in Pharmacology and Therapeutics, Vol. 3 (Eds. Yoshida H., Hagihara Y. and Ebashi S. E.) pp. 217—225. Pergamon Press, Oxfort, New York.

Final version accepted October 26, 1990