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Biochimica et Biophysica Acta 1419 (1999) 343–352

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

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TTX-sensitive Na^+ and nifedipine-sensitive Ca^{2+} channels in rat vas deferens smooth muscle cells

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Received 6 January 1999; received in revised form 21 April 1999; accepted 3 May 1999

Abstract

The inward currents in single smooth muscle cells (SMC) isolated from epididymal part of rat vas deferens have been studied using whole-cell patch-clamp method. Depolarising steps from holding potential −90 mV evoked inward current with fast and slow components. The component with slow activation possessed voltage-dependent and pharmacological properties characteristic for Ca^{2+} current carried through L-type calcium channels (I_{Ca}). The fast component of inward current was activated at around −40 mV, reached its peak at 0 mV, and disappeared upon removal of Na ions from bath solution. This current was blocked in dose-dependent manner by tetrodotoxin (TTX) with an apparent dissociation constant of 6.7 nM. On the basis of voltage-dependent characteristics, TTX sensitivity of fast component of inward current and its disappearance in Na-free solution it is suggested that this current is TTX-sensitive depolarisation activated sodium current (I_{Na}). Cell dialysis with a pipette solution containing no macroergic compounds resulted in significant inhibition of I_{Ca} (depression of peak I_{Ca} by about 81% was observed by 13 min of dialysis), while I_{Na} remained unaffected during 50 min of dialysis. These data draw first evidence for the existence of TTX-sensitive Na^+ current in single SMC isolated from rat vas deferens. These Na^+ channels do not appear to be regulated by a phosphorylation process under resting conditions.

Keywords: TTX-sensitive Na^+ channel; L-type Ca^{2+} channel; Vas deferens; Smooth muscle; Regulation; Phosphorylation; Patch-clamp

1. Introduction

It is commonly accepted that action potential (AP) generation in skeletal and cardiac muscles is due to activation of both voltage-gated Na^+ and Ca^{2+} channels. In contrast, voltage-gated inward current in smooth muscle cell (SMC) is mainly carried through Ca^{2+} channels. There are only few studies indicating the existence of voltage-gated Na^+ channels in SMCs. By using the technique of whole-cell patch-clamp, highly TTX-sensitive I_{Na}, resembling innervated skeletal muscle I_{Na}, has been observed in SMCs isolated from rat portal vein [1], ileum [2,3], vena cava [4], pregnant rat uterus [5], guinea pig ureter [6], human colon [7] and sheep lymphatic ducts [12]. The I_{Na} observed in SMCs from rat stomach fundus [6] and colon [7] displayed less sensitivity to TTX (IC_{50} of 870 nM in fundus and 130 nM in

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0005-2736/99/$ – see front matter
PII: S0005-2736(99)00075-9

colon). Considering that cardiac Na^+ channels have low TTX sensitivity (IC_{50} of about 10 μM), the Na^+ channel type in SMCs from rat stomach fundus and colon is intermediate between innervated skeletal and cardiac muscle Na^+ channel types.

Based on radioligand binding and on functional studies, it is known that drug effects on SMCs of rat vas deferens are strikingly dependent on L-type Ca^{2+} channels [8,9]. In the present study we investigated the pharmaco-biophysical characteristics of voltage-gated inward current in SMCs freshly isolated from epididymal part of adult rat vas deferens. It was demonstrated that voltage-gated inward current is carried through both TTX-sensitive Na^+ and L-type Ca^{2+} channels. An attempt was made to test the involvement of phosphorylation processes in regulation of TTX-sensitive Na^+ channel activity. A preliminary report of some of the results has been presented in abstract form [10,11].

2. Materials and methods

2.1. Cell isolation

Experiments were performed on single smooth muscle cells enzymatically isolated from epididymal part of rat vas deferens. Albino Wistar male rats, 8–9 weeks old, were anaesthetised with ether or stunned, followed by bleeding. Isolated strips (5–10 mm long) from the epididymal part of rat vas deferens were placed in low Ca^{2+} physiological salt solution (PSS) with the following composition (mM): NaCl 135; KCl 6; $CaCl_2$ 0.1; $MgCl_2$ 1.2; D-glucose 5; HEPES 10; pH 7.4 adjusted with NaOH, and cut into small segments. The segments were then transferred into a nominally Ca^{2+}-free PSS containing 0.2% collagenase (Type XI, Sigma) and 0.3% bovine serum albumin. After 30–40 min of incubation at 35°C, segments were carefully washed out with nominally Ca^{2+}-free PSS, and single cells obtained by gentle agitation with a Pasteur pipette. Dispersed cells were stored in PSS at 4°C, to be used during experiment.

2.2. Electrical recording

The cells were voltage- or current-clamped using standard whole-cell patch-clamp technique [13]. Patch pipettes were polished, achieving a resistance of 1–3 MW when filled with a pipette solution. The signals were recorded and stored in an IBM-compatible computer, using a List EPC-7 patch-clamp amplifier (List Medical Electronic, Germany) and a Digidata 1200A AD/DA converter (Axon Instruments, Foster City, CA, USA). When I_{Na} was under investigation, series resistance was partially compensated electronically and capacitative transients were removed either by applying electronic compensation or by subtracting currents obtained in a solution containing TTX (1 μM) from those obtained under control conditions. Leak subtraction was performed using Clampfit utilities (pCLAMP 5.6, Axon Instruments). The experiments were done at room temperature (21–24°C).

2.3. Solutions

The main external solution for electrophysiological recordings of inward currents had the following composition (mM): NaCl 120; CsCl 6; $CaCl_2$ 2.5; $MgCl_2$ 1.2; glucose 5; TEA-Cl 10; 4-AP 4; HEPES 10; pH 7.4 adjusted with CsOH. When I_{Ca} was under investigation, I_{Na} was blocked by either addition of TTX (1 μM) or by replacement of NaCl with TEA-Cl. The basic pipette solution for I_{Ca} study contained (mM): CsCl 140, $MgSO_4$ 1; Na_2ATP 1; Li_2GTP 0.2; EGTA 1; HEPES 10; pH 7.2 adjusted with CsOH. To eliminate I_{Ca}, 2.5 mM $CaCl_2$ were substituted by 2.5 mM $CoCl_2$. In the presence of 2 mM Na^+ in the pipette solution, the amplitude of I_{Na} varied from −8 to −224 pA with average density -1.3 ± 0.8 pA/pF ($n = 7$). In order to facilitate I_{Na}, the pipette solution had its concentration of Na^+ reduced from 2 mM to 0.2 mM, and I_{Na} was studied using the pipette solution of the following composition (mM): CsCl 140, NaCl 0.2; MgATP 1; Li_2GTP 0.2; EGTA 1; HEPES 10. In experiments, when metabolic dependence of I_{Ca} and I_{Na} was under investigation, we used a pipette solution containing (mM): CsCl 140, NaCl 0.2; $MgSO_4$ 1; EGTA 1; HEPES 10.

2.4. Statistics

All results were expressed as means ± S.E.M.; n represents the number of cells.

3. Results

3.1. Properties of the voltage-gated Na^+ current

When vas deferens isolated myocytes were voltage-clamped at −90 mV, voltage stepping positive to −50 mV resulted in the development of inward currents, showing fast activating followed by slow components (Fig. 1A). The clear difference in time-course of activation and inactivation of these two components is best observed at test potentials −10 and 0 mV (Fig. 1A(b)). At these potentials, the initial fast component reached its peak and decayed to more than one-half during the first 3 ms of depolarisation step, while the subsequent slow component reached its maximal value only after 6–7 ms of depolarisation. This observation prompted us to suggest that the fast component of inward current can represent current through voltage-gated Na^+ channels (I_{Na}), whereas the slow component seems to be a Ca^{2+} channel current (I_{Ca}), previously reported in SMCs of rat vas deferens [14]. Indeed, equimolar substitution of Ca^{2+} (2.5 mM) for Co^{2+} in external solution completely prevented the development of the slow component, while the fast component of inward current remained unaffected (Fig. 1B). Therefore, the further study of fast component was performed using a bath solution containing $CoCl_2$ instead of $CaCl_2$. Under such condition the fast component appeared at voltage steps from holding potential −90 to −40 mV, peaked at voltage steps in the range −10–0 mV, and completely disappeared upon replacement of Na^+ by TEA^+ (Fig. 1C).

To further characterise I_{Na}, its sensitivity to TTX has been studied. Addition of TTX in bath solution caused dose-dependent inhibition of I_{Na}. A slight reduction of current was observed at 0.3 nM TTX, whereas a concentration of 1 μM completely blocked Na^+ channel current. The apparent dissociation constant was determined to be 6.7 nM. Fig. 2 represents the effect of cumulative application of TTX on I_{Na} and dose-response curves for the inhibition exerted by TTX.

As can be seen from Fig. 3A, the decay of I_{Na} was

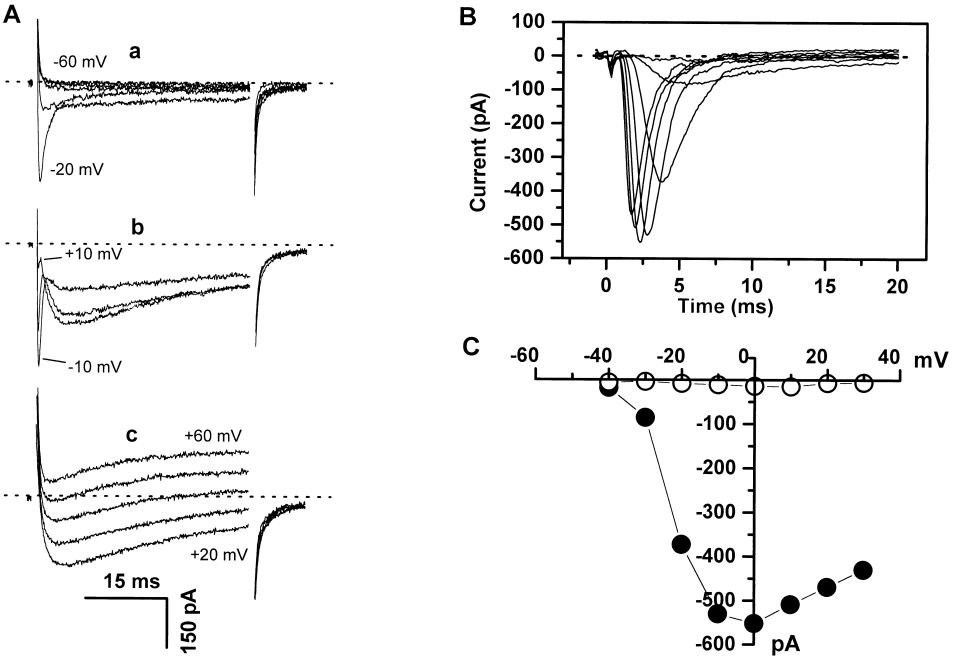

Fig. 1. Fast and slow components of voltage-gated inward current in rat vas deferens SMCs. Inward currents obtained by 40 ms depolarisation steps from holding potential −90 mV to −60, −50, −40, −30, −20 mV (A, a), to −10, 0, +10 mV (A, b), and to +20, +30, +40, +50, +60 mV (A, c). Transient inward currents produced by 40 ms pulses from holding potential −90 mV to −40–+30 mV with 10 mV increment (B). Slow component was blocked by replacement of Ca^{2+} with Co^{2+}. Corresponding peak *I/V* relationship (C) for current shown in B (filled circles) and for current obtained with similar voltage protocol after equimolar substitution of Na^+ with TEA^+ (open circles).

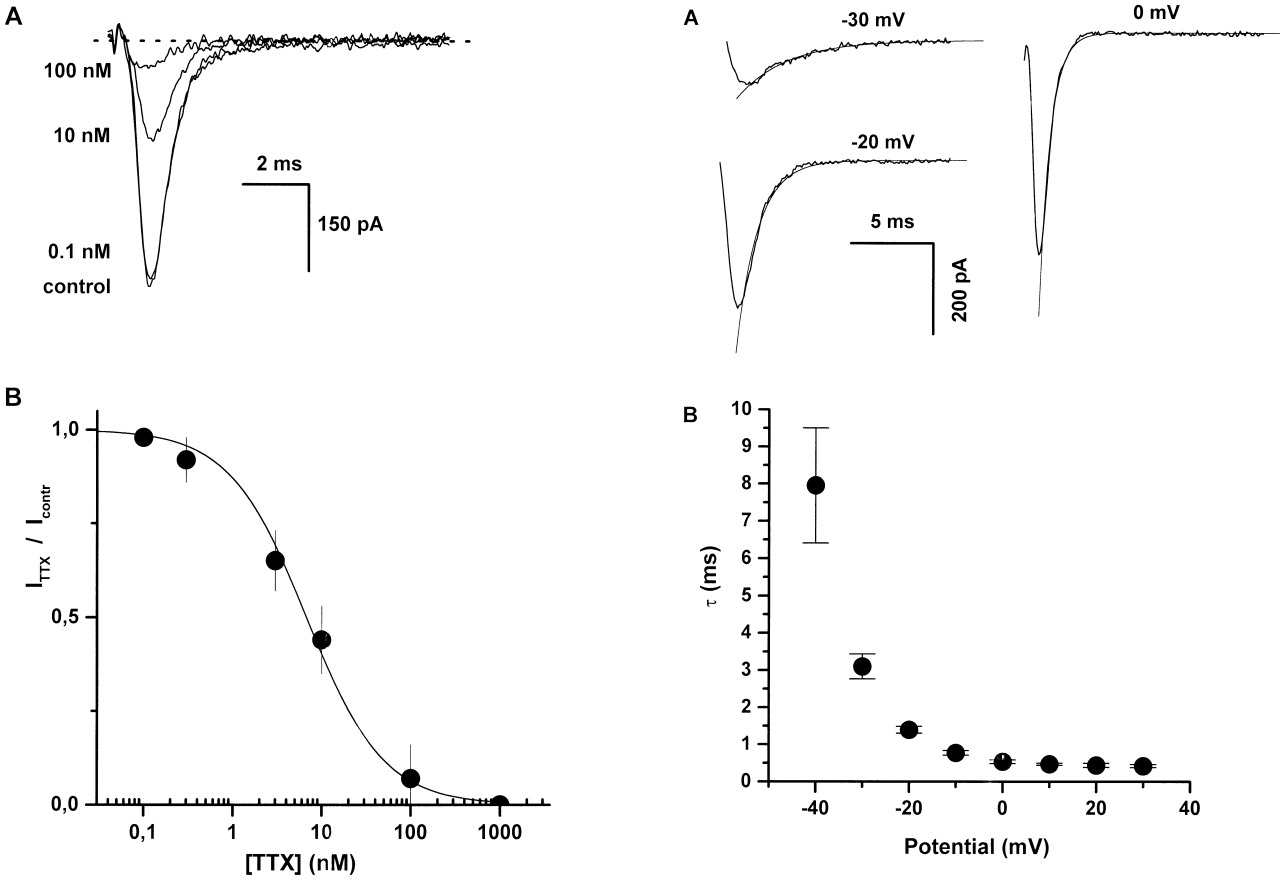

Fig. 2. Dependence of I_{Na} on TTX added to the bath solution. Current traces evoked by 8 ms depolarisation steps from −90 to 0 mV under control condition and in the presence of 0.1, 10, and 100 nM of TTX (A). Dose-response relationship for TTX block of I_{Na} (B). The current amplitude in the absence of TTX was normalised as 1.0 (I_{contr}). The smooth curve was fitted using a non-linear least squares minimisation algorithm according to the equation: $I_{TTX}/I_{contr} = (1 + [TTX]/K_d)^{-1}$, with the dissociation constant $K_d = 6.7$ nM.

well fitted by a single exponential in the whole range of potentials studied. The time constant of I_{Na} decay decreased monotonically from 7.95 ± 1.55 ms ($n = 4$) at −40 mV to 0.42 ± 0.04 ms ($n = 4$) at 30 mV (Fig. 3B). Recovery of I_{Na} from inactivation was studied after 30 ms depolarisation to 0 mV at two different holding potentials: −70 and −90 mV. At both holding potentials, recovery of I_{Na} from inactivation was well approximated by single exponential (Fig. 3C,D), and time constant at more negative potentials was significantly smaller. The average time constant of I_{Na} recovery at the holding potential −90 mV was 12.4 ± 3.2 ms ($n = 3$), whereas at −70 mV it was 32.3 ± 5.7 ms ($n = 3$).

Fig. 3. Inactivation and recovery from inactivation of I_{Na}. The declining phase of I_{Na} elicited by pulses from −90 to −30, −20, 0 mV was reasonably fitted by single exponentials with inactivation time constant (t) of 3.0, 1.3, 0.7 ms, respectively (A). Dependence of t_h on membrane potential (B). Data derived from four cells. Recovery of I_{Na} from inactivation at two different holding levels (C, D). The continuous lines are single exponentials with approximate time constants of 12 ms (C) and 32 ms (D). Schematic voltage protocol is shown at the bottom of each panel.
←

Voltage dependence of I_{Na} inactivation was studied using the following voltage protocol: 400 ms conditioning prepulse (V_c), ranging from −120 to −10 mV with 10 mV increment and 1 ms returning to holding potential −90 mV were applied followed by the 10 ms test pulse to 0 mV to elicit I_{Na}. A dependence of the real and normalised amplitude of I_{Na} on conditioning prepulse is shown in Fig. 4. This dependence was well fitted to the Boltzmann equation:

$$I_{Na}/I_{Na(max)} = \{1 + \exp[V_c - V_{0.5}]/k\}^{-1} \quad (1)$$

where $V_{0.5} = -62.7$ mV is a half-inactivation potential and $k = 7.7$ mV is the slope factor.

Fig. 4B also shows average values for the voltage dependence of I_{Na} activation. Mean activation curve was obtained after converting peak current to conductance g_{Na} according to equation:

$$g_{Na} = I_{Na}/(V - V_{Na}) \quad (2)$$

where g_{Na} is membrane conductance at potential V, I_{Na} is the peak Na^+ current, V_{Na} is the Na^+ reversal potential. Then it was normalised to maximum conductance ($g_{Na(max)}$) and plotted as function of potential. V_{Na} estimated by interpolation of linear portion of I–V curves was 101.1 ± 3.4 mV ($n = 4$). This value is close to 103.4 mV, predicted on the basis of the Nernst equation for purely Na^+-selective conductance, under the experimental condition used. Activation curve was well fitted by the equation:

$$g_{Na}/g_{Na(max)} = 1/(1 + \exp[V_{0.5} - V]/k) \quad (3)$$

with $V_{0.5}$, the membrane potential of half-maximal activation, of −19.4 mV and k of −6.4 mV.

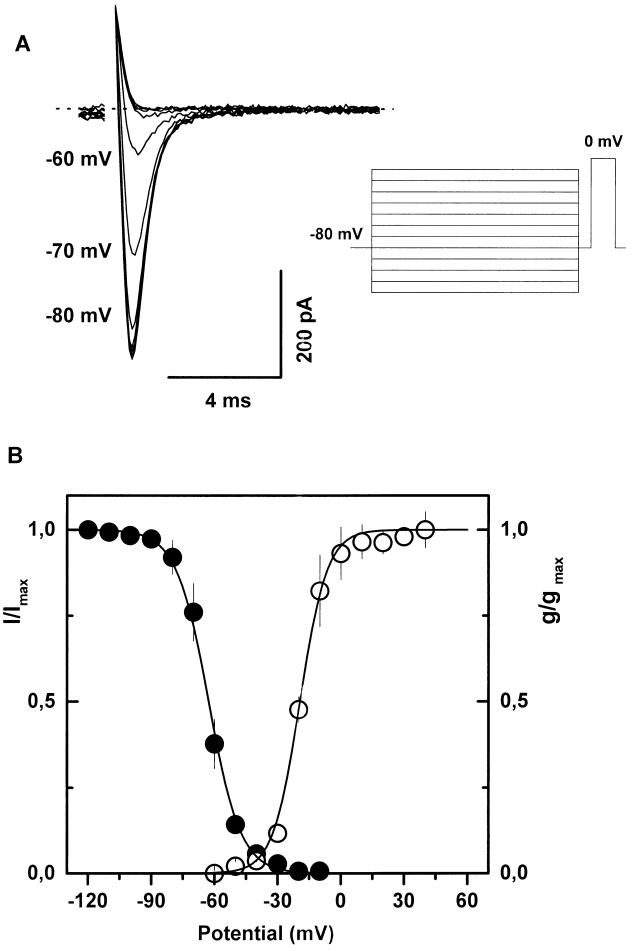

Fig. 4. Activation and steady-state inactivation of I_{Na}. Superimposed current traces were elicited by test pulse to 0 mV following to conditioning pulses (A). Schematic voltage protocol shown beside (see text for the details). Numbers beside some traces indicate potentials of conditioning prepulses. Mean inactivation (closed circles, $n = 6$) and activation (open circles, $n = 6$) curves (B). Inactivation curve was plotted as the normalised peak current elicited at 0 mV against the potential of the conditioning prepulse. Activation curve represents normalised conductance (g/g_{max}) plotted as a function of potential. Solid lines are non-linear least squares fittings of Boltzmann equations Eqs. 1 and 3 to the data.

3.2. Properties of I_{Ca}

In experiments carried out to study the characteristics of I_{Ca}, Na^+ conductance was eliminated by either substitution of Na^+ with TEA^+ or by addition to bath solution of TTX (1 μM). Under such conditions, depolarisation from a holding potential of −80 mV produced a I_{Ca} that was activated at about −40 mV and peaked at 0 or +10 mV. The inward

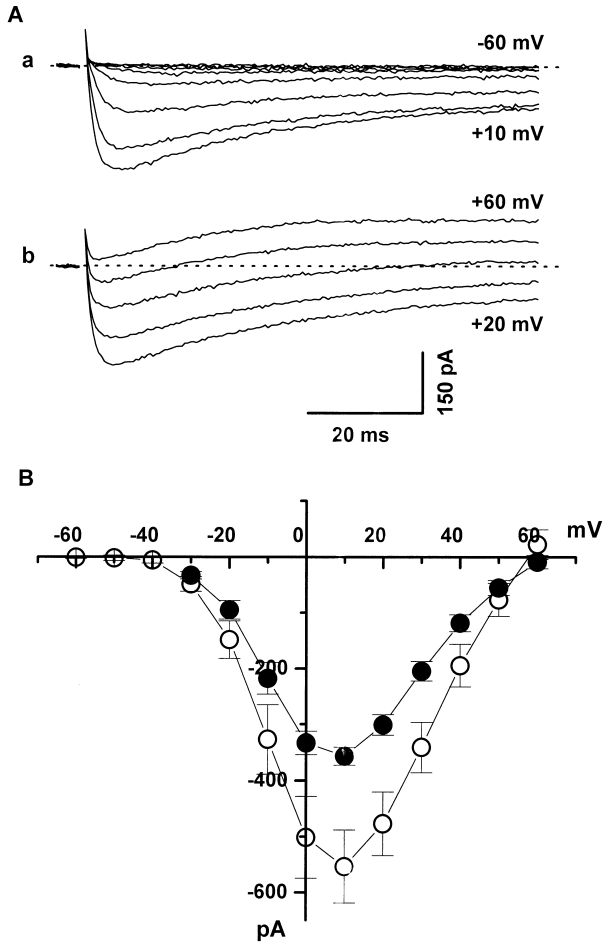

Fig. 5. Voltage-gated inward currents in the presence of 1 mM TTX. Current traces evoked by 80 ms depolarising steps from −80 mV to −60...+10 (A, a) and to +20...+60 (A, b) mV with 10 mV increment. Average ($n=6$) current-voltage relationship for amplitude of slow inward currents evoked from holding potential −80 (open circles) and −40 (closed circles) mV (B).

currents recorded on the rising portion of the *I–V* curve (from about −40 to 10 mV) increased smoothly to a single maximum value with no secondary 'hump' in their voltage dependence (Fig. 5). As shown in Fig. 5B, reduction of holding potential from −80 to −40 mV caused no shift in *I–V* relations. With a holding potential of −80 mV, the maximum I_{Ca} was recorded at depolarisation step to +10 mV and had average density -11.4 ± 2.3 pA/pF ($n=12$).

The I_{Ca}, elicited by depolarisation from holding potential −80 mV to −40...+40 mV, was completely blocked by nifedipine (10 μM), was not affected by $NiCl_2$ (25 μM) and increased about two-fold upon equimolar substitution of Ca^{2+} (2.5 mM) by Ba^{2+} at the whole range of potentials studied (data not shown). Thus, we suggested that I_{Ca} in SMCs from rat vas deferens carried mainly through L-type Ca^{2+} channels.

Recovery from inactivation of I_{Ca} was studied using a two-pulse protocol. A 1100 ms depolarising pulse to 0 mV eliciting I_{Ca} was followed, after a variable interpulse interval, by a test pulse to the same membrane potential. Fig. 6A demonstrates time dependence of I_{Ca} restoration at a holding potential of −60 mV. At this holding potential I_{Ca} recovery from inactivation well fitted to two exponentials with time constants (τ) 374 ± 50 and 2990 ± 746 ms ($n=7$).

Inactivation of I_{Ca}, evoked by 2000 ms depolarisation pulse from −80 to 10 mV was reasonably fitted to the sum of two exponentials: $\tau_1=42\pm5$ and $\tau_2=507\pm63$ ms ($n=5$, data not shown).

To study voltage dependence of I_{Ca} availability we used voltage protocol shown in Fig. 6B. Amplitudes of I_{Ca} evoked by 100 ms test pulses were normalised to that of I_{Ca} produced by control voltage steps from a holding potential −80 mV and plotted as a function of conditioning interpulse potentials ranging from −100 to +30 mV. The dependence of relative I_{Ca} amplitude on corresponding conditioning interpulse potential was well described by the Boltzmann equation of the form:

$$I/I_{max}=(1-C)/(1+\exp[V_c-V_{0.5}]/k)+C \qquad (4)$$

where $V_{0.5}=-32.5$ mV, $k=7.3$ mV, and $C=0.07$.

The I_{Ca} activation curve (Fig. 6B) was derived from *I–V* relation by calculating the Ca^{2+} conductance at each test potential used. The mean reversal potential for I_{Ca} estimated by interpolation of the linear portion of the *I–V* curves, was 58.4 ± 3.6 mV ($n=13$). The solid line through the activation curve (see Fig. 6B) is a least squares fit of the data to the Boltzmann equation:

$$g_{Ca}/g_{Ca(max)}=1/(1+\exp[V_{0.5}-V]/k) \qquad (5)$$

where $V_{0.5}$ was −6.4 mV, and $k=7.6$ mV.

3.3. Effect of intracellular nucleotides on I_{Ca} and I_{Na}

It is generally established that the basal activity of L-type Ca^{2+} channels in visceral smooth muscle cells

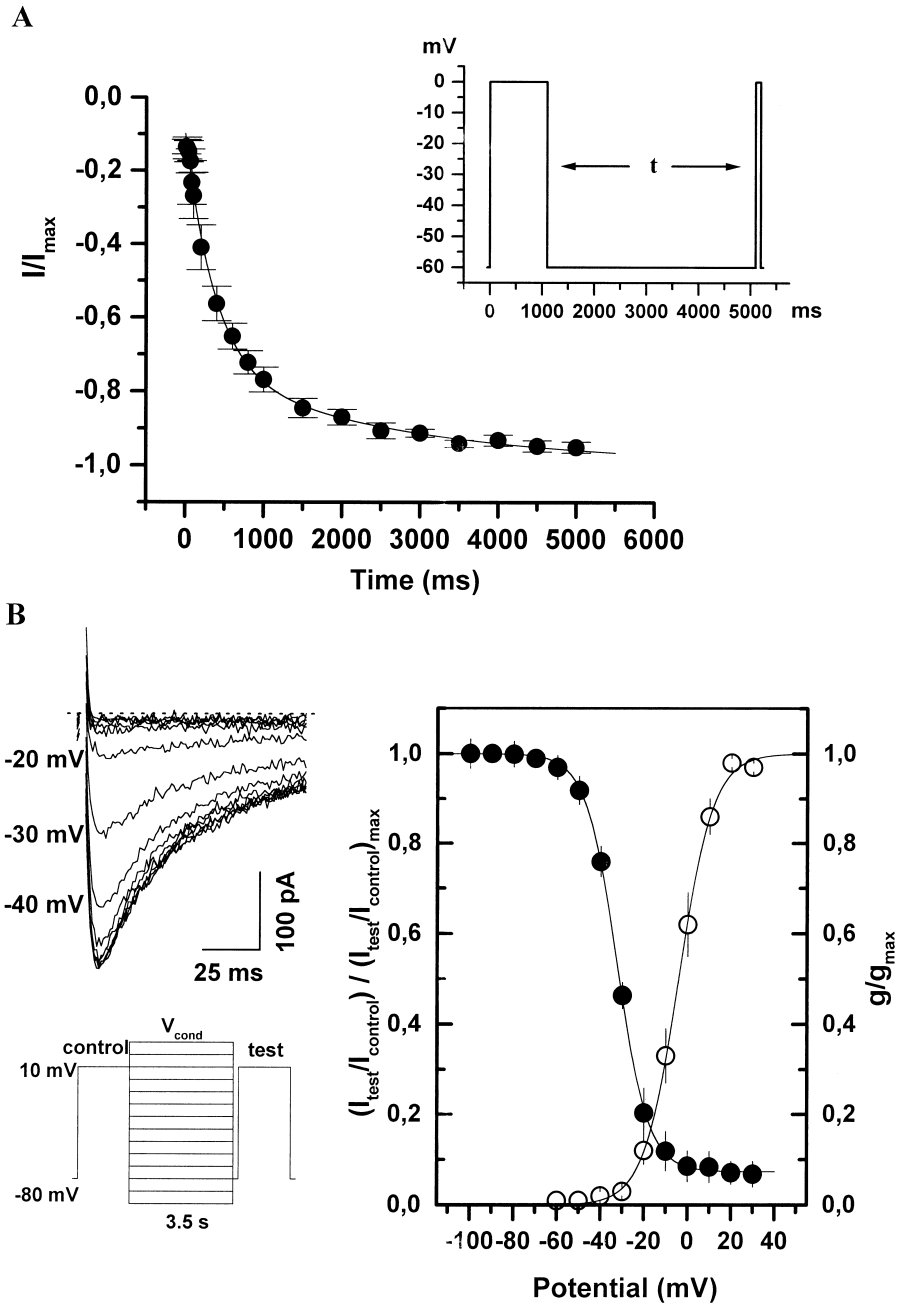

Fig. 6. Voltage-dependent characteristics of I_{Ca}. Average ($n=7$) time-course of I_{Ca} peak recovery from inactivation at holding potential −60 mV (A). The continuous line represents two exponentials fitting to data with time constants 374 ± 50 and 2990 ± 746 ms. Schematic voltage protocol is shown beside. Activation and steady-state inactivation of I_{Ca} (B). The activation curve (open circles) represents normalised conductance (g/g_{max}) plotted as a function of potential ($n=6$). The inactivation curve (closed circles) was calculated as a normalised ratio of I_{Ca} amplitudes evoked by test step to control at each conditioning potential (V_{cond}) and plotted against potential of conditioning pulse. Schematic voltage protocol and original traces of I_{Ca} activated in response to test pulse are shown on the left. Solid lines are non-linear least squares fittings of Boltzmann equations Eqs. 4 and 5 to the data.

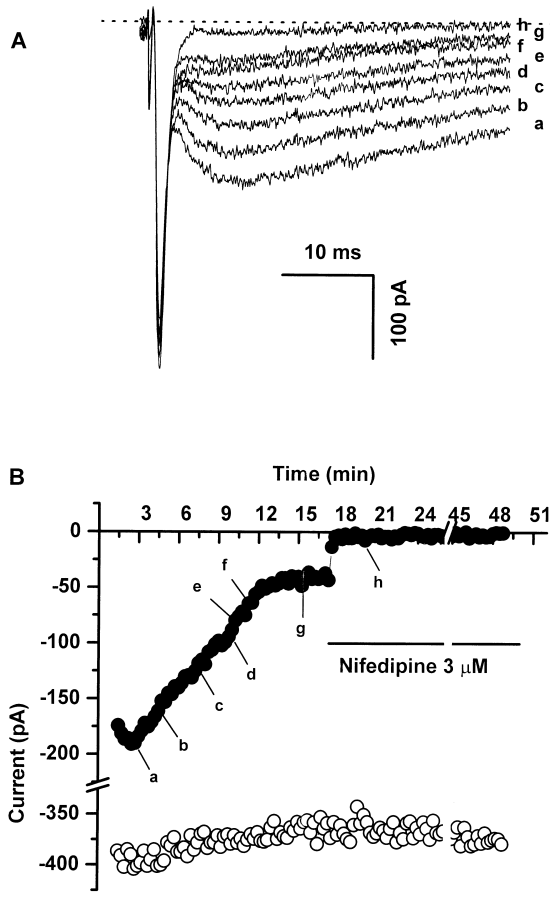

Fig. 7. Dependence of whole-cell I_{Ca} and I_{Na} on intracellular ATP and GTP. Inward currents were elicited by 40 ms depolarising steps from −90 to 0 mV (A). ATP and GTP were omitted from pipette solution (see Section 2). Letter near each trace corresponds to letter in B and indicates time after seal breaking. Time-courses of amplitudes of fast (I_{Na}, open circles) and slow (I_{Ca}, closed circles) components of inward current during cell dialysis. Peaks of the currents were measured every 15 s. Ordinate axe has break region from −325 to −225 pA; abscissa axe breaks from 26th to 45th min.

significantly depends on phosphorylation processes [15]. Under our experimental conditions, with 1 mM ATP and 0.2 mM GTP in a pipette solution, I_{Ca} displayed slight (< 10%) decrease during 20–30 min of intracellular dialysis. To study possible contribution of phosphorylation processes in basal I_{Ca} and I_{Na}, ATP and GTP were omitted from pipette solution. Under such conditions, a significant reduction of I_{Ca} amplitude has been observed during the first 15 min after establishing the whole-cell configuration: it decreased by 81.3 ± 4.6% ($n = 4$) by 12 min compared to maximal I_{Ca} amplitude recorded at the third min after seal breaking. In contrast, I_{Na} has not been drastically changed during 25 min of cell dialysis. Moreover, in two cells, in which steady recordings of both inward currents have been performed during more than 50 min, I_{Na} amplitude, measured at the fiftieth min, changed by less than 10% compared to the amplitude recorded at the third min after seal breaking (Fig. 7).

4. Discussion

In the present study we demonstrated that voltage-gated inward current in single SMCs from epididymal part of rat vas deferens consists of a fast activating followed by a slow activating component. The fast component of inward current appears to be carried through voltage-gated TTX-sensitive Na^+ channels. This conclusion is based on the following characteristics of the fast component: tolerance to substitution of Ca^{2+} by Co^{2+}; elimination upon removal of Na^+ from bath solution; high sensitivity to TTX ($K_d = 6.7$ nM); fast kinetics of activation and inactivation. In addition, it had a reversal potential close to that predicted by Nernst equation for purely Na^+-selective conductance. Moreover, the fast component was not sensitive to 10 μM of nifedipine and to 50 μM Ni^{2+} ($n = 4$, data not shown). It is worthwhile to note that the presence of fast Na^+ channel currents have been reported in a number of isolated SMCs from visceral tissues, such as rat stomach fundus and guinea pig ureter [6], rat ileum [3], rat and human colon [7], and pregnant rat uterus [16]. Recently TTX-sensitive I_{Na} has been reported in sheep lymphatic SMCs [12].

By high sensitivity of Na^+ channels in rat vas deferens SMCs to TTX, they can be related to the group of Na^+ channels observed in rat ileum ($K_d = 4.5$ nM) [3], guinea pig ureter ($K_d = 11$ nM) [6], pregnant rat uterus ($K_d = 27$ nM) [16], human colon ($K_d = 14$ nM) [7] and sheep lymphatic ducts ($K_d = 17$ nM) [12]. Key voltage-dependent characteristics of I_{Na} obtained in our study, such as: threshold of activation −40 mV, maximum −10 or 0 mV, potential of half-inactivation −62.7 mV are also in a good agreement with data reported by other authors for TTX-sensitive I_{Na} e.g. [3,7].

The slow component of voltage-gated inward cur-

rent in SMCs of rat vas deferens was shown to be Ca^{2+} channel current, since it was abolished upon substitution of Ca^{2+} with Co^{2+} and completely blocked by nifedipine (10 μM). When I_{Na} was eliminated by TTX or by Na^+ removal from bath solution, I_{Ca} activated at −40...−35 mV and had single maximum at +10 mV at both holding level used (−80 and −40 mV). Potentials of half-inactivation and half-activation of I_{Ca} were found to be −32.5 and −4.2 mV respectively. Taking into account the fact that I_{Ca} was not affected by Ni^{2+} (25 μM), we suggested that I_{Ca} in SMCs of epididymal portion of rat vas deferens is carried mainly through L-type Ca^{2+} channels. This is in a good consistency with previous studies of I_{Ca} in rat vas deferens SMCs performed by Nakazawa et al. [14,17,18] where no indication of T-type Ca^{2+} channels has been observed. It should be noted that in initial studies of electrophysiological properties of single SMCs from rat vas deferens, by Nakazawa et al. [14], the existence of I_{Na} has not been reported. This contradiction can be accounted for by several reasons: (1) holding potential used in that study was reported to be −60 mV at which more than 50% of Na^+ channels are in inactivated state; (2) gradient for Na^+ used by Nakazawa et al. was (in mM): $140_{out}/5_{in}$ or $140_{out}/7_{in}$ versus $120_{out}/2_{in}$ or $120_{out}/0.2_{in}$ used in our study. Even when we used Na^+ gradient (in mM) $120_{out}/2_{in}$ I_{Na} density was found to be -1.3 ± 0.8 pA/pF, while I_{Ca} had average density -11.4 ± 2.3 pA/pF. So, in many cases I_{Na} was significantly masked by I_{Ca} and could not be clearly observed in total voltage-gated inward current.

It is commonly accepted that phosphorylation of ion channel proteins by protein kinases can significantly affect the activity of voltage-gated Ca^{2+} and Na^+ channels (reviewed in [19]). But in major cases, experimental evidences about regulation of I_{Na} by phosphorylation were derived from studies performed on neurones and cardiomyocytes. To the best of our knowledge, for now, there are no available data concerning the effect of phosphorylation processes on the activity of TTX-sensitive Na^+ channels in SMCs. In contrast, strong metabolic dependence of L-type Ca^{2+} channels activity in SMCs has been extensively studied (see review [15]). For example, it has been shown that basal Ca^{2+} current through L-type Ca^{2+} channels in *Taenia coli* SMCs is stimulated by protein kinase C (PKC)- and protein kinase A (PKA)-dependent phosphorylation processes [20]. Tyrosine kinases also were reported to be involved in tonic stimulation of L-type Ca^{2+} channels activity in SMCs obtained from pregnant rat uterus [21]. In the present study, in controls, using basic pipette solution (see Section 2), a steady I_{Ca}, with slight ($< 10\%$), if any, rundown, could be usually recorded for 20–30 min after seal breaking. But omission of ATP and GTP from pipette solution resulted in a significant (more than 80%) rundown of I_{Ca} amplitude by 15 min of cell dialysis, while I_{Na} displayed no changes within 50 min 'wash out' of intracellular content. It has been previously reported by Nakazawa et al. [14] that I_{Ca} in SMCs from rat vas deferens almost completely disappeared within 10 min of myocytes dialysis by pipette solution containing no macroergic compounds. Such drastic changes in I_{Ca} can serve as an indicator that phosphorylation processes are indeed inhibited when pipette solution does not contain both ATP and GTP. This allowed us to conclude that basal I_{Na} in SMCs from rat vas deferens does not depend upon phosphorylation processes. This is in contrast with the finding that Na^+ channels in primary culture of rat brain neurones are substantially phosphorylated under basal condition [22]. It should be mentioned that while in neurones a capital contribution of Na^+ influx through Na^+ channels to generation of action potential is well documented, in SMCs the physiological implication of TTX-sensitive Na^+ channels has not been clearly defined. Using microelectrode technique, Holman et al. [23] suggested that 'the activation of a TTX-sensitive channel carrying inward current can lead to a regenerative response following depolarisation in the mouse vas deferens'. But this observation, as was stressed by authors, was made during impalements of some but not all SMCs. Muraki and coworkers [6] demonstrated that I_{Na} can facilitate triggering of AP and accelerate its development by rapid activation of I_{Ca} in SMCs from guinea pig ureter and rat stomach fundus. Only in a recent paper, Hollywood et al. [12] have reported that I_{Na} alone was sufficient to maintain AP generation in lymphatic SMCs. Using current-clamp mode, we found that the resting potential of SMCs from epididymal part of rat vas deferens is -43 ± 5 mV ($n = 6$, author's unpublished observation). At this membrane poten-

tial more than 90% of Na^+ channels are in inactivated state (Fig. 4B), while about 80% of Ca^{2+} channels are available for activation (Fig. 6B). Considering that the I_{Ca} density is about 10 times higher than that of I_{Na}, it is unlikely that Na^+ influx through TTX-sensitive Na^+ channels contribute to AP generation. In present, to get clear inside into the ionic mechanism of rat vas deferens SMCs excitation the contribution of Na^+, Ca^{2+} and K^+ conductances in AP generation is under investigation.

In summary, we found that voltage-gated inward current in SMCs isolated from epididymal part of rat vas deferens is carried through both TTX-sensitive Na^+ channels and L-type Ca^{2+} channels. In contrast to I_{Ca}, basal I_{Na} does not depend upon phosphorylation processes. And though basal I_{Na} in SMCs from rat vas deferens does not seem to be regulated by phosphorylation, we feel that further investigation should be directed to clarify whether this is true under conditions of plasma membrane receptor stimulation.

Acknowledgements

This work was supported by National Academy of Sciences of Ukraine, and FAPESP (Brazil).

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