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Non-selective cation channels in cerebrovascular smooth muscle cells of adult rats

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Abstract

Inside-out patch clamp recordings have revealed the presence of a novel, large conductance channel of the non-selective, cation permeable type in smooth muscle cells dispersed from the cerebral arteries of adult rats. In physiologically appropriate ionic gradients, current flow in these channels reversed polarity at a membrane potential of about −42 mV. Single channel conductance in symmetrical 140 mM K^+ salines was 211 pS. The channel was permeable to both K^+ and Na^+, with a ratio $P_{Na}/P_K = 0.15$, while Cl^- was effectively impermeant. Calcium ions were weakly permeant ($P_{Ca}/P_K = 0.03$, $P_{Ca}/P_{Na} = 0.20$). Channel open probability increased with membrane depolarization and was weakly dependent on the concentration of free intracellular Ca^{2+}. This channel would contribute outward membrane current at potentials more positive than about −42 mV. In concert with outward potassium currents, it may serve to limit membrane depolarization during action potential activity in cerebrovascular smooth muscle cells.

Keywords: Cerebral artery; Smooth muscle; Cation permeable channel; Patch clamp

1. Introduction

Blood flow to the brain and other organs is partly determined by the contractile state of vascular smooth muscle cells (VSMCs) in the walls of arteries and arterioles. The contractile state of VSMCs reflects in part the balance of inward and outward current across the VSMC membrane [1–4]. In general, inwardly directed currents depolarize the VSMC membrane and promote the influx of Ca^{2+} through voltage-operated calcium channels (VOCCs). The attendant increase in the concentration of free sarcoplasmic calcium ions, $[Ca^{2+}]_i$ activates the contractile apparatus of VSMCs [5,6].

Voltage-dependent sodium channels, though common in many other excitable tissues, are rarely found in VSMCs [7]. Rather, inward membrane current in VSMCs is carried by calcium-selective channels [8,9], by chloride channels [10,11] and by a variety of non-selective cation (NSC) channels, which exclude anions but are permeable in some degree to both monovalent and divalent cations [12–14].

NSC channels of large conductance (200 pS) occur in the membrane of VSMCs isolated from rat portal veins [15]. These channels are highly permeable to Ca^{2+} and are strongly activated by increases in $[Ca^{2+}]_i$. They may aid in the replenishment of intracellular Ca^{2+} stores during contractile activity, and may also play a role in the activation of VSMCs by some vasoconstrictors [15].

In contrast, the VSMCs of porcine coronary artery contain a small conductance (30 pS) type of NSC channel which is activated when the muscle cell membrane is stretched [16]. Mechanosensory channels of this type may play a role in the myogenic response [2,9,16] of arteries to passive distension.

Previous studies on vascular NSC channels have been carried out on vessels of the systemic circulation. In consequence, little is presently known about the properties and function of NSC channels in cerebrovascular smooth muscle cells (CVSMCs). This study describes a large conductance NSC channel present in the membrane of rat CVSMCs and differing in important respects from NSC channels reported in systemic vessels.

2. Materials and methods

CVSMCs were dissociated from basilar, middle and posterior cerebral arteries of adult Wistar rats, 250–300 g

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[17–19]. Under pentobarbital anesthesia (30 mg/kg), rats were decapitated and the arteries removed using aseptic techniques. Arteries were cut into small pieces and incubated for 10 min at 37° C in 0.1% trypsin (Type 3, Sigma, St. Louis, MO) dissolved in potassium glutamate (KG) solution containing (in mM): 140 potassium glutamate; 16 $NaHCO_3$; 16.5 dextrose; 0.5 NaH_2PO_4 and 25 Hepes, pH 7.4.

Artery fragments were washed in KG solution containing 0.5% trypsin inhibitor (Sigma) and incubated for 15 min at 37° C in KG saline with 0.3% collagenase (Type 1A, Sigma). On dispersion, cells were washed in Minimum Essential Medium (Gibco, Burlington, Ontario) and resuspended in a balanced salt solution containing (in mM): 130 NaCl; 5 KCl; 0.8 $CaCl_2$; 1.3 $MgCl_2$; 5 glucose; 10 Hepes; pH 7.4. Cells were plated onto glass coverslips pre-coated with poly(D-lysine) and laminin (Sigma), kept at 4° C and used within 48 h of dispersion. CVSMCs were identified using a monoclonal antibody (Sigma) directed against smooth muscle α-actin [19].

Patch clamp recordings took place at room temperature (21–23° C) using a List EPC-7 amplifier and patch electrodes (10–20 MΩ resistance when filled with 140 mM KCl). The use of room temperature for recording facilitated comparison of single channel currents with previous data obtained under similar conditions. Standard methods were used to obtain inside-out membrane patches from dispersed CVSMCs [18,20]. The external membrane face was typically exposed to a standard external saline of composition 140 NaCl; 5 KCl; 1.8 $CaCl_2$; 1.2 $MgCl_2$; 10 Hepes, pH 7.4. The cytoplasmic membrane face was usually bathed in standard internal saline of composition: 140 KCl; 5 NaCl; 3 EGTA; 1.72 $CaCl_2$; 10 Hepes, free calcium concentration, $[Ca^{2+}]_i = 50$ nM [21]. All solutions were brought to pH 7.4 using *N*-methylglucamine (Sigma) to avoid changes in K^+ or Na^+ concentrations. Membrane voltages were corrected for the effects of liquid junction potentials arising during solution changes, and these potentials did not exceed 5 mV in amplitude.

Patch current and voltage were recorded on FM Wideband tape, digitized at 8 kHz and subjected to a Gaussian digital filter of bandwidth DC-2 kHz. Off-line data analysis was performed on an Atari Mega 4 computer using software devised by Instrutech Corporation, New York. The threshold for event detection was set at 50% of the mean current in open NSC channels. The probability, P_o of a single NSC channel being open during a recording of duration T_{tot} was calculated from the expression $P_o = (T_1 + 2.T_2 + ... + NT_N)/NT_{tot}$ where N is the number of functional NSC channels in the patch. T_1, $T_2 ... T_N$ are the times for which at least 1,2.... N channels were open. N was estimated at a membrane potential of +60 mV. All data were required to reach a level of significance of $P < 0.05$ using the Student's *t*-test for paired data, or the ANOVA test for unpaired data, as appropriate.

For those patches containing a single NSC channel, open and closed time distributions were plotted using a $\log_{10}$ time axis, transforming the exponential function $y = Ae^{-t/TAU}$ into a curve with peak amplitude at the time constant, *TAU* [22]. Frequency response correction was applied to channel openings shorter than $1/2\pi f_c$, where f_c was the −3 dB frequency of the signal filter, 2 kHz [23]. These distributions were fitted by sums of exponential terms using Simplex maximization of likelihood [23]. Simplex methods were used to fit Gaussian terms to frequency distributions of single channel current amplitudes.

3. Results

Fig. 1 shows the typical appearance of currents flowing in single NSC channels, as recorded from an inside-out patch of CVSMC membrane bathed in standard external and internal salines. The membrane potential of this patch was stepped to the values shown at the left of each trace. From these data, it can be seen that current flow in NSC channels reversed polarity at a membrane potential of about −45 mV.

Inspection of these traces also showed that the conductance of single NSC channels was large, and that channel

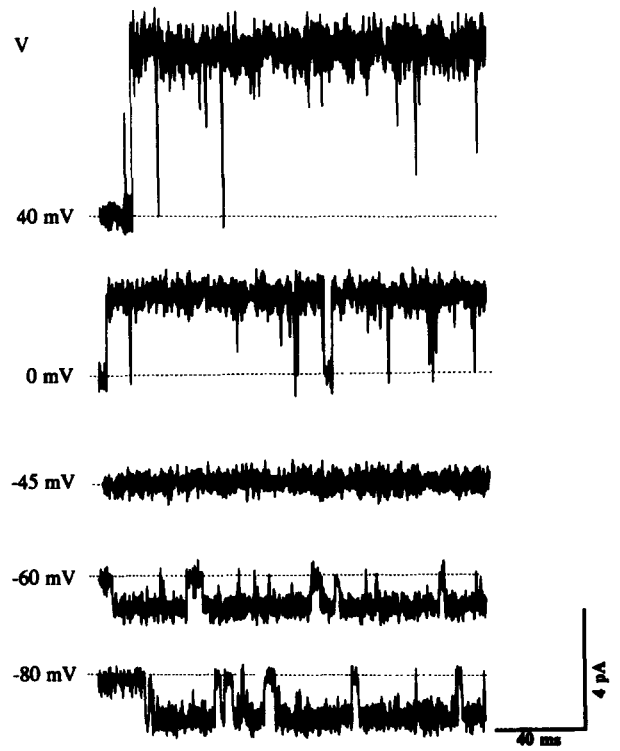

Fig. 1. Currents flowing in a single NSC channel at the membrane potential, *V* indicated to the left of each trace. These traces were obtained from an inside-out patch exposed to the standard external and internal salines defined in Materials and methods. The zero current levels at each potential are denoted by the dashed lines, which indicate when the channel was closed. Note that current flow reversed polarity from outward (upward deflection from baseline) to inward (downward deflection) at a membrane potential of about $V = -45$ mV.

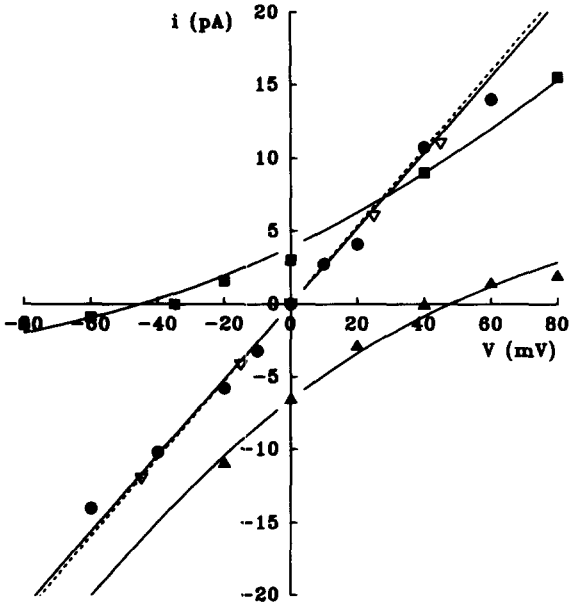

Fig. 2. Current–voltage (I–V) relations of NSC channels recorded in inside-out patches under the following ionic conditions. Standard external and internal salines (filled squares). Data were fitted by the constant field current equation, assuming $P_{Na}/P_K = 0.18$ (solid line through data points). With $[Na^+]_o = 5$ mM, $[K^+]_o = 140$ mM, $[Na^+]_i = 140$ mM and $[K^+]_i = 5$ mM (filled triangles). Data again fitted by the constant-field equation, assuming that $P_{Na}/P_K = 0.12$ (solid line). Symmetrical 140 mM KCl salines with no Na^+ present (filled circles). The least squares regression fit (solid line) yielded a single channel conductance of 211 pS and a reversal potential of 0 mV. Reducing the concentration of internal Cl^- to 5 mM (replacement with equimolar potassium gluconate) did not significantly alter the conductance or reversal potential (data, open triangles and least squares regression fit, dashed line).

open probability was high and increased slightly at depolarized membrane potentials. Furthermore, channel activation did not require application of suction or pressure to the membrane patch, showing that this NSC channel was not of the stretch-activated type. Currents showing these properties were seen in 32/126 patches examined. In most cases, active patches contained a single NSC channel, and the maximum number of functional NSC channels seen in one patch was four.

In the standard, physiologically appropriate salines used in the previous figure, the current–voltage relation of single NSC channels showed outward rectification around a mean reversal potential of -42 ± 3.7 mV ($n = 7$, see Fig. 2, closed squares). When the normal sodium and potassium gradients were inverted, so that $[Na^+]_o = 5$ mM, $[K^+]_o = 140$ mM, $[Na^+]_i = 140$ mM and $[K^+]_i = 5$ mM, the current–voltage relation showed inward rectification around a reversal potential of +45 mV (Fig. 2, closed triangles).

In symmetrical 140 mM KCl salines with no Na^+ present, the current–voltage relation was linear with a reversal potential of 0 mV and a single channel conductance of 211 ± 10.7 pS, (Fig. 2, filled circles). Reducing the concentration of internal Cl^- to 5 mM (replacement with equimolar potassium gluconate) did not significantly alter the conductance or reversal potential measured in symmetrical 140 mM K^+ solutions (Fig. 2, open triangles).

Currents which flowed in single NSC channels under the various ionic conditions pertaining in Fig. 2 were well predicted by Goldman-Hodgkin-Katz constant-field equations [24] (Fig. 2, continuous lines). For data obtained in physiological or inverted Na^+/K^+ gradients, these lines were drawn to the equation

$$i = VF^2P_K/RT\left(([K^+]_o + P_{Na}/P_K[Na^+]_o) - ([K^+]_i + P_{Na}/P_K[Na^+]_i)e^{VF/RT}\right) / (1 - e^{VF/RT})$$

Here, i is single channel current, V is membrane voltage, P_{Na}/P_K is the ratio of sodium to potassium permeabilities through the open channel, and R, T and F have their normal meanings. In physiological ionic gradients, the best fit to the observed currents was obtained when $P_{Na}/P_K = 0.18$. In the inverted sodium and potassium gradients described previously, a best fit ratio of $P_{Na}/P_K = 0.12$ was obtained, yielding an average estimate of $P_{Na}/P_K = 0.15$ for normal and inverted ionic gradients.

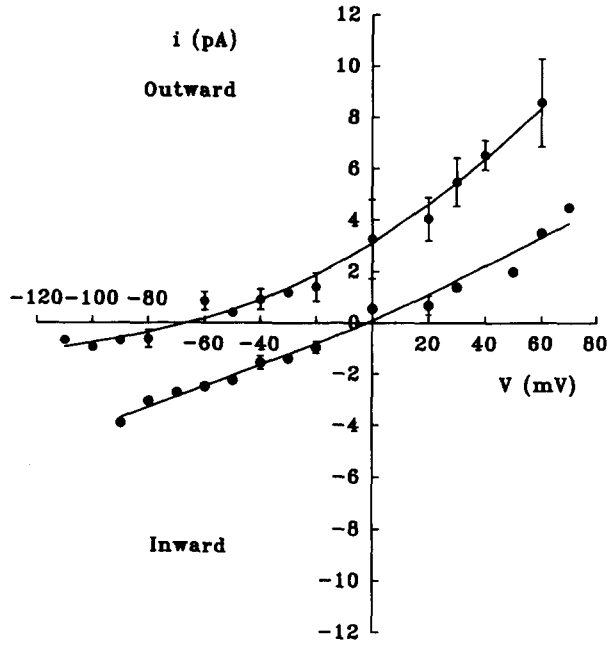

Fig. 3. Permeability of NSC channels to Ca^{2+} relative to K^+ (upper data) and to Na^+ (lower data). For the upper data, the external membrane face was bathed in a saline of composition (mM): 93 $CaCl_2$; 10 Hepes, while the cytoplasmic membrane face was exposed to (in mM) 140 KCl; 10 Hepes and 50 nM $CaCl_2$. Data points represent mean ± S.E. for five patches. The smooth curve fitted by least-squares regression to these points was drawn to the constant field expression assuming $P_{Ca}/P_K = 0.03$. For the lower data, the external membrane face was bathed in a saline containing (in mM): 93 $CaCl_2$; 10 Hepes, while the cytoplasmic membrane face was exposed to (mM): 140 NaCl; 10 Hepes and 50 nM $CaCl_2$. Data points represent the mean ± S.E. for four patches. The smooth curve fitted by least-squares regression was drawn to the constant field relation assuming $P_{Ca}/P_{Na} = 0.27$.

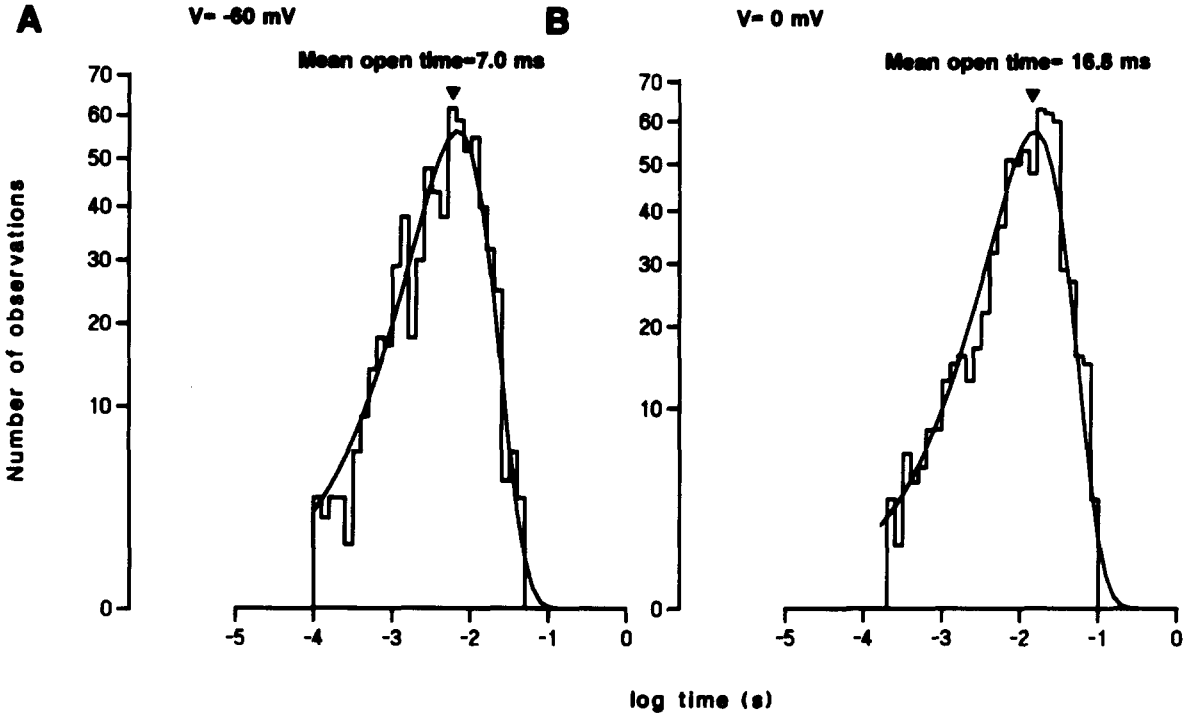

Fig. 4. The distribution of open times for a single NSC channel studied in a membrane patch voltage-clamped to a potential, V of -60 mV (A) or 0 mV (B). Note the use of a $\log_{10}$ time axis in these plots, which were constructed from 658 and 677 channel openings in (A) and (B), respectively. Both distributions were well described by a single exponential function. For the distribution in (A), the best fit was obtained with an exponential (smooth curve) of time constant 7.0 ms (arrow), while for that in (B) the smooth fit curve had a time constant of 16.8 ms. Data were recorded in standard internal and external solutions at 21° C.

The ratio P_{Cl}/P_K was estimated from data obtained when the internal chloride concentration was reduced to 5 mM, in the absence of sodium. This estimate was made using the constant-field relation

$$i = VF/RT\left(([K^+]_o + P_{Cl}/P_K[Cl^-]_i) - ([K^+]_i + P_{Cl}/P_K[Cl^-]_o)e^{VF/RT}\right) /(1 - e^{VF/RT})$$

A value of $P_{Cl}/P_K < 0.01$ was obtained. It was concluded that NSC channels are significantly permeable to both sodium and potassium ions, while their chloride permeability is negligible.

The relative permeability of NSC channels for Ca^{2+} and K^+ was determined in five patches exposed to 140 mM KCl at the cytoplasmic membrane face and 93 mM $CaCl_2$ at the external membrane face (Fig. 3, upper data). Under these conditions, inward currents were observed at membrane potentials more negative than -67 mV, showing that NSC channels were measurably permeable to Ca^{2+}. The current–voltage relation was well described by the equation $i = i_K + i_{Ca}$, where

$$i_K = F^2P_KV/RT\left(([K^+]_o - [K^+]_ie^{VF/RT}) /(1 - e^{VF/RT})\right)$$

and

$$i_{Ca} = 4F^2P_{Ca}V/RT\left(([Ca^{2+}]_o - [Ca^{2+}]_ie^{2VF/RT}) /(1 - e^{2VF/RT})\right)$$

The best fit to the observed currents was obtained when $P_{Ca}/P_K = 0.03$.

In order to estimate the ratio P_{Ca}/P_{Na}, a further series of four patches was studied with 140 mM NaCl at the cytoplasmic face and 93 mM $CaCl_2$ at the external membrane surface (Fig. 3, lower data). Under these conditions, inward membrane current was seen at potentials negative to -2 mV. The observed currents were well described by the equation $i = i_{Na} + i_{Ca}$, where these terms were as given above for the P_{Ca}/P_K determination. The best fit to the data was obtained when $P_{Ca}/P_{Na} = 0.27$, in reasonable agreement with the value of 0.20 calculated from the measured P_{Ca}/P_K and P_{Na}/P_K ratios.

As shown in Fig. 4, frequency distributions for NSC channel open times were well fitted by single exponential

Table 1
Voltage dependence of mean open time and open probability of NSC channels studied in six inside-out membrane patches exposed to standard external and internal salines

Membrane potential (V, mV)	Open probability	Mean open time (ms)
−60	0.50 ± 0.09	9.8 ± 1.2
0	0.82 ± 0.07 [b]	12.6 ± 1.5 [b]
+60	0.86 ± 0.05 [a]	16.9 ± 2.0 [a]

[a] Significantly different from value at $V = -60$ mV ($P < 0.05$, ANOVA).
[b] Not significantly different from value at $V = -60$ mV ($P > 0.05$, ANOVA).

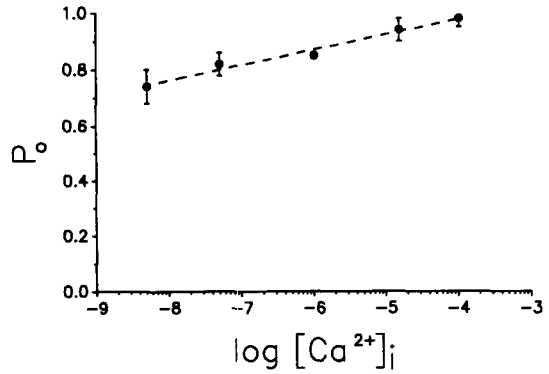

Fig. 5. Dependence of the open probability of NSC channels at $V = +60$ mV on the concentration of free calcium ions, $[Ca^{2+}]_i$ in the internal solution. Data were obtained from eight patches bathed in standard external saline, and in otherwise standard internal saline containing the indicated levels of $[Ca^{2+}]_i$. The straight line was fitted to the data points by least-squares regression.

functions, indicating that these channels displayed one kinetically resolvable open state [23]. The distribution of open times was shifted to longer durations on changing the membrane voltage from −60 mV to 0 mV, indicating that the gating of NSC channels was voltage dependent. For the distribution acquired at −60 mV, the best fit was obtained with an exponential of time constant 7.0 ms (Fig. 4A, smooth curve), while at 0 mV the fit curve had a time constant of 16.8 ms (Fig. 4B, smooth curve).

Table 1 shows the dependence of mean channel open time on membrane potential averaged from 6 patches studied in standard external and internal salines. In addition, Table 1 shows that the open probability, P_o of NSC channels increased significantly on membrane depolarization, averaging 0.50 ± 0.09 at $V = -60$ mV and 0.86 ± 0.05 at $V = +60$ mV ($P < 0.05$, ANOVA).

P_o also increased weakly on raising the concentration of free calcium ions at the cytoplasmic membrane surface, $[Ca^{2+}]_i$ (Fig. 5). However, P_o remained high, even when $[Ca^{2+}]_i$ was reduced well below physiological levels (at $[Ca^{2+}]_i = 5$ nM and $V = +60$ mV, $P_o = 0.74 \pm 0.06$, $n = 8$ patches). Therefore, this channel did not require physiological levels of $[Ca^{2+}]_i$ for opening to occur.

4. Discussion

The present study has revealed the presence of a large conductance, non-selective cation (NSC) channel in the membrane of rat cerebrovascular smooth muscle cells. NSC channels were found to be permeable to sodium, potassium and, to a small degree, calcium ions, while Cl^- was essentially impermeant. NSC channels clearly differed from the large conductance, Ca^{2+}-dependent potassium channels (BK channels) also present in these cells in their much higher Na^+ permeability and much weaker activation by intracellular Ca^{2+} [18]. In addition, analysis of open time distributions showed that NSC channels exhibit only one kinetically resolvable open state, while the BK channels of cerebrovascular smooth muscle cells follow a more complex gating scheme [18].

The open probability of NSC channels increased on membrane depolarization and the single channel current displayed outward rectification when measured in physiologically relevant solutions. These channels are therefore distinct from the non-selective, cation permeable pores which mediate inward rectification in smooth muscle cells [25].

In terms of its high conductance, the present channel closely resembles non-selective cation channels reported in smooth muscle cells isolated from two systemic vessels, namely rat portal vein [15] and porcine coronary artery [26]. Despite this similarity, however, there is evidence that these three NSC channels are in fact not identical.

Firstly, the present NSC channel was appreciably more permeable to K^+ than to Na^+ ($P_{Na}/P_K = 0.15$), while the portal vein and coronary artery channels did not discriminate between these two cations [15,26]. Secondly, both the present channel and the coronary artery channel were weakly permeable to Ca^{2+}, while the portal vein channel exhibited a very high calcium permeability relative to sodium ions ($P_{Ca}/P_{Na} = 21$) [15].

Thirdly, the present NSC channel was much less sensitive to changes in intracellular free Ca^{2+} concentration, $[Ca^{2+}]_i$ than was the case for the rat portal vein channel [15]. In relaxed CVSMCs, $[Ca^{2+}]_i$ has been estimated at 40 nM [19], increasing to a few micromolar during muscle contraction [5]. The open probability of the present NSC channel showed only an 11% increase between these two values. Non-selective cation channels which exhibit a marked dependence on $[Ca^{2+}]_i$ can be regulated by vasoactive agents which release Ca^{2+} from intracellular stores in vascular smooth muscle, such as noradrenaline [10,27] and endothelin [28]. In view of its low sensitivity to changes in $[Ca^{2+}]_i$, it seems unlikely that the present NSC channel is also regulated in this fashion.

The high conductance, non-selective cation channels found in rat portal veins may serve to replenish Ca^{2+} released from intracellular stores during muscle contraction [15]. In contrast, the low Ca^{2+} permeability and marked outward rectification exhibited by the present NSC channels suggest that their role in directly mediating influx of Ca^{2+} is relatively minor.

The present NSC channel also differs in major respects from a stretch-activated, cation permeable channel described in porcine coronary arteries [16]. The latter channel discriminated relatively poorly between Na^+, K^+ and Ca^{2+}, displayed only a moderate single channel conductance (30 pS) and required application of membrane stretch for activation [16]. The present NSC channel displayed a high open probability even in the absence of membrane stretch, suggesting that this channel does not play a major,

direct role in the myogenic response [2,9,16] of cerebral vessels.

Rodent CVSMCs display resting membrane potentials in the range of −40 mV to −60 mV and depolarizing currents elicit only small (15 mV) regenerative responses in these cells, due to the presence of strong outward currents [4,29]. NSC channels would contribute outward membrane current at potentials seen during regenerative responses in CVSMCs [4,29]. These channels may therefore serve to limit excessive membrane depolarization during action potential activity in CVSMCs, acting in concert with the outward potassium currents flowing in delayed rectifier [30,31] and calcium-dependent K^+ channels [4,18,31,32].

Acknowledgements

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