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Ca^{2+} entry blocking and contractility promoting actions of norbormide in single rat caudal artery myocytes

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1 Aim of the present study was to investigate the effects of norbormide, a selective vasoconstrictor agent of the rat peripheral vessels, on the whole-cell voltage-dependent L-type Ca^{2+} current ($I_{Ca(L)}$) of freshly isolated smooth muscle cells from the rat caudal artery, using either the conventional or the amphotericin B-perforated whole-cell patch-clamp method.

2 Norbormide decreased L-type Ca^{2+} current in a concentration- and voltage-dependent manner, without modifying the threshold and the maximum of the current-voltage relationship. Norbormide-induced $I_{Ca(L)}$ inhibition was reversible upon wash-out.

3 Norbormide both shifted the voltage dependence of the steady-state inactivation curve to more negative potentials by about 16 mV, without affecting the activation curve, and decreased the slope of inactivation. Norbormide, however, did not modify both the activation and the inactivation kinetics of the $I_{Ca(L)}$.

4 Norbormide decreased $I_{Ca(L)}$ progressively during repetitive step depolarizations, with inhibition depending on the stimulation frequency (use-dependent block) as well as on the holding potential.

5 Addition of 50 μM norbormide caused the contraction of all freshly isolated cells and also of those impaled with the perforated method, but not of those impaled with the conventional method (i.e. dialysed).

6 In conclusion, these results prove norbormide to be a vascular L-type Ca^{2+} channel inhibitor, which preferentially acts on the inactivated and/or open state of the channel. In rat caudal artery smooth muscle, however, this mechanism does not result in a vasodilating effect since it is overwhelmed by the mechanism underlying norbormide-induced vasoconstriction.

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Keywords: norbormide; L-type Ca^{2+} channel inhibitor; rat caudal artery smooth muscle; whole-cell patch-clamp

Abbreviations: G, conductance; $I_{Ca(L)}$, L-type Ca^{2+} current; PKC, protein kinase; PLC, phospholipase C; PSS, physiological salt solution; TEA, tetraethylammonium; V_h, holding potential

Introduction

Norbormide, 5-(α-hydroxy-α-2-pyridylbenzyl)-7-(α-2-pyridylbenzylidene)-5 norbormene-2,3-dicarboximide, is a synthetic compound introduced as a specific rat toxicant in 1964 (Roszkowski *et al*., 1964). This compound is endowed with unique pharmacodynamic properties since it induces, at the same concentrations, selective contraction of rat peripheral arteries and relaxation of arteries of several non-rat species so far tested, as well as rat aorta (Roszkowski, 1965; Bova *et al*., 1996). In the last years, several studies have been conducted in the attempt to identify the mechanisms underlying norbormide-induced vasoconstriction and vasorelaxation. Norbormide has been shown to elicit its selective vasoconstrictor effect by stimulating the phospholipase C (PLC)-protein kinase C (PKC) signalling cascade with promotion of Ca^{2+} influx mainly *via* verapamil-insensitive Ca^{2+} channels (Bova *et al*., 2001a). The mechanism(s) involved in norbormide-induced stimulation of PLC-PKC cascade is unknown; however, since this biochemical pathway is shared by most receptor-coupled vasoconstrictor agents, it has been hypothesized that norbormide selective vasoconstriction could be due to its interaction with a PLC-coupled receptor, uniquely expressed in the myocytes of rat terminal arteries (Bova *et al*., 2001a).

The mechanism involved in norbormide-induced relaxation of rat aorta and non-rat arteries has not been elucidated. On the basis of indirect evidences, it was proposed that the drug could induce vasorelaxation by reducing Ca^{2+} influx through voltage-dependent Ca^{2+} channels (Bova *et al*., 2001a). Such evidences come from patch-clamp studies performed on guinea-pig ventricular myocytes, in which norbormide has been shown to reduce L-type Ca^{2+} current (Bova *et al*., 1997), and from experiments conducted on guinea-pig isolated hearts (Langendorff preparation) in which it caused functional and electrocardiographic modifications similar to those induced by the Ca^{2+} entry blocker verapamil (Bova *et al*., 1997).

Albeit proposed as a safe compound to non-rat species (Roszkowski, 1965), norbormide, orally administered to healthy volunteers, only reduces systolic blood pressure (Pelfrene, 2001). In view of its possible blocking activity on vascular L-type Ca^{2+} channels, norbormide deserves further studies aimed at elucidating the mechanism of its myorelaxing effect. Consequently, this study investigated the electro-

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physiological effects of norbormide on voltage-dependent Ca^{2+} channels in single myocytes freshly isolated from the rat caudal artery. The present results show indeed that norbormide reduces $I_{Ca(L)}$, thus supporting the hypothesis that a block of L-type Ca^{2+} channels may be involved in its vasorelaxant activity and introducing norbormide as a prototype of a novel chemical class of Ca^{2+} antagonists.

Methods

Cell isolation procedure

Smooth muscle cells were freshly isolated from the tail main artery of male rats (350–450 g) by means of collagenase treatment, as previously described (Fusi *et al*., 2001). The cells were continuously superfused with physiological salt solution (PSS) using a peristaltic pump (LKB 2132) at a flow rate of 800 μl min^{-1}. Electrophysiological responses were tested at room temperature (22–24°C) only in those cells that were phase dense.

Whole-cell patch-clamp recording

Conventional (Hamill *et al*., 1981) and amphotericin B-perforated (Rae *et al*., 1991) whole-cell patch-clamp methods were employed to voltage-clamp smooth muscle cells. Recording electrodes were pulled from borosilicate glass capillaries (WPI, Berlin, Germany) and fire-polished to create a pipette resistance of 2–5 MΩ when filled with internal solution. A low-noise, high-performance Axopatch 200B (Axon Instruments, U.S.A.) patch-clamp amplifier driven by an IBM computer in conjunction with an A/D, D/A board (DigiData 1200 A/B series interface, Axon Instruments, U.S.A.) was used to generate and apply voltage pulses to the clamped cells and record the corresponding membrane currents. Current signals, after compensation for whole-cell capacitance, series resistance and liquid junction potential, were low-pass filtered at 1 kHz and digitized at 3 kHz prior to being stored on the computer hard disk. $I_{Ca(L)}$ was always recorded in 5 mM Ca^{2+}-containing PSS: this concentration was shown to cause maximal peak current in arterial smooth muscle cells (Bolton *et al*., 1988).

$I_{Ca(L)}$ was measured over a range of test potentials (250 ms) from −50 to 50 mV from a holding potential (V_h) of −50 mV. Data were collected once the current amplitude had been stabilized (usually 5–10 min after the whole-cell configuration had been obtained). $I_{Ca(L)}$ did not run down during the following 30–40 min under these conditions.

Steady-state inactivation curves, recorded twice from the same cell (in the absence and presence of the drug, respectively), were obtained using the double-pulse protocol. Once various levels of the conditioning potential had been applied for 5 s, followed by a short (5 ms) return to the V_h, a test pulse (250 ms) to 10 mV was delivered to evoke the current. Under control conditions, the 50% inactivation potentials evaluated by Boltzmann fitting of the first curve (-23.20 ± 0.27 mV, $n=3$) was not significantly different from that of the second curve recorded after 10 min (-23.96 ± 0.37 mV).

Activation curves were derived from the current-voltage relationships (see Figure 1b). Conductance (G) was calculated from the equation $G=I_{Ca}/(E_m-E_{Ca})$, where I_{Ca} is the peak current elicited by depolarizing test pulses to the various potentials and E_{Ca} is the equilibrium potential. G_{max} is the maximal Ca^{2+} conductance (calculated at potentials above 10 mV). The points for G/G_{max} were plotted against the membrane potential as relative amplitude.

To examine the use-dependent inhibition of $I_{Ca(L)}$, a train of test pulses (10 mV from a V_h of −50 or −80 mV for 50 ms) was applied at different pulse intervals, ranging from 1 to 5 s (1, 0.5, 0.33 and 0.2 Hz) before and after bath application of the drug.

K^+ currents were blocked with 30 mM tetraethylammonium (TEA) in the PSS and Cs^+ in the internal solution. Values were corrected for leakage using the P/4 protocol or 300 μM Cd^{2+} which was assumed to completely block $I_{Ca(L)}$.

Solutions and chemicals

Ca^{2+}-free PSS contained (in mM): NaCl 110, KCl 5.6, HEPES 10, taurine 20, glucose 20, $MgCl_2$ 1.2, Na-pyruvate 5 (pH 7.4). The 5 mM Ca^{2+}-containing PSS was prepared by replacing NaCl with equimolar $CaCl_2$.

The internal solution for the conventional method (pCa 8.4) consisted of (in mM): CsCl 100, HEPES 10, EGTA 11, $MgCl_2$ 2, $CaCl_2$ 1, Na-pyruvate 5, succinic acid 5, oxalacetic acid 5, Na_2ATP 3 and phosphocreatine 5, pH was adjusted to 7.4 with CsOH. For the perforated method, the internal solution (pCa 8.4) contained (in mM): CsCl 125, HEPES 10, EGTA 11, $MgCl_2$ 2, $CaCl_2$ 1, amphotericin B (200 μg/ml), pH was adjusted to 7.4 with CsOH.

Amphotericin B (100 mg/ml) was first dissolved in dimethylsulphoxide and then added to the internal solution.

The osmolarity of PSS was adjusted to 335 mosmol and that of the internal solution to 310 mosmol (Stansfeld & Mathie, 1993) by means of an osmometer (Osmostat OM 6020, Menarini Diagnostics, Italy).

The chemicals used were: collagenase (type XI), TEA, bovine serum albumin, trypsin inhibitor, amphotericin B and $CdCl_2$ (Sigma Chimica, Italy). Norbormide was a kind gift of I.N.D.I.A. S.p.a. (Padova, Italy). Norbormide dissolved directly in dimethylformamide, was diluted at least 1000 times in PSS, prior to use. The resulting concentrations of dimethylformamide (below 0.1%) failed to alter the current (data not shown). Final drug concentrations are presented in the text.

Following control measurements, each cell was exposed to drug by perfusing the experimental chamber with a drug-containing PSS.

Statistical analysis

Acquisition and analysis of data were accomplished using pClamp 8.0.1.12 software (Axon Instruments, U.S.A.) and GraphPad Prism version 3.02 (GraphPad Software, U.S.A.). Data are reported as mean±s.e.mean; n is the number of animals (indicated in parentheses). Statistical analyses and significance as measured by the Student's t test for unpaired and paired samples, as appropriate, were obtained using GraphPad InStat version 3.02 (GraphPad Software, U.S.A.). In all comparisons, $P<0.05$ was considered significant.

The current-voltage relationships were calculated on the basis of the peak values from the original currents.

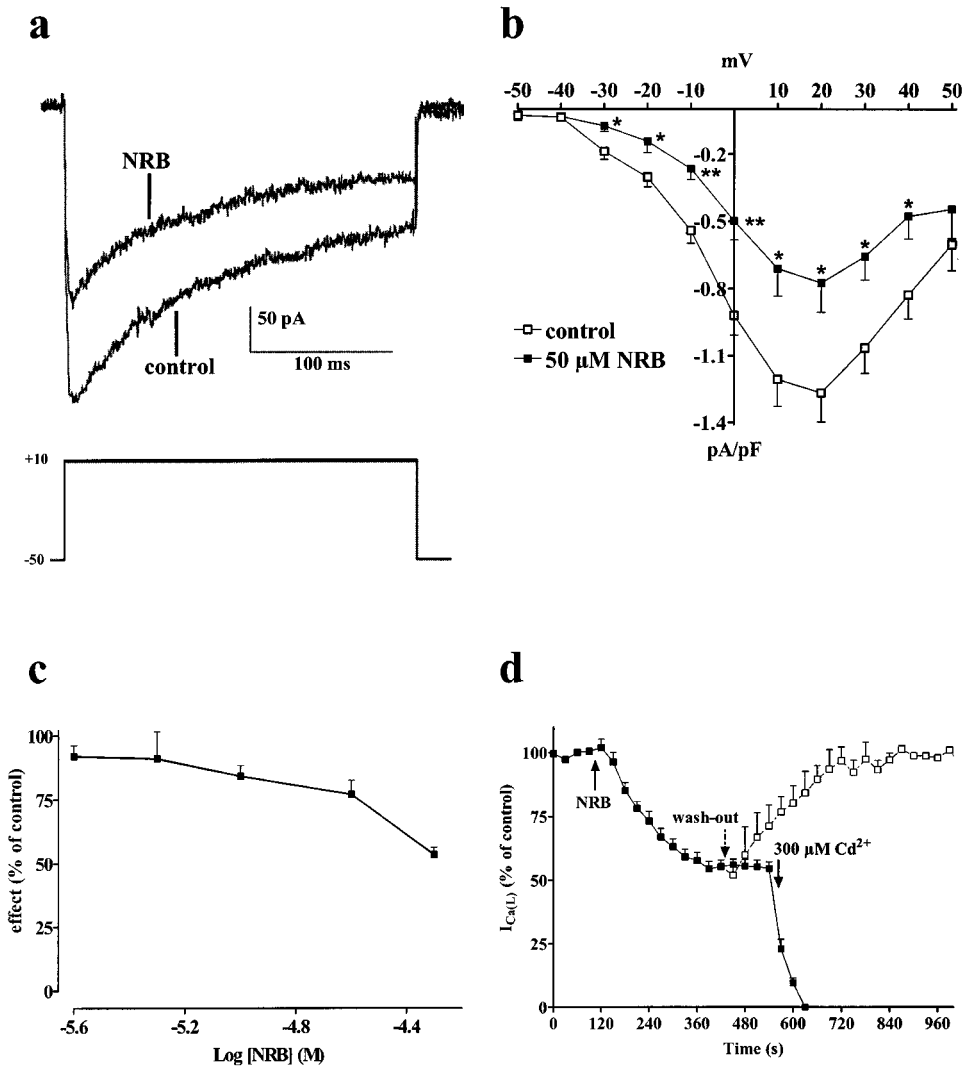

Figure 1 Inhibition by norbormide of $I_{Ca(L)}$ in rat tail artery myocytes. (a) Original recordings of conventional whole-cell $I_{Ca(L)}$ elicited with 250-ms clamp pulses to 10 mV from a V_h of -50 mV, measured in the absence (control) or presence of 50 μM norbormide (NRB). (b) Current-voltage relationships constructed prior to the addition of norbormide (control) and in the presence of 50 μM norbormide. Data points are mean$\pm$s.e.mean of six cells ($n=3$). *$P<0.05$, **$P<0.01$, Student's t test for paired samples. (c) Concentration-dependent effect of norbormide on $I_{Ca(L)}$. On the ordinate scale, response is reported as a percentage of control. Data points are mean$\pm$s.e.mean of three to 18 cells ($n=7$). (d) Time course of $I_{Ca(L)}$ inhibition induced by norbormide. Norbormide (50 μM) was applied, at the time indicated by the arrow, and peak currents were recorded during a typical depolarisation from -50 to 10 mV, applied every 30 s, and subsequently normalized according to the current recorded just prior to norbormide application. Drug wash-out (open symbols) allows for full recovery of the current. $I_{Ca(L)}$ suppression by 300 μM Cd^{2+} is also shown. Data points are mean$\pm$s.e.mean of 7–10 cells ($n=7$).

Results

Inhibitory action of norbormide on $I_{Ca(L)}$

The mean membrane capacitance of caudal artery smooth muscle cells was 54.3 ± 1.6 pF ($n=46$) and was not affected by application of norbormide.

Figure 1 shows the effect of norbormide on $I_{Ca(L)}$. Norbormide (50 μM) significantly inhibited the peak inward current at all potentials tested; this inhibition, however, was not accompanied by a significant shift of the current-voltage curve along the voltage axis. Furthermore, norbormide inhibited $I_{Ca(L)}$ in a concentration-dependent manner.

Figure 1d shows the time course of the effects of norbormide on the current. After $I_{Ca(L)}$ had reached steady values, 50 μM norbormide was added to the bath solution. This produced a gradual decrease of the current (over 5 min) that was reversible upon wash-out. A close correlation was found between maximal inhibition of $I_{Ca(L)}$ by 50 μM norbormide and both pulse frequency and V_h. In fact, $I_{Ca(L)}$ decreased to $54.9\pm4.5\%$ ($n=6$) and to $37.9\pm0.6\%$ ($n=3$, $P<0.05$) of control when test pulses were given, from V_h of -50 mV, every 30 s (i.e. at 0.033 Hz) and every 3 s (0.33 Hz), respectively. Furthermore, at 0.033 Hz, norbormide inhibited $I_{Ca(L)}$ evoked from V_h -80 mV to $75.0\pm5.5\%$ ($n=3$, $P<0.05$) of control.

Effect of norbormide on steady-state inactivation and activation curves for $I_{Ca(L)}$

The voltage dependence of norbormide inhibition was analysed by determining the steady-state inactivation curve of $I_{Ca(L)}$. Norbormide (50 μM) significantly shifted the steady-state inactivation curve to more negative potentials (Figure 2a,b). The 50% inactivation potentials evaluated by Boltzmann fitting were -22.71 ± 1.39 mV (control, $n=6$) and -38.26 ± 3.56 mV (norbormide, $n=6$, $P<0.01$, Student's t test for paired samples). Also the slope factor (-7.04 ± 0.79 mV, control) was significantly decreased by norbormide (-8.69 ± 0.96 mV, $P<0.05$). Noticeably, the blockade of $I_{Ca(L)}$ by norbormide was still evident even at negative conditioning potentials. In fact, after conditioning pulses to -80 mV for 5 s, $I_{Ca(L)}$ was still reduced to $59.6\pm4.9\%$ by 50 μM norbormide (Figure 2b).

Activation curves, obtained from the current–voltage relationships of Figure 1b and fitted to the Boltzmann equation, are shown in Figure 2a. The 50% activation potential as well as the slope factor obtained from individual experiments (0.99 ± 0.54 and 8.15 ± 0.48 mV, control, $n=5$) were not affected by 50 μM norbormide (2.45 ± 0.78 and 7.31 ± 0.37 mV, $n=4$).

Effect of norbormide on $I_{Ca(L)}$ activation and inactivation rates

The effect of norbormide on the kinetic of $I_{Ca(L)}$ activation and inactivation was analysed. Activation and inactivation rates were measured under control conditions as well as at the plateau of norbormide inhibition. The current evoked at 10 mV from V_h -50 mV activates and then declined with time courses that could be fitted by mono-exponential equations, with a τ of activation of 1.72 ± 0.11 ms and a τ of inactivation of 74.34 ± 6.57 ms ($n=6$) (Figure 2c). The addition to the perfusion medium of 50 μM norbormide had no effects on both activation and inactivation rates (τ of activation of 1.88 ± 0.21 ms and τ of inactivation of 74.21 ± 4.26 ms, $n=6$).

Use-dependent blockage of $I_{Ca(L)}$ by norbormide

To examine the possible use-dependent inhibition of $I_{Ca(L)}$ by norbormide, a train of test pulses (-50 to 10 mV for 50 ms) was applied at different pulse intervals, ranging from 1 to 5 s (namely 1, 0.5, 0.33 and 0.2 Hz) (Figure 3). In the absence of norbormide, $I_{Ca(L)}$ remained stable at 0.2 and 0.33 Hz whereas a progressive inhibition of the current amplitude during repetitive stimulation was observed at 0.5 and 1 Hz. With 50 μM norbormide, the current inhibition showed an accumulation during the repetitive stimulations at all frequencies, the higher frequency causing the greater inhibition. The use-dependent block, evaluated with the current amplitude evoked by the 20th applied stimulus was significantly lower than that observed under the corresponding control condition.

The use-dependent block of $I_{Ca(L)}$ by norbormide depended upon the V_h applied. In fact, $I_{Ca(L)}$ evoked at 0.5 Hz from V_h of -50 mV was reduced to $57.6\pm2.2\%$ of control ($n=5$) whereas that from V_h of -80 mV to $78.6\pm2.9\%$ ($n=4$, $P<0.001$, Student's t test for unpaired samples).

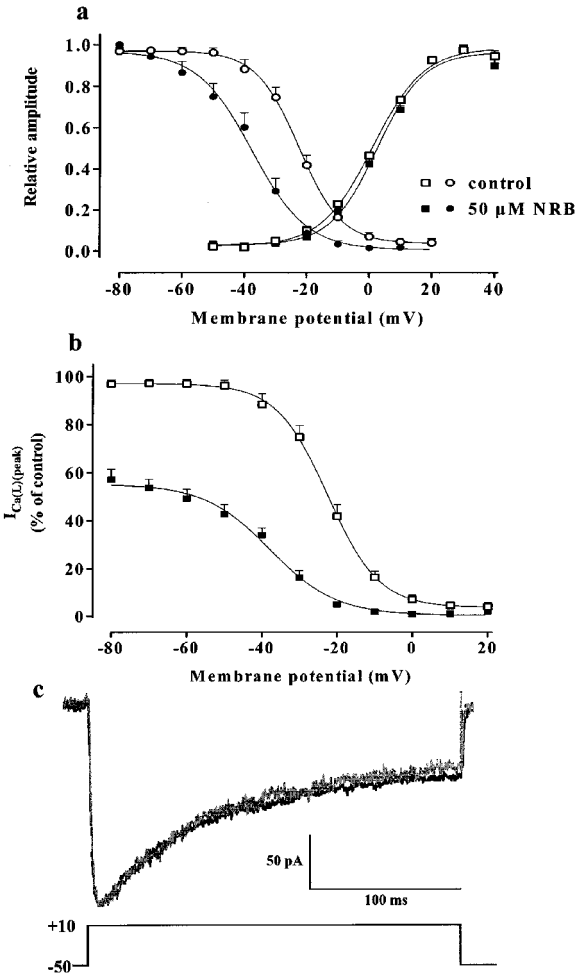

Figure 2 The effects of norbormide on both the activation and inactivation curves, and $I_{Ca(L)}$ activation and inactivation kinetics. (a,b) Steady-state inactivation curves, obtained in the absence (control) and presence of 50 μM norbormide (NRB), were fitted to the Boltzmann equation. Peak current values were used. The steady-state inactivation curve was obtained using the double-pulse protocol (see Methods section). The current measured during the test pulse is plotted against membrane potential and expressed as relative amplitude (a) and in per cent of control (b). Activation curves were obtained from the current–voltage relationships of Figure 1b and fitted to the Boltzmann equation (see Methods section). Relative amplitude is plotted against membrane potential. Each point represents the mean$\pm$s.e.mean of six cells ($n=5$). (c) Average traces measured from six cells ($n=6$) of conventional whole-cell $I_{Ca(L)}$ in rat tail artery myocytes elicited with 250 ms clamp pulses to 10 mV from a V_h of -50 mV, measured in the absence (control, black) or presence of 50 μM norbormide (grey). The drug trace is magnified (magnification is 1.84) so that the peak amplitudes of the traces before and after drug are matched. Vertical bar refers only to control trace.

Contractile effect of norbormide with conventional and perforated method

The contractile effect of norbormide was evaluated by recording the morphological changes of the cells (shortening with formation of membranous evaginations; see Ives *et al.*, 1978) with conventional as well as perforated method. As shown in Figure 4, the addition of 50 μM norbormide caused the contraction of the cells impaled only with the perforated

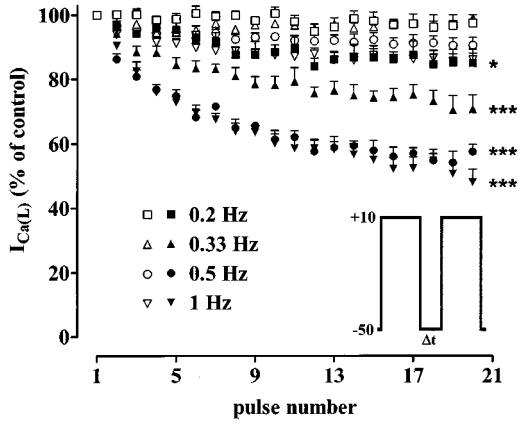

Figure 3 Use-dependent inhibition of $I_{Ca(L)}$ by norbormide. The peak current amplitude of $I_{Ca(L)}$ was measured during repetitive stimulation at intervals (Δt) of 5, 3, 2 and 1 s (0.2, 0.33, 0.5 and 1 Hz, respectively), before (control, open symbols) and 5 min after bath application of 50 μM norbormide (NRB, closed symbols). The peak current amplitudes were normalized to that induced by the first step pulse. $*P<0.05$, $***P<0.001$ (Student's *t*-test for unpaired samples) refers to the 20th pulse. Data points are mean ± s.e.mean of 4–5 cells ($n=4$).

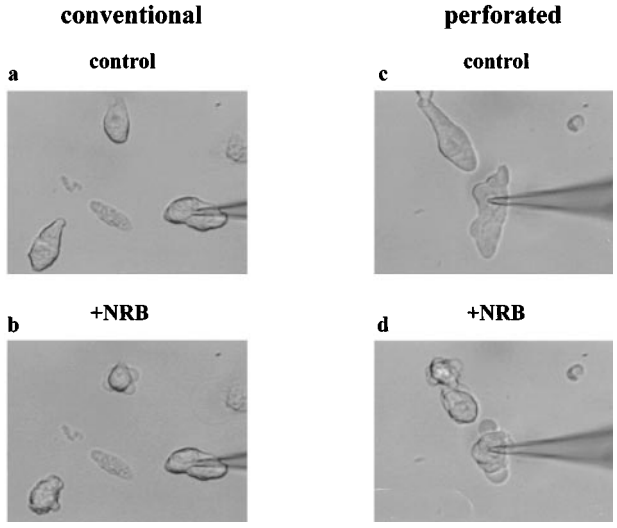

Figure 4 Contractile effect of norbormide with conventional and perforated method. Video images of rat caudal artery cells in resting state (control) and after 1 min exposure to 50 μM norbormide (NRB) recorded with the conventional (a,b) and perforated method (c,d). Images are representative of at least five experiments on five animals.

method. This determined the loss of the seal thus preventing $I_{Ca(L)}$ from being recorded in the presence of the drug. Noticeably, with the conventional method, all the cells but those impaled (i.e. dialysed) contracted after the addition of norbormide.

Discussion

This study provides the first electrophysiological evidence that norbormide is an inhibitor of vascular L-type Ca^{2+} channels, as demonstrated by the concentration-dependent inhibitory effect elicited by the drug on $I_{Ca(L)}$. This finding is in accordance with previous data obtained in cardiac tissues (Bova *et al*., 1997) and possibly accounts, at least in part, for the relaxant effect of the drug observed in rat aorta and in several non-rat arteries (Bova *et al*., 1996). In fact, we can not rule out that norbormide myorelaxing activity originates from additional target sites since the 50% inhibition of $I_{Ca(L)}$ observed at 50 μM concentration does not correlate to the full relaxation of rat aorta obtained with the same concentration (see Bova *et al*., 1996). Additional experiments on single cells isolated from vascular beds fully relaxed by norbormide are in progress in order to clarify this point.

The Ca^{2+} entry blocker effect of norbormide in vascular myocytes shows electrophysiological features which are partially shared by other well known Ca^{2+} channel inhibitors such as verapamil and nicardipine. In fact, norbormide, like verapamil (McDonald *et al*., 1994), inhibited $I_{Ca(L)}$ in a frequency-dependent fashion (use-dependent block), i.e. the repetitive depolarization with high frequency potentiated inhibition of $I_{Ca(L)}$; also, norbormide, like nicardipine (Kuriyama *et al*., 1995), shifted the voltage dependence of the inactivation curve to more negative potentials, i.e. the inhibition of $I_{Ca(L)}$ was more marked at a more depolarized holding potential. These effects indicate that norbormide stabilises Ca^{2+} channels in their inactivated state (see Bean, 1984) and that norbormide Ca^{2+} entry blocking effect is voltage-dependent, that is the extent of $I_{Ca(L)}$ inhibition is modified by changes in membrane potential. Another feature of norbormide effect is that the blockade of $I_{Ca(L)}$ was only weakened but not abolished even at negative V_h (Wegener *et al*., 2000), indicating that the blocked channels can not be restored to the available pool by hyperpolarization that normally permits drug release from the weakly binding closed–state (McDonald *et al*., 1994); this suggests that, in caudal artery myocytes, norbormide rapidly binds to the open/inactivated channel, preferentially stabilising it in the inactivated state.

The characteristics of norbormide Ca^{2+} channel antagonism described here, such as the extent of inhibition, time for maximal effect development, depression of current-voltage relationship, use-dependency of the effect as well as the lack of effect on $I_{Ca(L)}$ activation and inactivation rates, looked similar to those observed previously in guinea-pig cardiac myocytes (Bova *et al*., 1997). On the contrary, rat caudal artery myocytes displayed a reversibility of the inhibition upon wash-out that was only partially observed in cardiac myocytes. Norbormide caused a significant decrease in the slope of the steady-state inactivation curve, thus indicating that the drug alters the sensitivity of the channel inactivation mechanism to membrane voltage. The same conclusion, however, can not be drawn for the voltage dependence of the channel activation, since norbormide did not modify the corresponding curve.

Strikingly enough, norbormide inhibits $I_{Ca(L)}$ in the same cells where it induces its selective contractile effect (Bova *et al*., 1996; Figure 4, present manuscript). This indicates that the drug is able to activate, in the same cell, both vasoconstriction and vasorelaxant mechanisms and that the selective myotonic effect of norbormide in the rat peripheral vessels originates from its interaction with a target, beyond the L-type Ca^{2+} channel, which hierarchically prevails over the decrease in the Ca^{2+} influx to be expected from $I_{Ca(L)}$

inhibition. This idea is further supported by the observation that norbormide causes contraction, but not relaxation, also in caudal artery rings depolarized with high K^+ (Bova, unpublished observations), i.e. under experimental conditions similar to those represented by voltage-clamp pulses of depolarization applied to evoke $I_{Ca(L)}$. This second and hierarchically predominant target for norbormide action may be represented by PKC, since this enzyme, which plays a key role in the maintenance of tonic contraction of vascular smooth muscle (Rasmussen *et al*., 1987), is activated by norbormide *via* a PLC pathway (Bova *et al*., 2001b). The predominance of PKC-mediated events over Ca^{2+} influx *via* L-type Ca^{2+} channels in the hierarchy of the mechanisms responsible for vascular smooth muscle tone has been recently observed also for quercetin, a natural polyphenolic flavonoid, which, although activating $I_{Ca(L)}$, induces vasorelaxation as a consequence of PKC inhibition (Saponara *et al*., 2002). The involvement of distinct target sites in norbormide-induced effects in the rat caudal artery is also supported by the data obtained from amphotericin B-perforated cells (i.e. with minimal wash-out of the intracellular components), which demonstrated that an extensive dialysis of the cytoplasm (as in the case of the conventional method) prevented norbormide-induced cell contraction but not norbormide inhibition of $I_{Ca(L)}$. This observation suggests that diffusible intracellular factors are necessary in order that norbormide contracts vessels (Bova *et al*., 2001a,b). On the other hand, norbormide inhibition of $I_{Ca(L)}$ seems to be related to a direct interaction of the drug with the channel protein, although the possible involvement, in this effect, of intracellular signalling pathways can not be ruled out.

The present electrophysiological data point to norbormide as a vascular Ca^{2+} channel inhibitor characterized by a voltage- and use-dependent profile, strongly supporting the view of a Ca^{2+} entry blocking agent endowed with a peculiar vasoconstrictor activity in rat terminal arteries. It is concluded that in rat caudal artery myocytes, at least two target sites are independently activated by norbormide: one, represented by L-type Ca^{2+} channels, which is responsible for the Ca^{2+} entry blocking effect; the other, presumably a membrane receptor, which causes a PKC-dependent activation of store-operated Ca^{2+} channels, increase of Ca^{2+} influx and the ensuing contraction. The synthesis of novel norbormide derivatives would be very helpful to either foster a novel chemical class of Ca^{2+} channel antagonists or identify a norbormide recognition site on the plasma membrane responsible for its selective vasocontracting action.

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