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Ca^{2+} channel activation and membrane depolarization mediated by Cl^- channels in response to noradrenaline in vascular myocytes

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1 The effects of noradrenaline (NA) were studied on vascular smooth muscle cells isolated from rat portal vein.

2 Two types of single-Ca^{2+} channel currents with conductances of 17 pS and 8 pS were obtained in cell-attached configuration. Bath application of NA increased the open probability of both channels during depolarizing pulses without a change of background membrane conductance. However, NA did not open Ca^{2+} channels when the membrane patch potential was held at -50 mV, which is about the resting potential in physiological conditions.

3 In the whole-cell configuration, studies of voltage-dependent Ca^{2+} channel currents showed that the peak conductance curve was not shifted to more negative potentials by NA.

4 Measurements of internal Ca^{2+}-concentration ($[Ca^{2+}]_i$) with Indo-1 indicated that NA increased $[Ca^{2+}]_i$ at a holding potential of -50 mV and evoked a Ca^{2+}-activated Cl^- current. These effects were blocked when heparin was included in the pipette solution.

5 A Cl^- channel blocker without effect on Ca^{2+} channels (anthracene-9-carboxylic acid) inhibited the contractions of portal vein strips induced by NA in a manner similar to that produced by a Ca^{2+} channel inhibitor (isradipine). The NA-induced contraction was completely suppressed in the presence of ryanodine which depletes intracellular Ca^{2+} stores.

6 The present study suggests that activation of Cl^- channels by Ca^{2+} release produces a membrane depolarization which is a prerequisite for enhanced opening of voltage-dependent Ca^{2+} channels in response to NA in venous smooth muscle.

Keywords: Noradrenaline; chloride channel; calcium channel; vascular smooth muscle cells; rat portal vein; anthracene-9-carboxylic acid; isradipine; ryanodine

Introduction

Noradrenaline (NA) has powerful effects on the mechanical and electrical activity of vascular smooth muscle cells (Bolton, 1979; Bülbring & Tomita, 1987). In portal vein smooth muscle, stimulation of α_1-adrenoceptors produces contraction that involves release of Ca^{2+} from intracellular stores and Ca^{2+} influx through voltage-dependent Ca^{2+} channels (Mironneau & Gargouil, 1979; Dacquet *et al.*, 1987). The NA-induced contraction is associated with a depolarization and an increase in membrane conductance (Takata, 1980; Nanjo, 1984). Recent results obtained from single venous smooth muscle cells indicated that the NA-induced depolarization is mainly mediated by an increase in chloride conductance (Byrne & Large, 1988a; Van Helden, 1988; Pacaud *et al.*, 1989). However, an increase in a non-specific cation channel (Byrne & Large, 1988b) and a decrease in potassium conductance (Suzuki, 1981) could also be involved.

In addition, NA enhances the amplitude of the Ca^{2+} channel current of vascular smooth muscle cells (Benham & Tsien, 1988; Pacaud *et al.*, 1989). We have recently shown the involvement of a G-protein regulating the phospholipase C activity in the electrophysiological effects of NA in portal vein smooth muscle cells (Loirand *et al.*, 1990). D-*myo*-Inositol 1,4,5-trisphosphate ($InsP_3$) may be responsible for Ca^{2+} release and subsequent activation of Ca^{2+}-activated Cl^- current, while diacylglycerol may be responsible for the stimulation of the Ca^{2+} channel current, probably through activation of protein kinase C (Loirand *et al.*, 1990).

The present work was undertaken to determine whether NA acted by producing a shift to more negative voltages of the activation curve for Ca^{2+} channels so that the open state probability was greater at any given membrane potential and that depolarization would not be necessary to cause a substantial increase in Ca^{2+} entry through voltage-dependent channels. The alternative hypothesis is that NA induced only an increase in the open state probability without change in the voltage-dependence of the Ca^{2+} channels so that depolarization would be required to activate Ca^{2+} channels. It will be shown that NA activates an inward current which drives the membrane potential into the activation range of voltage-dependent Ca^{2+} channels as has already been suggested (Byrne & Large, 1988a), and that the contraction to NA was not brought about by direct activation of Ca^{2+} channels as was proposed by Nelson *et al.* (1988, 1990).

Methods

Single cell isolation and short term primary culture

Wistar rats (150 g) were stunned and then killed by cervical dislocation. Portal veins were dissected free of connective tissue and single cells were obtained by a dispersal procedure similar to that described previously (Loirand *et al.*, 1986). The cells were plated on collagen-coated cover-slips in medium M199 (Flow Laboratories) containing 10% foetal bovine serum, 2 mM glutamine, 20 u ml^{-1} penicillin and 20 μg ml^{-1} streptomycin (Gibco) and kept in an incubator gassed with 95% O_2, 5% CO_2 at 37°C. The cells were used between 2 h and 20 h after isolation.

Contraction measurements

Contractions of portal vein longitudinal strips were measured with an isometric force transducer (Akers 801) as previously described (Dacquet *et al.*, 1987). The bath solution (reference solution) contained (mM): NaCl 130, KCl 5.6, $CaCl_2$ 2, $MgCl_2$ 0.24, HEPES 10, glucose 11, pH 7.4 with NaOH.

Electrophysiological recordings

Membrane currents were measured by use of standard patch-clamp techniques (Hamill *et al.*, 1981). Whole-cell Cl^- currents were recorded in response to 10^{-5} M NA applied from a glass pipette with a pressure ejector. The cells were bathed in the reference solution and the basic pipette solution contained (mM): CsCl 130, HEPES 10, pH 7.3 with NaOH. For the measurements of whole-cell Ca^{2+} channel current, the bath solution contained (mM): $BaCl_2$ 90, glucose 10, HEPES 10, pH 7.4 at 30°C and the pipette was filled with a solution containing (mM): CsCl 130, HEPES 10, EGTA 5, pH 7.3 with NaOH. Single Ca^{2+} channel currents were measured in the cell-attached patch configuration. The patch pipette solution was (mM): $BaCl_2$ 90, glucose 10, HEPES 10, pH 7.4. Under these experimental conditions, unitary Cl^- current could not be recorded since they could not be activated by Ba^{2+}. The unitary currents were filtered at 0.6 kHz, digitized at 10 kHz, stored and analysed with a Plessey 6200 computer. Capacitive and leakage currents were digitally subtracted using the average currents of blank sweeps. Records were displayed on an X-Y plotter (Hewlett-Packard 7470A).

Chemicals used were: noradrenaline and heparin (Sigma); anthracene-9-carboxylic acid (9-AC) and caffeine (Merck); Bay K 8644 (methyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl)-pyridine-5-carboxylate, Bayer); isradipine (Sandoz); ryanodine (Calbiochem).

The values given in the text are means ± s.e.mean with *n* as the sample size. Significance was tested by means of Student's *t* test.

Fluorescence measurements

For these experiments, cells were plated on glass cover-slips and bathed in the reference solution. Combined microfluorimetric and electrophysiological experiments were performed by use of the whole-cell recording configuration. Cells were loaded passively with Indo-1 in the patch pipette filled with the basic pipette solution containing 0.06 mM Indo-1 pentasodium salt (Calbiochem). The dual emission microspectrofluorimeter was constructed from a Nikon Diaphot inverted microscope fitted with epifluorescence (× 40 oil-immersion objective). For excitation of the Indo-1, light from a 100 W mercury lamp (Osram) was reduced by use of two neutral density filters (ND 16, Nikon) and then filtered at 360 ± 5 nm and reflected off a dichroic mirror (380 nm). The emitted fluorescence signal was passed through a pinhole diaphragm slightly larger than an average cell and was directed to another dichroic mirror (455 nm). Transmitted light was filtered at 480 ± 10 nm and reflected light at 405 ± 5 nm. The intensities were recorded by two photometers (P1, Nikon), and single photon currents were converted to voltage signals. Before each experiment, the background fluorescence was determined in the cell-attached mode and cancelled by offsetting the output level of each photometer. Voltage signals at each wavelength were sampled at 17 Hz and stored in an IBM-PC computer for subsequent analysis. The ratio ($R = F405/F480$) was calculated on-line and displayed with the two voltage signals on a monitor. Intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) was estimated from the ratio R of the fluorescence measured at the two wavelengths by the equation $[Ca^{2+}]_i = K_D(F_o/F_s)(R - R_{min})/(R_{max} - R)$ (Grynkiewicz *et al.*, 1985) where K_D is the effective dissociation constant of Indo-1, F_o and F_s are the 480 nm signals in the absence and in the presence of saturating Ca^{2+} respectively. R_{min} and R_{max} are the 405/480 ratios in the absence and in the presence of saturating Ca^{2+} respectively. Since the values of R_{min} and R_{max} *in vitro* and *in vivo* were notably different (Almers & Neher, 1985) intracellular calibration was made. R_{min}, R_{max} and $K_D(F_o/F_s)$ were determined as described by Benham (1989) and Pacaud & Bolton (1991). The means value for R_{max}, R_{min} and $K_D(F_o/F_s)$ were 0.850 ± 0.007 ($n = 17$), 0.050 ± 0.001 ($n = 7$) and 1072 ± 29 nM ($n = 6$).

Results

Single-channel recordings

Figure 1 illustrates some features that distinguish two types of Ca^{2+} channel in isolated smooth muscle cells from rat portal vein in the same cell-attached patch. When the holding potential was held at −70 mV, single channel currents typified by brief openings of about −1 pA in amplitude were recorded upon stepping to a test potential of −32 mV in the presence of 1 μM Bay K 8644 (Figure 1a). At more depolarized test potentials a second type of channel activity of about −1.5 pA in amplitude was seen with long-lasting openings (Figure 1b). When the same patch was depolarized from a holding potential of −40 mV only large amplitude single-channel currents were observed (Figure 1c). Figure 1d shows the current-voltage relationships for both types of current. The slope conductance was 8 pS for the small conductance channel (T-type) and 17 pS for the large conductance channel (L-type). The extrapolated reversal potentials were near +80 mV for both Ca^{2+} channels. In Figure 1b separate L-type events (sweeps 1, 2, 4), T-type events (sweep 3), simultaneous occurrence of L-type and T-type events with a closing of the L-type channel (sweep 5) and simultaneous occurrence of L-type and T-type events with a closing of the T-type channel (sweep 6) can be observed in the same single L-type channel patch. In a total of 100 sweeps obtained in the absence of Bay K 8644, 9 sweeps only show L-type openings, in 24 sweeps only T-type openings are observed and 4 sweeps show both L-type and T-type events. Therefore the probability of the L-type channel and of the T-type channel being open are $P_f(L) = 0.13$ and $P_f(T) = 0.28$ respectively and the probability of the two types of channel being open in the same sweep is $P_f(L \wedge T) = 0.040$. The product $P_f(L)xP_f(T) = 0.036$ is approximately equal to $P_f(L \wedge T)$, indicating that L-type and T-type currents appear to be independent.

Single-channel current recordings provide the most direct approach for the identification of the NA-sensitive Ca^{2+} channel and to characterize the modulatory effect. Ca^{2+} influx is proportional to $N_T.P_f.P_o.i$, where N_T is the total number of Ca^{2+} channel proteins per cell, P_f is the probability that the channel is functional i.e. that it responds to depolarization at all, P_o is the probability of a functional channel being open for a given time at a given membrane potential and i is the amplitude of the single-channel current. Figure 2a shows records of large conductance channel activity in the cell-attached mode at +10 mV from a holding potential of −70 mV in the absence (Figure 2a(i)) and in the presence of 10 μM NA (Figure 2a(ii)), without Bay K 8644 in the bath solution. NA did not significantly change single-channel current amplitude (+1 ± 0.5%, $n = 7$, $P > 0.05$). By contrast, there was a marked change in the probability of the large conductance Ca^{2+} channel being open (Figure 2b). This is seen as an increase of P_f from 0.12 ± 0.03 (control) to 0.22 ± 0.04 in 10 μM NA, and P_o from a mean value of 0.085 ± 0.004 (control) to 0.287 ± 0.011 in 10 μM NA ($n = 7$, $P < 0.01$). Similarly, NA increased the small conductance Ca^{2+} channel activity recorded at +10 mV from a holding potential of −70 mV (Figure 3a). The probability of the channel being open was enhanced by NA (Figure 3b) which acted by increasing P_f from 0.25 ± 0.02 (control) to 0.44 ± 0.04 and P_o from a mean value of 0.026 ± 0.005 (control) to 0.044 ± 0.009 ($n = 7$, $P < 0.01$).

These increases in the probability of the Ca^{2+} channels being open induced by NA could result from a shift in the activation curve of Ca^{2+} channels to more negative voltages and/or from an increase in the maximal open probability. In the absence of Bay K 8644, in cells where Ca^{2+} channel activity was recorded during depolarizing pulses, no evidence of Ca^{2+} channel activity was observed when the membrane patch potential was maintained at −50 and −30 mV ($n = 20$) without voltage steps, for recording times as long as 1 h. Under these conditions, addition of 10 μM NA in the bath

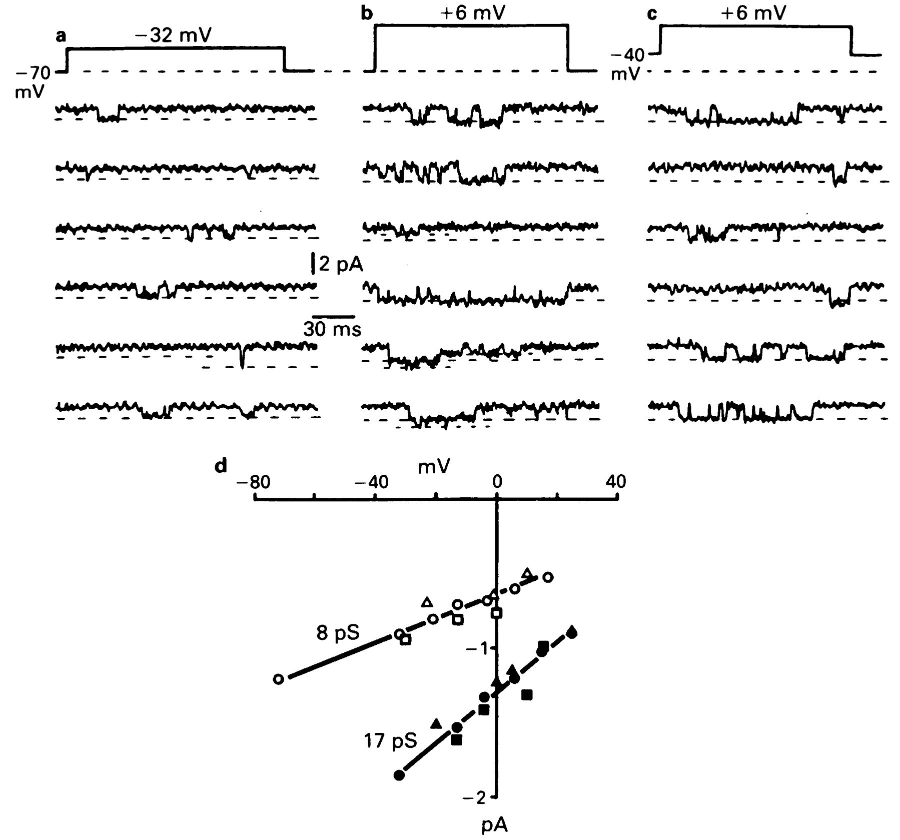

Figure 1 Voltage-dependence of single Ca^{2+} channels in a cell-attached patch. The holding potential was set to −70 mV (a,b) or −40 mV (c). The bath solution contained 1 μM Bay K 8644 and the pipette solution 90 mM Ba^{2+}. (a) At −32 mV, openings of the small conductance channel were evenly distributed throughout the test pulse whereas openings of the large conductance channel were very rare. (b) At +6 mV, the open-state probability of the large conductance channels was increased. Separate large conductance channel (sweeps 1, 2, 4), small conductance channel (sweep 3), closing of large conductance channel with small conductance channel still open (sweep 5) and closing of the small conductance channel with large conductance channel still open (sweep 6) can be observed. (c) Only the large conductance channel was recorded upon stepping the patch potential from −40 mV to +6 mV. Sweeps with no detectable openings were not shown. Dashed lines indicate the mean single-channel current level obtained from amplitude histograms. (d) Single channel current-voltage relationships for the two channel types obtained from amplitude histograms (3 examples for each channels). Low conductance channel = 8 pS; high conductance channel = 17 pS.

never induced Ca^{2+} channel openings ($n = 15$), contrary to that expected if NA produced a change in the voltage-gating of Ca^{2+} channel activation. To confirm this result, Ca^{2+} conductance voltage relationship was established from macroscopic Ca^{2+} channel currents recorded in experimental conditions similar to those used for single channel recordings (90 mM Ba^{2+}) in the absence and in the presence of NA.

Whole-cell currents and $[Ca^{2+}]_i$ *measurements*

NA (10 μM) induced an increase in the Ca^{2+} channel current amplitude. Steady-state stimulatory effect of NA was obtained within 2–3 min. Figure 4 shows the mean peak conductance curves against voltage obtained in control conditions and in 10 μM NA. The two curves were superimposed and the potentials corresponding to half-activation were not significantly different: 15.31 ± 2.24 mV (control, $n = 8$) and 16.17 ± 1.66 mV (10 μM NA, $n = 6$). These results agreed with the absence of Ca^{2+} channel opening observed in the cell-attached condition at a holding potential of −30 mV, even in the presence of 10 μM NA. Under the ionic conditions of these experiments (90 mM external Ba^{2+}) the voltage-dependence of Ca^{2+}-channel activation will be shifted toward positive potentials by around 20 mV relative to its position under physiological conditions (Benham *et al.*, 1987; Fox *et al.*, 1987). The potential corresponding to half-activation, determined from the mean peak conductance curve obtained for the Ca^{2+} channel current recorded in the reference solution (Figure 4) was −5.7 ± 0.3 mV ($n = 3$), a value 21 mV more negative than that obtained in 90 mM Ba^{2+}. The foot of the activation curve was −50 mV, a value that corresponds to the resting potential of portal vein cells (Nanjo, 1984; Yamamoto & Hotta, 1985). Taken together, these results suggest that NA could not induce a significant Ca^{2+} influx through voltage-dependent Ca^2 channels if the membrane potential was maintained at −50 mV. Changes in intracellular Ca^{2+} concentration induced by NA were estimated using the emission from the dye Indo-1 to verify this hypothesis.

In control conditions (Figure 5a), ejection of 10 μM NA at a holding potential of −50 mV in the reference solution evoked an initial peak in $[Ca^{2+}]_i$ followed by a smaller sustained rise. These changes in $[Ca^{2+}]_i$ were still observed in the presence of 10 μM desmethoxyverapamil (not shown). The initial increase in $[Ca^{2+}]_i$ induced activation of Ca^{2+}-dependent Cl^- channels. Heparin has been shown to block $InsP_3$ receptors (Hill *et al.*, 1987; Ghosh *et al.*, 1988) and to inhibit Ca^{2+} release via these receptors in smooth muscle cells (Komori & Bolton, 1990; 1991; Pacaud & Bolton, 1991). When 1 mM heparin was added to the pipette solution, the rise in $[Ca^{2+}]_i$ normally seen upon applying NA (10 μM) was completely inhibited as well as the Ca^{2+}-activated Cl^- current (Figure 5b) while activation of Ca^{2+} channels by a depolarization from −50 mV to +10 mV was still able to produce an increase in $[Ca^{2+}]_i$ (Figure 5c). Under these conditions, the amplitude of the Ca^{2+} channel current and of the corresponding $[Ca^{2+}]_i$ transient evoked by repetitive depolarizations were increased during NA (10 μM) application. This result indicated that NA did not produce a detectable Ca^{2+} entry through voltage-dependent Ca^{2+} channels at a holding potential of −50 mV.

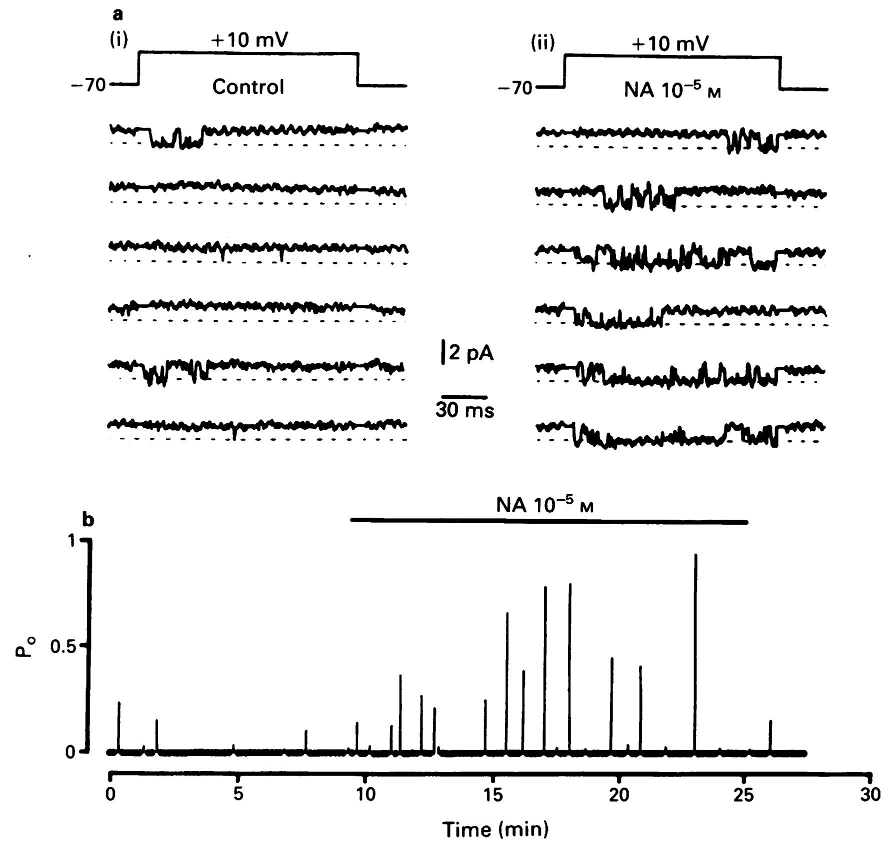

Figure 2 Effect of noradrenaline (NA) on the large conductance Ca^{2+} channel. (a) Activity of the large conductance Ca^{2+} channel was recorded from a holding potential of −70 mV to +10 mV, in the absence of Bay K 8644, before (i) and during bath application of 10 μM NA (ii). Dashed lines indicate the mean single-channel current level obtained from amplitude histograms. (b) Channel open probability for individual sweeps plotted against time. The open-state probability P_o was determined by dividing the time that the channel spends in the open state during a depolarization by the total time of the depolarization. Sweeps with no detectable openings were assigned a P_o value of 0. P_f corresponds to the ratio of the number of sweeps with at least one opening over the total number of sweeps in the ensemble. The NA effect is shown as an increase in P_o and P_f.

Contraction measurements

Our results suggest that NA does not alter the voltage-gating of Ca^{2+} channel activation. Consequently, opening of voltage-dependent Ca^{2+} channels has to be promoted by a membrane depolarization. Increase in Cl^- conductance can depolarize the membrane potential to between −20 and −30 mV (Pacaud *et al.*, 1989) at which values the open-state probability of Ca^{2+} channels is high. The role of both Ca^{2+} channel activation and membrane depolarization evoked by Cl^- current in NA-induced contractions is illustrated by the effects of isradipine (a dihydropyridine derivative), 9-AC and ryanodine. Isradipine (0.5 μM) completely blocked voltage-dependent Ca^{2+} channels in venous muscle (Loirand *et al.*, 1989). In contrast, 9-AC (5 mM) completely inhibited Ca^{2+}-activated Cl^- current but had no significant effects on Ca^{2+} channel current amplitude (Boton *et al.*, 1989; Baron *et al.*, 1991). Figure 6 shows that inhibition of Ca^{2+} channels by 0.5 μM isradipine (Figure 6a) or inhibition of Ca^{2+}-activated Cl^- channels by 5 mM 9-AC (Figure 6b(i)) led to a similar blockade of the NA-induced contraction: 57.1 ± 9% ($n = 5$) and 55 ± 7% ($n = 4$) respectively. The NA-induced contraction which is resistant to both 9-AC and isradipine is likely to be due to activation of agonist-sensitive intracellular Ca^{2+} stores since its amplitude is similar to that of NA-induced contraction obtained in Ca^{2+}-free solutions (Dacquet *et al.*, 1987). The contraction induced by 60 mM KCl was not significantly inhibited in the presence of 9-AC (5.3 ± 3.1%, $n = 4$, Figure 6b(ii)).

Ryanodine is known to deplete Ca^{2+} stores in smooth muscle cells (Rousseau *et al.*, 1987; Sakai *et al.*, 1988). In the presence of 10 μM ryanodine in the reference solution, the contractile response to 10 μM NA was completely suppressed (Figure 6c(i)). This effect seems to be specifically due to the depleting action of ryanodine since the caffeine-induced contraction was also completely inhibited whereas the contractile response induced by 130 mM KCl was only reduced by 9.0 ± 3.7% ($n = 4$; Figure 6c(ii)).

Discussion

The single channel data provide further insight into the cellular effects of NA. The fact that NA acts on Ca^{2+} channels after application to the bath in cell-attached mode agrees with the existence of an intracellular coupling mechanism between α_1-adrenoceptors and Ca^{2+} channels (G-protein, phospholipase C, protein kinase C; Loirand *et al.*, 1990). The stimulatory effect of NA on Ca^{2+} channels is brought about by two mechanisms. The first is through a decrease in the percentage of nulls; this means that the probability of the channel being in a closed state decreases. The second mechanism is an increase in open-state probability of the available channel. This effect is similar to that observed after β-adrenoceptor stimulation of neonatal rat cardiocytes (Tsien *et al.*, 1986), thrombin application on frog ventricular cells (Markwardt *et al.*, 1990), stimulation of mesenteric artery cells by NA (Nelson *et al.*, 1988) and action of endothelin on guinea-pig portal vein cells (Inoue *et al.*, 1990).

One mechanism by which NA increases the Ca^{2+} channel current could be a simple leftward shift of the steady-state activation curve. Our results do not provide any support for such a mechanism; NA cannot induce a significant Ca^{2+} influx through Ca^{2+} channels at a holding potential corresponding to the foot of the activation curve for Ca^{2+} channels and to the resting potential of portal vein cells (−50 mV), con-

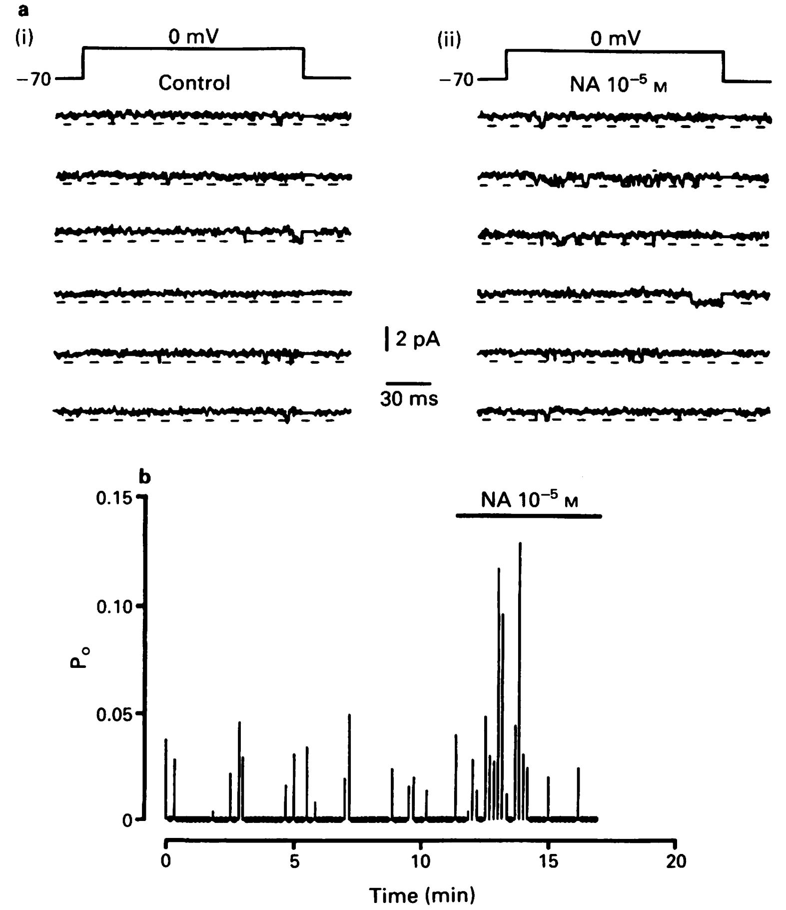

Figure 3 Effect of noradrenaline (NA) on the small conductance Ca^{2+} channel. (a) Activity of the small conductance Ca^{2+} channel was recorded from a holding potential of −70 mV to 0 mV, in the absence of Bay K 8644, before (i) and during bath application of 10 μM NA (ii). Dashed lines indicate the mean single-channel current level obtained from amplitude histograms. (b) Channel open probability for individual sweeps plotted against time. The open-state probability P_0 and the probability that the channel is functional, P_f, were determined as in Figure 2. The NA effect is shown as an increase in P_0 and P_f.

trary to that expected if a shift of the voltage-dependence of Ca^{2+} channel activation occurred upon NA application. Nevertheless, the strong inhibition produced by Ca^{2+} antagonists left no doubt about the important role played by the voltage-dependent Ca^{2+} channels in the NA-induced contraction of the portal vein (Figure 6a; Dacquet *et al.*, 1987).

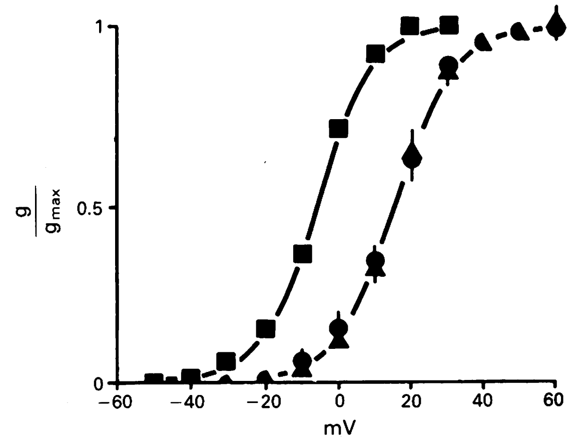

Figure 4 Effect of noradrenaline (NA) in the whole-cell configuration. Peak conductances calculated using the equation $g = I_{Ca}/(V_m - E_{rev})$ from whole-cell Ca^{2+} channel currents, recorded in the reference solution (■) and in a 90 mM Ba^{2+}-containing external solution before (●) and after bath application of 10 μM NA (▲). These are expressed as a fraction of maximal conductance and plotted against membrane potential.

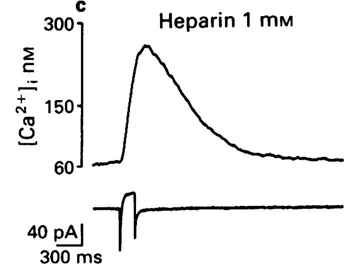

Figure 5 (a) In the reference solution, application of 10 μM noradrenaline (NA) evoked a transient increase in $[Ca^{2+}]_i$ followed by a smaller sustained component (upper trace). The internal Ca^{2+} concentration was determined using the emission from the dye Indo-1. This increase in $[Ca^{2+}]_i$ induced the activation of Ca^{2+}-dependent Cl^- channels (lower trace). (b–c) In the presence of 1 mM heparin in the pipette solution, no change in $[Ca^{2+}]_i$ and in whole-cell current was observed upon application of 10 μM NA; the holding potential was −50 mV (b). Activation of Ca^{2+} current by a depolarization to +10 mV from −50 mV produced an increase in $[Ca^{2+}]_i$ (c).

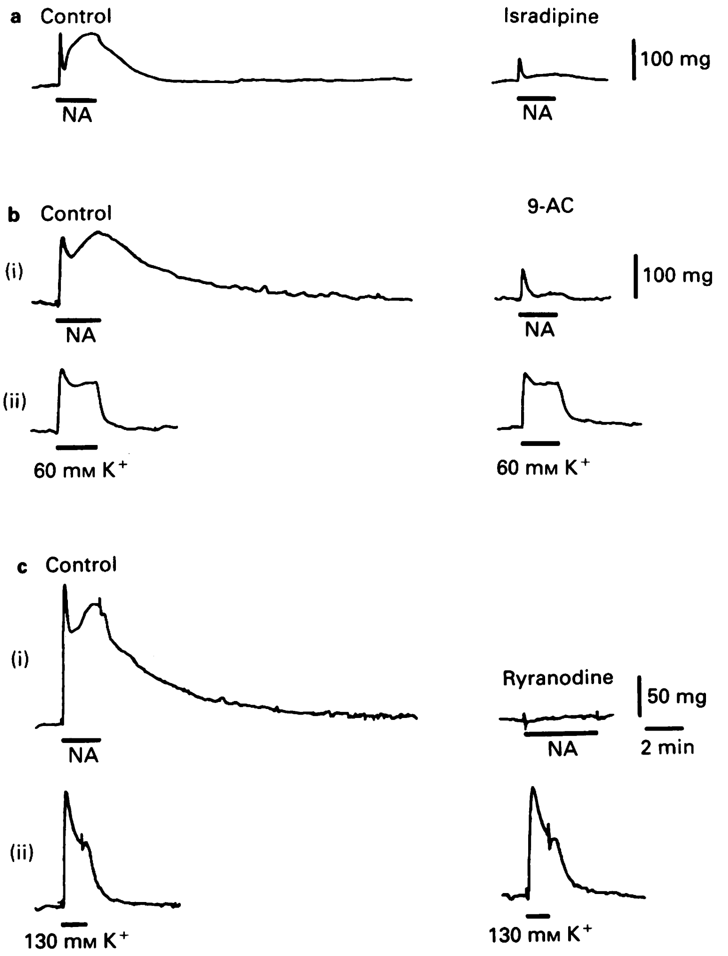

Figure 6 Effect of various pharmacological agents on noradrenaline (NA)-induced contractions. Contractions were induced by 10 μM NA (a, b(i), c(i)), by 60 mM KCl (b(ii)) and by 130 mM KCl (c(ii)) in the absence (left column) and in the presence (right column) of 0.5 μM isradipine (a), 5 mM anthracene-9-carboxylic acid (b) or 10 μM ryanodine (c).

Blockade of Ca^{2+}-activated Cl^- channels led to an inhibition of the NA-induced contraction similar to that produced by Ca^{2+} antagonists, suggesting that inhibition of Cl^- channels prevented the involvement of voltage-dependent Ca^{2+} channels during NA application. Experiments done with heparin and ryanodine also indicate that when Ca^{2+} release from intracellular store could not occur, NA was not able to induce Ca^{2+} influx through voltage-dependent Ca^{2+} channels. On portal vein smooth muscle cells, the NA-induced Ca^{2+} release is responsible for Ca^{2+}-dependent Cl-channel activation triggering membrane depolarization (Pacaud *et al.*, 1989). These results suggest that the depolarization produced by activation of Ca^{2+}-activated Cl^- channels is a prerequisite to opening of voltage-dependent Ca^{2+} channels in response to NA in portal vein cells. In addition, NA increased the open-state probability of both types of Ca^{2+} channels leading to an increased Ca^{2+} influx. For the large conductance Ca^{2+} channel corresponding to the slow calcium current (L-type), the change in the open-state probability was induced by NA through a protein kinase C-dependent pathway (Loirand *et al.*, 1990). For the small conductance Ca^{2+} channel, the intracellular mechanism involved in the increase in the open-state probability induced by NA, is not known and may use the same or a different pathway. However, the NA effect on the small conductance Ca^{2+} channel seems to be less sustained than on the large conductance Ca^{2+} channel, suggesting that the intracellular mechanisms involved may differ for the two channels. Thus, the sequence of events induced by NA could be summarized as follows: (i) NA releases intracellular Ca^{2+} stores through $InsP_3$ production; (ii) the Ca^{2+} released opens Cl^- channels producing inward current and membrane depolarization; (iii) this depolarization produces Ca^{2+} entry through voltage-dependent Ca^{2+} channels and their open probability is enhanced by NA.

It is possible that this mechanism of a required depolarization might represent a common mechanism involved in many agonist-mediated contractions of smooth muscle. However, the nature of the channels responsible for the depolarization could differ from one tissue to another. In vascular smooth muscle, Ca^{2+}-activated Cl^- channels seem to play the major role: in the portal vein in response to NA (Byrne & Large, 1988b; Pacaud *et al.*, 1989), in the mesenteric vein in response to NA (Van Helden, 1988), in the coronary and mesenteric arteries in response to endothelin (Klockner & Isenberg, 1991); whereas in visceral smooth muscle, Ca^{2+}-activated cation channels seem to be more important: in the ileum in response to acetylcholine (Inoue & Isenberg, 1990a, b) and in the jejunum in response to acetylcholine (Pacaud & Bolton, 1991).

Thus, Ca^{2+} store release probably has an important role in determining indirectly the final size of the contractile response to receptor activation by influencing strongly the size of the depolarization which occurs; this in turn will determine the extent of Ca^{2+} channel activation which is produced by the receptor agonist.

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