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Voltage-dependent calcium current and the effects of adrenergic modulation in rat aortic smooth muscle cells

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SUMMARY

Smooth muscle cells from rat aorta were cultured in defined, serum-free medium and studied using whole-cell patch-clamp techniques. Under conditions designed to isolate currents through Ca channels, step depolarizations produced inward currents which were fast in onset and inactivated rapidly, with little sustained inward current being observed. Both Ni and Cd blocked these currents, with Ni being effective at 50 μM. Removal of external Na or addition of 1 μM tetrodotoxin had no effect. Peak inward currents were attained at about −15 mV, with half-maximal activation at −41 mV using −80 mV holding potentials. The transient inward currents were reduced by depolarized holding potentials, with half-maximal steady-state inactivation at −48 mV. In three of the 98 cells studied, small maintained inward currents were observed with a −40 mV holding potential. The Ca channel antagonist nicardipine (5 μM) blocked the transient inward current while neither of the dihydropyridine Ca channel agonists S(+)202 791 and (−)BAY K 8644 produced a significant augmentation of sustained inward current. At 10 μM, both noradrenaline and adrenaline but not phenylephrine decreased the peak inward current. This inhibition was unaffected by a variety of adrenoceptor antagonists and was also observed when internal solutions having high Ca buffering capacity were used, but was absent when GDP-β-S instead of GTP was included in the pipette solution. The main conclusions from this study are that under our cell culture conditions, rat aortic smooth muscle cells possess predominately a transient, low-threshold-activated inward Ca current and that this Ca current is inhibited by certain adrenoceptor agonists but with a quite atypical adrenoceptor antagonist pharmacology.

1. INTRODUCTION

Pathways for calcium entry are of evident interest in the understanding of excitation-contraction coupling in vascular smooth muscle cells (Bolton 1979). Three types of calcium-permeable channels have been found (Benham & Tsien 1987): voltage-dependent, receptor-operated and leak channels. With the development of the patch-clamp technique together with methods allowing isolation of single cells and cell culture (see Tomita 1988), it is only relatively recently that voltage-dependent Ca currents in single vascular smooth muscle cells have been extensively characterized. In several reports where cultured myocytes or smooth muscle-derived cell lines were studied at varying numbers of passages, differences as to the relative presence of sustained, high-voltage-activated, L-type and transient, low-voltage-activated, T-type Ca channels have been found (Friedman *et al.* 1986; Loirand *et al.* 1986, 1989; Sturek & Hermsmeyer 1986; Toro *et al.*, 1986; Toro & Stefani 1987; Fish *et al.* 1988; Rusch & Hermsmeyer 1988; Akaike *et al.* 1989; Kuga *et al.* 1990; Marks *et al.* 1990; Molleman *et al.* 1991; Bodin *et al.* 1991). At least part of this variability may be associated with cell culture conditions, as changes in phenotype of smooth muscle cells in culture (for example, expressed actin or myosin isoforms and contractile responses to various agonists) are well-documented (see Chamley-Campbell *et al.* 1979, 1981). While the use of freshly isolated smooth muscle cells essentially circumvents this methodological problem associated with cell culture, a large diversity in the characteristics of Ca channels nevertheless has been described in freshly isolated vascular smooth muscle cells (Bean *et al.* 1986; Worley *et al.* 1986; Benham *et al.* 1987; Yatani *et al.* 1987; Aaronson *et al.* 1988; Ohya *et al.* 1988; Klöckner *et al.* 1989; Nelson & Worley 1989; Ohya & Sperelakis 1989; Wang *et al.* 1989; Ganitkevich & Isenberg 1990; Matsuda *et al.* 1990; Clapp & Gurney 1991; Simard 1991). Thus, one of our interests was to study voltage-dependent Ca currents in cultured vascular myocytes that retain some characteristics associated with a contractile or differentiated phenotype (Bodin 1988; Bodin *et al.*

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1991; André *et al.* 1991). As differing effects of noradrenaline on Ca currents in vascular smooth muscle cells have been described in the literature (Bean *et al.* 1986; Droogmans *et al.* 1987; Pacaud *et al.* 1987, 1989; Yatani *et al.* 1987; Benham & Tsien 1988; Nelson *et al.* 1988; Declerck *et al.* 1990), a second point of interest was to examine the effects of this agonist on Ca currents in our cells. A preliminary report of some of these results has been made (Serebryakov & Takeda 1991).

2. METHODS

Primary cultures of smooth muscle cells were prepared from aortae of 10-week-old male Wistar–Kyoto rats essentially as described elsewhere (Bodin *et al.* 1987, 1991). Briefly, rats were stunned, exsanguinated and aortae were dissected out under sterile conditions. The aortae were cleaned of adhering fat and connective tissue and placed in a defined, serum-free culture medium (Eagle's MEM/Ham's F-10 (1:1 by volume; Eurobio), supplemented with 2% (by volume) synthetic serum substitutes Ultroser SF/Ultroser G (3:1 by volume; I.B.F.), 100 U ml^{-1} penicillin G, 0·05 mM vitamin C and 2 mM glutamine). A first digestion was carried out using collagenase (CLS II, 80 U ml^{-1}; Worthington) in Hank's balanced salt solution (Eurobio) for 0.5 h in a 37°C incubator. Tissues were then replaced in culture medium and the adventitia and intima gently removed. The media of the vessels thus obtained were kept overnight in a CO_2 incubator at 37 °C and then incubated in a second digestion medium containing collagenase (CLS II, 60 U ml^{-1}; Worthington) and elastase (ultra pure grade, 5 U ml^{-1}; Biosys) for 60–90 min at 37°C. Single cells were dispersed by trituration using flame-polished Pasteur pipettes. The cell suspension was diluted with culture medium and following centrifugation (120 *g* for 7 min), pelleted cells were resuspended in culture medium and then plated at 1500–2000 cells per square centimetre onto collagen-coated glass coverslips in 35 mm Petri dishes.

Single, fusiform cells were studied usually 5–10 days after plating using standard whole-cell patch-clamp techniques (Hamill *et al.* 1981). In some cases, cells from first or second passages were also used; cells were passaged using a Ca-free/EDTA salt solution (Gibco) every 12–14 days. As shown previously in our laboratory, cells cultured under these conditions can be classed as contractile in phenotype, at least when judged by their changes in shape and in internal Ca concentration in response to vasoactive agonists or KCl depolarization and by their immunoreactivity to antibodies specific for smooth muscle α-actin and smooth muscle myosin (see Bodin *et al.* 1987, 1991; Travo *et al.* 1987; Bodin 1988; André *et al.* 1991).

Pipettes of 2–4 MΩ resistance when filled with Cs internal solution (in millimoles per litre: 100 Cs glutamate, 40 CsCl, 2 $MgCl_2$, 3 EGTA, 5 MgATP, 0·2 GTP, 20 HEPES, pH 7.3 with CsOH; 2 mM TEA Cl was sometimes included) were made from thin-wall, borosilicate capillaries (Vitrex BRI/E). Initial seal resistances were greater than 5 GΩ and acceptable whole-cell input resistances were at least several GΩ. When the internal Ca buffer capacity was increased, 5.5 mM BAPTA/0.5 mM $CaCl_2$ or 10 mM EGTA/1 mM $CaCl_2$ were used. Normal K internal solution contained KCl in place of Cs glutamate and CsCl (pH adjusted with KOH). Pipettes were coated with beeswax and dipped in Sigmacote (Sigma) to minimize associated capacitance. Compensation for series resistance and capacitance was made using the inbuilt circuitry of the patch amplifier (List EPC-7). The normal Na external bath solution contained (in millimoles per litre): 140 NaCl, 5 KCl, 2 $MgCl_2$, 2 $CaCl_2$, 11 glucose, 10 HEPES (pH 7.3 with NaOH). External 10 Ca or 20 Ba solutions contained *inter alia* 10 mM $CaCl_2$ or 20 mM $BaCl_2$ with NaCl being reduced isoosmotically. Whole-cell currents through Ca channels were elicited by step depolarizations, usually from a holding potential of −80 mV, once every 10 or 20 s. Rundown of Ca currents was usually between 15–25% and no greater than 40% over 15–20 min. Peak current amplitudes were measured with respect to the steady state current at the end of the pulse. Adrenoceptor agonists were locally microperfused using pressurized (10 kPa), wide-bore puffer pipettes positioned ≈50 μm from the cell (in controls, puffer application of normal external solution alone had no effect). Other drugs were preincubated or directly added to the bath as required. Experiments were made at room temperature (20–24°C).

The data were analysed using a digital oscilloscope (Nicolet 3091) following appropriate filtering and then plotted using an X–Y plotter (Hewlett-Packard 7470A). For steady-state activation (m_∞) and inactivation (h_∞) curves respectively, data were fitted using a Marquardt nonlinear least-squares algorithm to a standard Boltzmann-type equation having the form: $I/I_{max} = 1/[1 + \exp(V - V_h/k)]$, where V_h is the voltage for half-maximal activation or inactivation and k is the slope factor. The data illustrated are non-leak subtracted. Where appropriate, data are expressed as mean ± s.e.m., for n cells. Calcium channel data analysed from 98 cells from experiments done over a three year period are presented here.

S(+) 202 791 was from Sandoz; (−)BAY K 8644 was from Bayer; prazosin was from Pfizer; WB 4101 and phentolamine were from Research Biochemicals Inc.; propranolol, nicardipine, phenylephrine, adrenaline and noradrenaline were from Sigma. All of the above drugs were routinely used in parallel contractile experiments on rat isolated aortic rings. All other chemicals were of the best quality commercially available.

3. RESULTS

Under whole-cell current-clamp conditions using normal Na external bath and K internal pipette solutions, the resting or zero current potential of our cells was quite variable, ranging from 0 to −50 mV, with as a consequence, quite variable holding currents at the normally used holding potential of −80 mV. With Na external (2 mM Ca) and K internal solutions, large outward whole-cell K currents were evoked by

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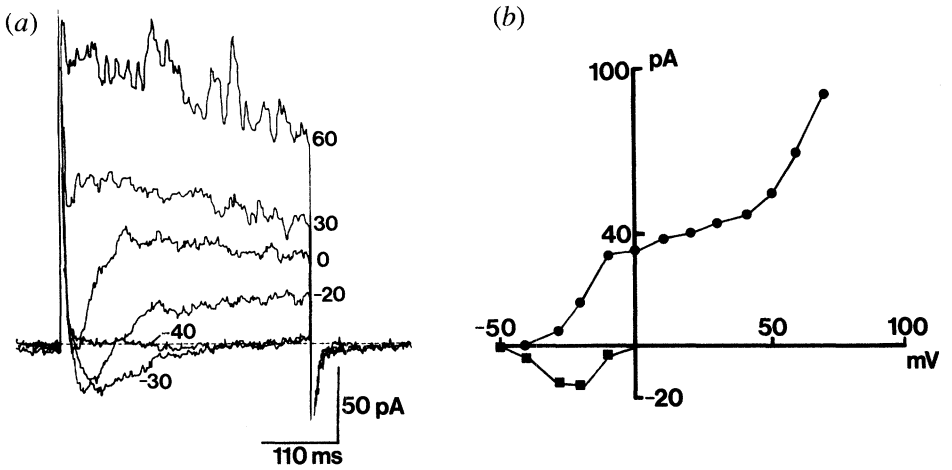

Figure 1. (*a*) Whole-cell currents from a rat aortic myocyte (external 10 Ca and internal K solutions). The numbers indicate the command voltages; holding potential, −80 mV. The interrupted line indicates the zero current level in this and subsequent figures. (*b*) Corresponding *I–V* relationship for the cell in (*a*) (squares, peak inward current; circles, peak outward current).

depolarizing steps to potentials more positive than −30 mV from a −80 mV holding potential, while inward-going whole-cell currents in the voltage range −50 to 20 mV were very small (less than 5 pA peak amplitude) or indistinguishable (not shown). When the external Ca concentration was raised to 10 mM, small transient inward currents were observed in addition to the large outward K currents seen for strong depolarizations (figure 1). These inward currents were affected neither by 1 μM external tetrodotoxin ($n=4$) nor by replacement of external Na by choline ($n=3$; not shown) and seem thus likely to be due to influx through Ca channels. When K currents were blocked using Cs internal and 20 Ba external solutions, depolarization-activated whole-cell inward Ba currents having a fast onset and a rapid timecourse of inactivation were recorded (figure 2*a*). After the decay of the initial transient inward current, no perceptible net sustained inward current was present. From the current–voltage (*I–V*) relationship (figure 2*b*), the threshold for activation was about −50 mV with peak inward Ca channel current occurring between −10 and −20 mV, while no 'shoulder' either on the descending or ascending phases of the *I–V* curve was apparent. Similar *I–V* relationships for whole-cell Ba currents were obtained from 64 cells.

The peak amplitude of the transient Ca channel currents was strongly dependent on the holding potential, as illustrated for two holding potentials of −80 and −40 mV (figure 3*a*). Average steady-state inactivation data were fitted to a Boltzmann-type equation (see Methods), with half-inactivation occurring at −48.3 mV and a slope factor k of 5.6 mV (figure 3*b*). The average voltage-dependence of activation of inward Ba currents was also fitted to a Boltzmann-type equation, with a half-activation at −41.3 mV and a slope factor k of 4.3 mV (figure 3*c*, *d*).

Both external Cd and Ni blocked inward Ba currents (figure 4). Most of the inward current was blocked by Cd at 1 mM (figure 4*a*; $n=4$) but not at 50 μM (figure 4*b–e*; $n=6$), whereas 50 μM Ni was capable of rapidly and reversibly blocking most of the inward current (figure 4*c–e*; $n=6$). As described in other cell types (see Bean 1989; Hess 1990; Pelzer *et al.* 1990), this differential blocking potency of Cd and Ni suggests that the inward Ba current is flowing though low-voltage-activated, transient T-type Ca channels. The dihydropyridine antagonist nicardipine was

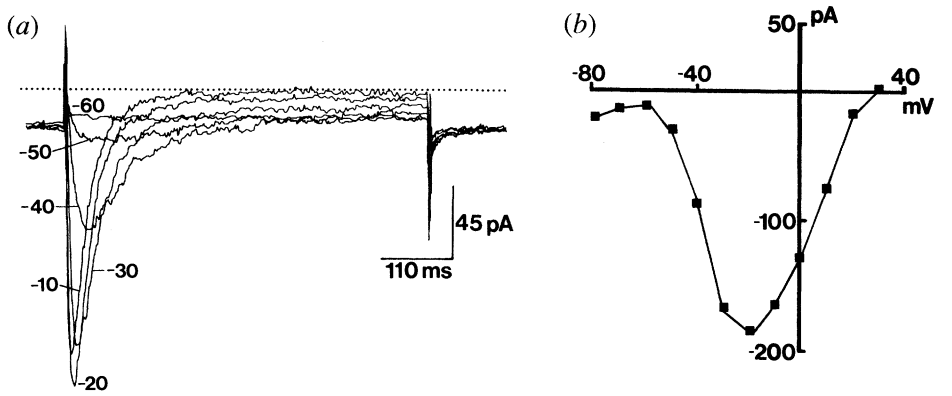

Figure 2. (*a*) Depolarization-activated, whole-cell, inward Ca channel currents (20 Ba external and Cs internal solutions). The numbers indicate the command voltages; holding potential, −80 mV. (*b*) Corresponding *I–V* relationship for the cell in (*a*).

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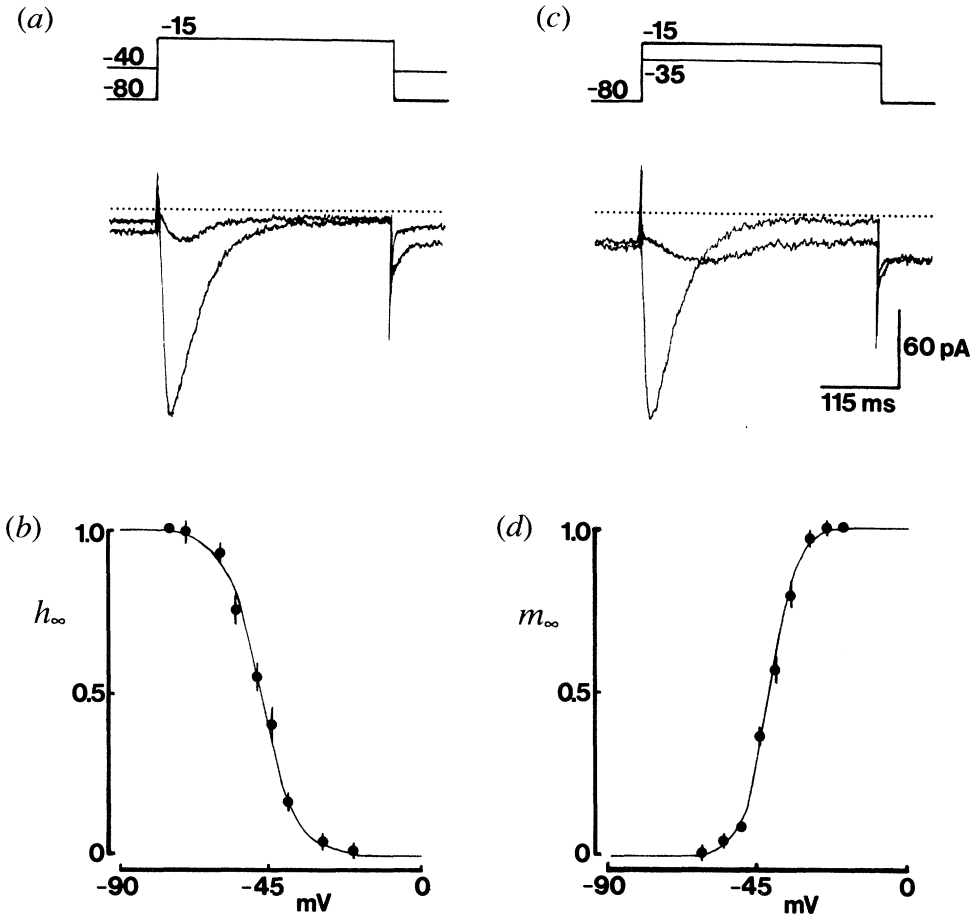

Figure 3. (*a*) Transient inward Ca channel currents are decreased at a holding potential of −40 mV. (*b*) Average steady-state inactivation data were fitted with a Boltzmann-type equation with $V_h = -48.3 \pm 0.3$ mV and the slope factor $k = 5.6 \pm 0.4$ mV. (*c*) The activation threshold for inward currents is about −50 mV with peak inward current around −15 mV. (*d*) Average activation data fitted with a Boltzmann-type equation with $V_h = 41.3 \pm 0.3$ mV and $k = 4.3 \pm 0.3$ mV. ((*a*) and (*c*) from the same cell; voltage clamp protocols are indicated above the current traces. The data in (*b*) and (*d*) are plotted as mean ± s.e.m. for $n = 7$–9 cells; 20 Ba external and Cs internal solutions.)

tested: at 0.5 μM, no effects were observable, whereas at 5 μM, a strong inhibition of the transient peak inward Ca channel current was seen (figure 5*a*; $n = 3$). This block was poorly reversible upon drug washout. At 10 μM, nicardipine produced complete block of inward current, which was irreversible over the time-course of our experiments (≈20 min; not shown). Because the apparent lack of sustained inward whole-cell current suggests the presence of few or no L-type Ca channels, the stereo-specific dihydropyridine Ca channel agonists (−)BAY K 8644 and S(+) 202 791 were bath applied in an effort to facilitate any possible L-type current. Both (−)BAY K 8644 (1 μM; $n = 4$; not shown) and S(+) 202 791 (1 μM; $n = 3$; figure 5*b–d*) were ineffective in producing clear-cut increases in sustained inward current. However, in three of 98 cells using 20 Ba external and Cs internal solutions in the absence of drugs, a small maintained inward current component was observed in addition to the initial transient current (figure 6). Unlike the transient current, this maintained current was little affected when a holding potential of −40 mV was used (figure 6).

When 10 μM noradrenaline was applied by local microperfusion, peak Ca channel current was decreased with a rapid onset and in a reversible fashion (figure 7). In nine cells, the average decrease in peak Ca channel current was 37 ± 9% with recovery to 87 ± 4% of control values. No shifts in holding current were observed during noradrenaline application. Because at least one action of noradrenaline is to cause release of Ca from internal stores, it might be that the noradrenaline-induced decrease in Ca channel current results from a calcium-dependent inactivation process. However, when the Ca buffering capacity of the pipette internal solution was increased from 3 to 10 mM EGTA (figure 8*a*; $n = 5$) or when 5.5 mM BAPTA was used (not shown; $n = 4$), reversible decreases in peak Ca channel current were still produced by 10 μM noradrenaline. Furthermore in three cells, noradrenaline (10 μM) was without effect on outward whole-cell K currents (evoked at test potentials where any changes in Ca current would be minimal; figure 8*b*) when the pipette internal solution was buffered with 3 mM EGTA, suggesting that at least under these recording conditions, noradrenaline-associated rises in internal Ca were insufficient to potentiate Ca-sensitive outward K current.

Various adrenoceptor antagonists were tested in an effort to characterize the type of adrenoceptor responsible for the noradrenaline-induced decrease in peak

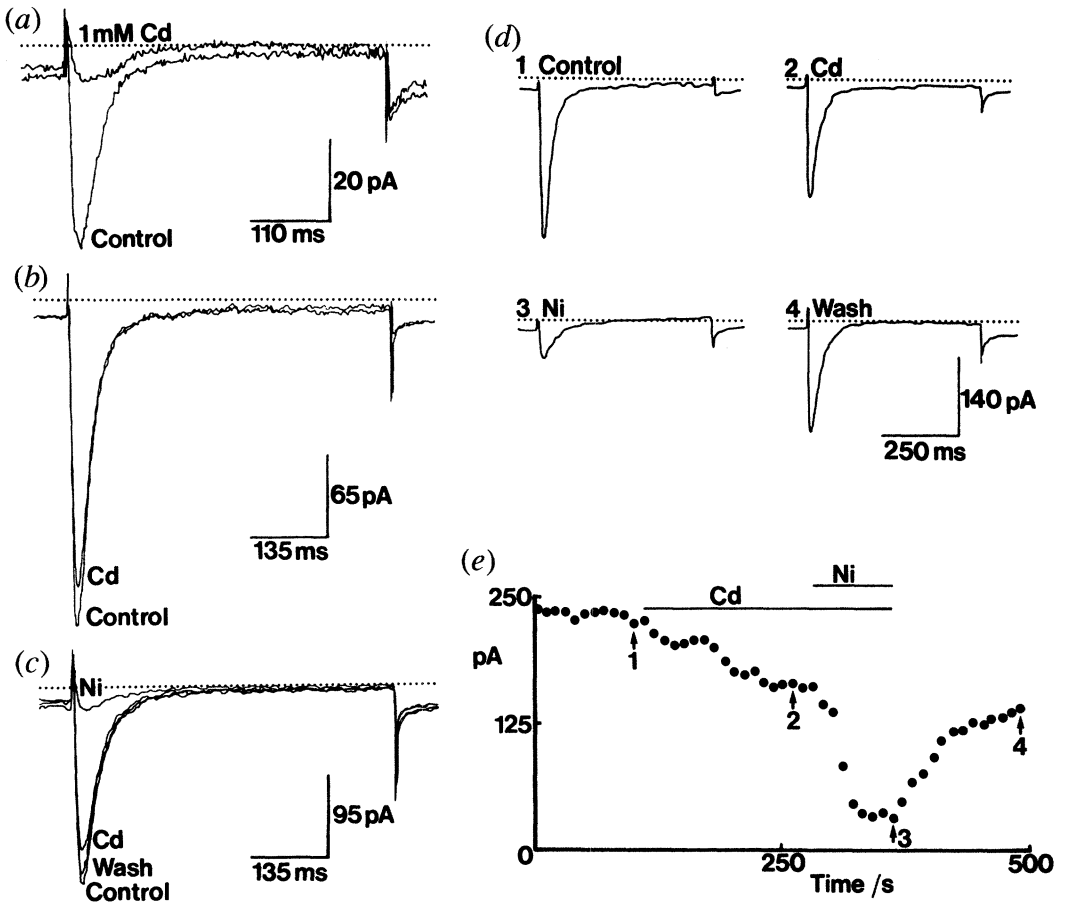

Figure 4. Cd and Ni block the transient inward Ca channel current. Locally applied Cd at a concentration of 1 mM (*a*) but not 50 μM (*b–e*) almost completely blocks inward current. 50 μM Ni blocks the inward current (*c–e*). The currents in (*d*) were obtained at the indicated times in (*e*). (Separate cells, (*a–d*); holding and test potentials were −80 and −10 mV, respectively; 20 Ba external and Cs internal solutions.)

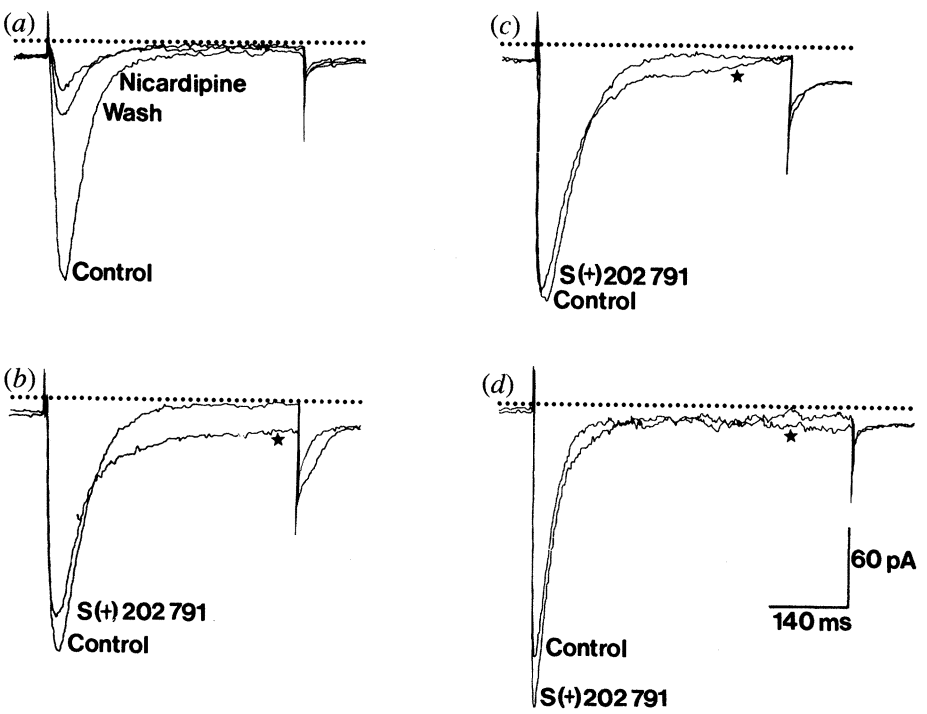

Figure 5. (*a*) Inhibition of peak inward Ca channel current by 5 μM nicardipine. Block was poorly reversible after 10 min washout. (*b–e*) Effects of the dihydropyridine Ca channel agonist S(+) 202 791 (1 μM). Stars at the end of the test pulses indicate the currents obtained in the presence of the Ca channel agonist. ((*a–d*), separate cells; holding and test potentials were −80 and −10 mV, respectively; 20 Ba external and Cs internal solutions.)

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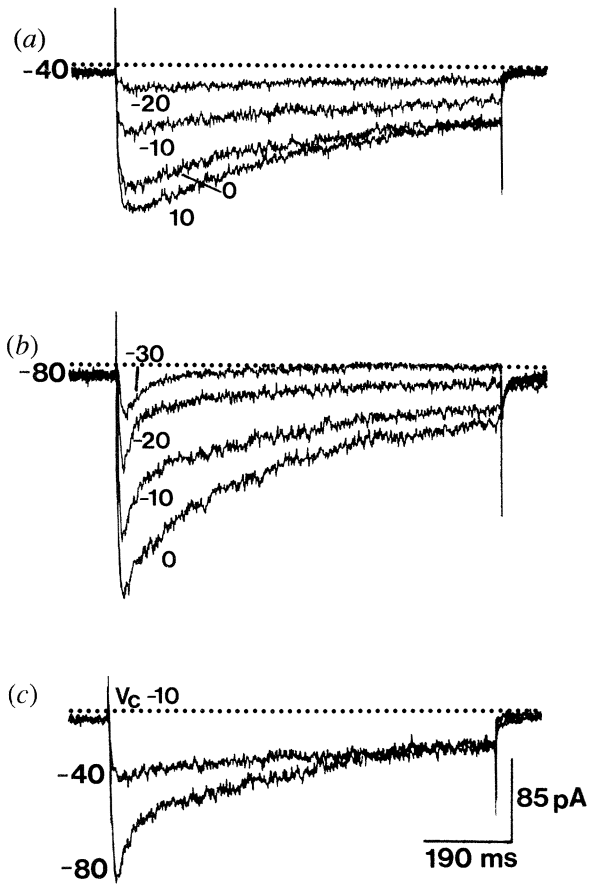

Figure 6. Presence of sustained inward Ca channel current in some cells (holding potential in (*a*) was −40 mV and in (*b*), −80 mV; test pulses are indicated). (*c*) Superimposed currents for a test pulse to −10 mV from the indicated holding potentials. ((*a–c*), same cell; 20 Ba external and Cs internal solutions.)

Ca channel current. Pre-exposure for at least 5 min with none of 1 μM prazosin or 0.1 μM WB 4101 (α_1-adrenoceptor antagonists; $n=2$ for both drugs), 1 μM phentolamine (a non-specific α-adrenoceptor antagonist; $n=3$) and 1 μM propranolol (a β-adrenoceptor antagonist; $n=2$) prevented the effect of noradrenaline on peak Ca channel current amplitude (figure 9). Other α-adrenoceptor agonists were also studied in another series of experiments, with adrenaline (10 μM; $n=4$) but not phenylephrine (10 μM; $n=3$) being capable of mimicking noradrenaline in decreasing peak Ca channel current (figure 10). Taken together, these data suggest that neither α- nor β-adrenoceptors are likely to be involved in the observed decreases in Ca channel current evoked by noradrenaline and adrenaline. A role for GTP-binding proteins in mediating the inhibitory effect of noradrenaline on Ca channel current was indicated in a further series of experiments where the 0.2 mM GTP usually contained in the pipette internal solution was replaced by GDP-β-S (which prevents GTP binding to G-proteins). Under these conditions, noradrenaline (10 μM) was without effect on Ca channel current (figure 11; $n=4$).

4. DISCUSSION

Multiple classes of Ca channels have been widely described in many different cell types (see Bean 1989; Hess 1990; Pelzer *et al.* 1990). Vascular smooth muscle cells, whether in culture or freshly isolated, are no exception with both L-type and T-type Ca channels being found, although much variability in the relative presence of these subtypes is apparent (see references cited in the Introduction). Part of this variability arises because the separation of whole-cell Ca currents

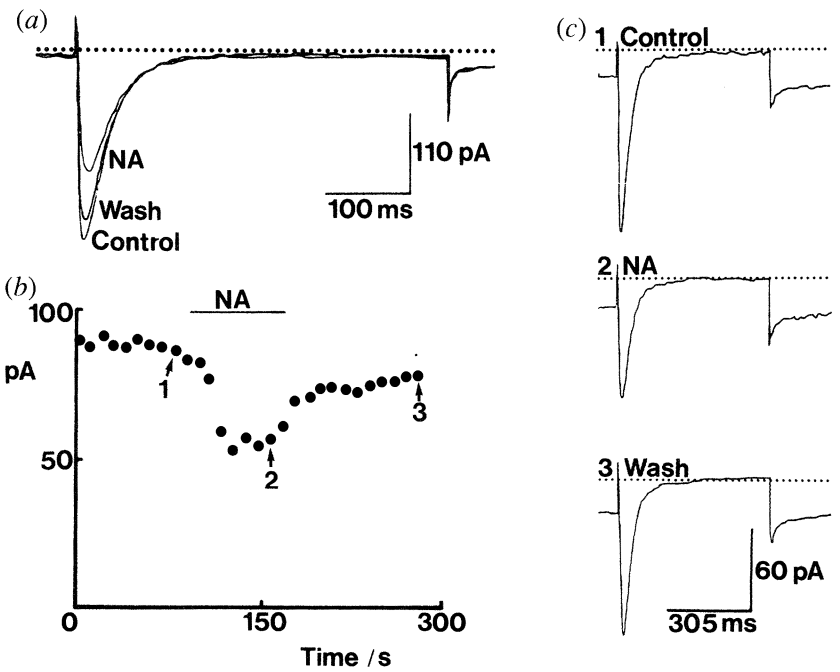

Figure 7 (*a*) Noradrenaline (10 μM) produces reversible inhibition of peak inward Ca channel current. (*b–c*) Timecourse of the action of noradrenaline (10 μM). The currents in (*c*) were obtained at the times indicated in (*b*). (Separate cells in (*a*) and (*c*); holding potential −80 mV, test potential −10 mV; 20 Ba external and Cs internal solutions).

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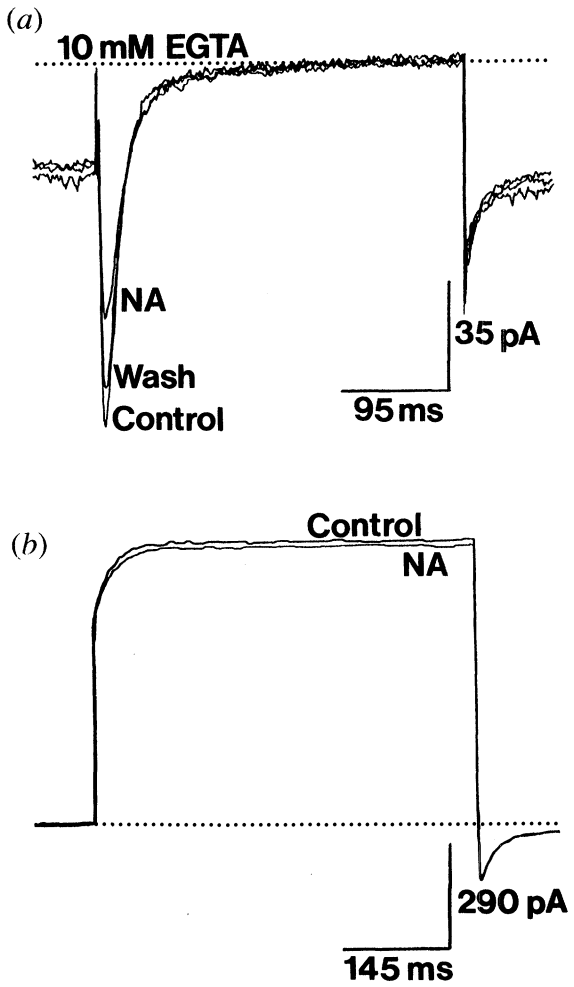

Figure 8. (*a*) Noradrenaline (10 μM) inhibition of peak inward Ca channel current with 10 mM EGTA in the pipette internal solution (20 Ba external and Cs internal solutions). (*b*) Noradrenaline (10 μM) does not increase outward K current (Na external (2 mM Ca) and K internal (3 mM EGTA) solutions). (Separate cells; holding potential −80 mV, test potential −10 mV in (*a*) and 80 mV in (*b*).)

into distinct subtypes is not always straightforward (Aaronson *et al.* 1988; Ohya *et al.* 1988; Klöckner *et al.* 1989), especially when only voltage-dependent kinetic or inactivation parameters are considered. A further difficulty arises from the small current density in vascular myocytes observed for physiological external Ca concentrations, with consequently high divalent ion concentrations (e.g. 10–100 mM Ba) being often employed. Aside from masking any internal Ca-dependence of physiologically relevant Ca currents, voltage-dependent parameters such as activation and inactivation kinetics are undoubtedly affected by surface potential shifts in high Ba external solutions (see Gilbert & Ehrenstein 1984).

Keeping in mind these caveats, our observations in rat aortic myocytes cultured in defined, serum-free medium indicate that the transient, low-voltage-activated, T-type Ca channel current was largely predominant, with the sustained L-type current being evident in only three of 98 cells. This was the case for cells from both primary cultures ($n=66$; three cells with L-type currents) and first ($n=18$) or second passages ($n=11$). The criteria used to classify the Ca current as being of the T-type include voltage-dependence of activation and steady-state inactivation, rapid kinetics of inactivation during depolarizing steps, high sensitivity to block by Ni but not Cd, and relative insensitivity to dihydropyridine Ca channel agonists. Recently, in the same type of cells cultured under almost identical conditions (Bodin *et al.* 1991), a preliminary demonstration of both L- and T-type Ca channel currents was made, although it was unclear what proportion of cells possessed either one or the other alone or both subtypes of currents together. In another study on rat aortic myocytes cultured in serum-containing medium (Akaike *et al.* 1989), both subtypes of Ca channel currents were described. Interestingly, in this study, there was a strong dependence of the number of cells expressing either L- or T-type Ca currents on time in culture, with 80% of cells possessing Ca currents before day 12 in culture and less than 10% of the cells after days 13–14 when the cells had reached confluence. Moreover, the relative proportion of cells expressing L- versus T-type Ca currents changed with time: after 6 days in culture, 60% of cells possessed L-type currents whereas after 12 days, only T-type currents were present, with less than 5% of the cells presenting L-type currents (Akaike *et al.* 1989). This diversity of observations made on a single cell type under almost identical experimental conditions only underlines the similar variety of Ca channel data that exists for smooth muscle cells obtained from different vascular beds. Careful consideration of cell isolation and culture procedures is required before comparison of Ca current data from different vascular smooth muscle cells can be made. The reasons why under our culture conditions T-type Ca current was predominantly expressed as compared to L-type Ca current are not clear, although an obvious possibility would be the presence of a factor in serum which was absent in the synthetic serum substitute used (see Methods). An interesting aspect of our work is the constant expression of T-type Ca current in myocytes from primary cultures and after first and second passages (that is, up to 2–3 weeks after initial plating) using defined, serum-free conditions. In any case, heterogeneity of Ca currents expressed in a single vascular bed would seem not unlikely, given the recent report of alternatively spliced forms of cDNAs encoding the α_1-subunit of the rat aortic Ca channel (Koch *et al.* 1990). Functional expression of these different Ca channel clones might allow a possible explanation for the observed diversity in Ca currents from rat aortic myocytes.

It is interesting to compare Ca channel current densities from different vascular smooth muscle cells, although this is complicated by the divalent cation and the concentration used, and also by the way in which membrane surface area is estimated. We did not routinely measure cell capacitances, but if we estimate that the visible surface of the cells typically used is approximately 100 μm by 5 μm, then the total surface area should be about 1100 μm^2 (assuming a factor of 2.2; see Klöckner & Isenberg 1985*a*), corres-

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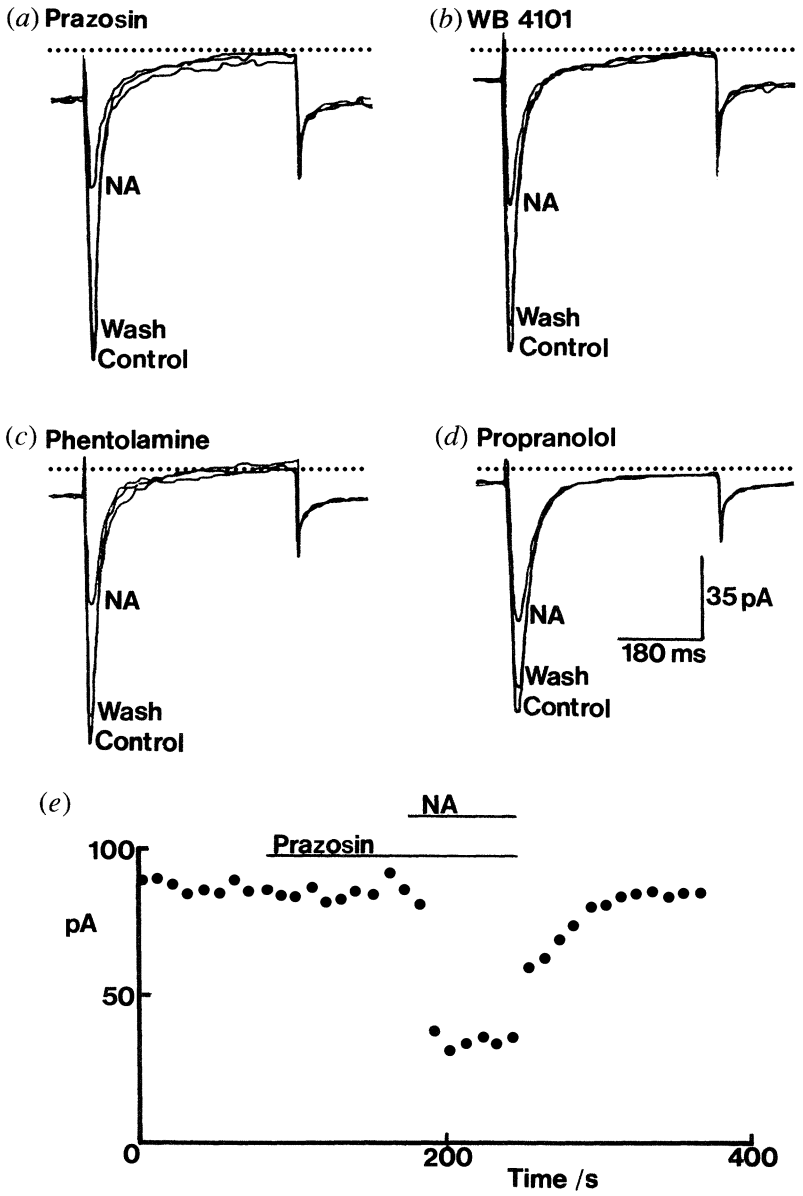

Figure 9. (*a–d*) Various adrenergic antagonists are ineffective in blocking the 10 μM noradrenaline-induced decrease in peak inward Ca channel current (prazosin, 1 μM; WB 4101, 0.1 μM; phentolamine, 1 μM; propranolol, 1 μM). (*e*) Timecourse of noradrenaline (10 μM) inhibition of peak inward Ca channel current in the presence of 0.5 μM prazosin. ((*a–e*), separate cells; holding potential −80 mV, test potential −10 mV; 20 Ba external and Cs internal solutions.)

ponding to a cell capacitance of 11 pF with a specific capacitance of 1 μF cm^{-2}. If the volume:visible surface area ratio is 0.6×10^{-4} cm (see Jones 1981), then a cell volume of about 0.3 pl can be estimated. Assuming that our average peak current in 20 mM Ba solutions was about 100 pA, a current density of 9 μA cm^{-2} is found. By comparison, in isolated rabbit ear artery cells (Aaronson *et al.* 1988), measured cell capacitance was 40 pF (corresponding to a visible surface area of about 1800 μm^2 and a cell volume of 1.1 pl), and average peak current was 30 pA in 1.5 mM Ca solution, corresponding to a current density of 0.8 μA cm^{-2} (cell input resistances in two cases can be estimated as 9 and 2 GΩ). In the same preparation in another study (Benham *et al.* 1987), a cell volume of 0.4 pl was given, corresponding to a visible surface area of 670 μm^2, a cell capacitance of 15 pF and current densities of 1 and 4.3 μA cm^{-2} in 1.5 mM Ca and 110 mM Ba solutions respectively. In isolated rat mesenteric artery cells (Bean *et al.* 1986), cell capacitance was 18 pF (which does not match well the given cell dimensions of 20 μm × 10 μm or 13 μm in diameter if 1 μF cm^{-2} is used), input resistances were greater than 5 GΩ, with a current density of 1.1 μA cm^{-2} in 110 mM Ba solution. In isolated dog saphenous vein cells (Yatani *et al.* 1987), cell capacitance was 20 pF, input resistances 5–10 GΩ, and current density was 5 μA cm^{-2} in 20 mM Ba solution. In isolated guinea-pig basilar artery cells (Simard 1991), cell capacitance was 18 pF with a current density of 15 pA pF^{-1} or 15 μA cm^{-2} in 10 mM Ba solution. Finally in the A7r5 smooth muscle cell line (Fish *et al.* 1988), average current density was 7 pA pF^{-1} or 7 μA cm^{-2} in 20 mM Ba solution. In summary, estimates of

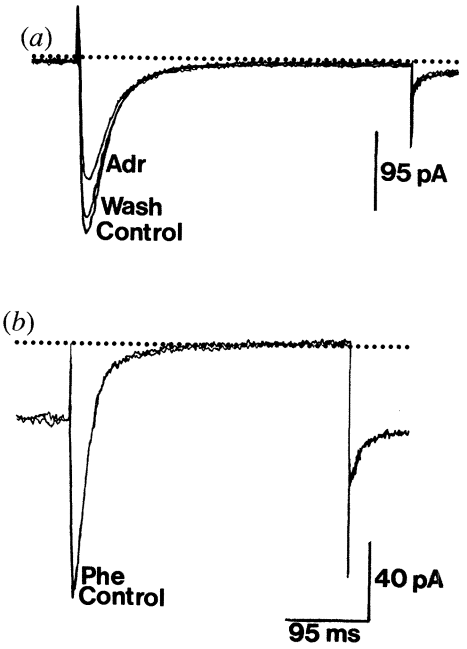

Figure 10. Adrenaline (10 μM; (*a*)) but not phenylephrine (10 μM; (*b*)) causes inhibition of peak inward Ca channel current. (Separate cells; holding potential −80 mV, test potential −10 mV; 20 Ba external and Cs internal solutions.)

peak Ca channel current densities for isolated or cultured vascular smooth muscle cells are quite variable, ranging from 0.8 to 15 μA cm^{-2} (but allowances need to be made for differences in charge carrier species and concentration and in surface area calculations). A value of 20 μA cm^{-2} was found in isolated bladder cells in 3.6 mM Ca solution (Klöckner & Isenberg 1985*b*).

Perhaps not surprisingly, a common effect of noradrenaline on Ca currents from vascular smooth muscle cells has not been found, despite noradrenaline clearly being a vasoconstrictor. For example, in one study (Droogmans *et al.* 1987), both noradrenaline and phenylephrine were reported to inhibit slow Ca currents in freshly isolated smooth muscle cells from rabbit ear artery, whereas in another study (Benham & Tsien 1988) using exactly the same preparation, noradrenaline, but not phenylephrine, caused increases in slow L-type Ca current without affecting the fast T-type Ca current. As well, in K-depolarized tissue strips from rabbit ear artery, phenylephrine reduced both internal Ca levels and ^{86}Rb efflux through Ca-activated K channels, both of these effects being attributed to an inhibition of Ca current by phenylephrine (Declerck *et al.* 1990). In freshly isolated smooth muscle cells from rabbit mesenteric artery, the open probability of single, depolarization-activated L-type Ca channels was greater in the presence of noradrenaline (Nelson *et al.* 1988). No effect of noradrenaline was found on whole-cell Ba currents in freshly isolated cells from rat mesenteric artery (Bean *et al.* 1986), as also was the case for isolated smooth muscle cells from dog saphenous vein (Yatani *et al.* 1987). Finally, in cultured smooth muscle cells from rat portal vein, noradrenaline increased T-type Ca current but decreased L-type Ca current (Pacaud *et al.* 1987, 1989).

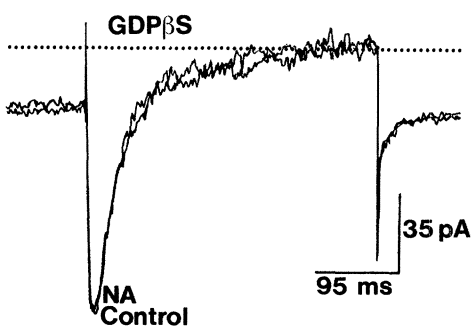

Figure 11. When GTP is replaced by GDP-β-S in the pipette internal solution, noradrenaline (10 μM) does not decrease peak inward Ca channel current. (Holding potential −80 mV, test potential −10 mV; 20 Ba external and Cs internal solutions.)

Our data show clearly that noradrenaline and adrenaline but not phenylephrine reversibly decrease T-type Ca current, and that this inhibition is not blocked by a variety of α- or β-adrenoceptor antagonists. This unusual pharmacological profile was also found by Benham & Tsien (1988) for the noradrenaline-mediated increase in L-type Ca current observed in myocytes isolated from rabbit ear artery, and it was suggested that the putative γ-type adrenoceptor postulated by Hirst & Neild (1980), which shares the same profile, might be implicated. By contrast, both the increase in T-type, and the decrease in L-type, Ca currents provoked by noradrenaline in cultured smooth muscle cells from rat portal vein were blocked by phentolamine and prazosin (Pacaud *et al.* 1987, 1989), with the conclusion that noradrenaline was acting via α-adrenoceptors. Similarly, α_1-adrenoceptor activation was proposed to underly the decrease in Ca current induced by noradrenaline in isolated myocytes from rabbit ear artery, as this decrease was mimicked by phenylephrine but not clonidine (Droogmans *et al.* 1987).

The cellular mechanisms linking noradrenaline action to modulation of Ca channels in vascular smooth muscle cells remain unclear. A role for G-proteins appears to be clearly indicated by our observation of the inhibitory effect of including GDP-β-S in the internal pipette solution on the noradrenaline-induced decrease in Ca current. A similar suggestion was made in isolated cells from rabbit ear artery, based on the irreversible inhibition of Ca current by noradrenaline when GTP-γ-S was present in the pipette solution (Droogmans *et al.* 1987) and on the potentiating effect of including GTP in the pipette solution on the noradrenaline-associated increase in slow Ca current (Benham & Tsien 1988). However, the underlying mechanism explaining the noradrenaline-induced increase in open probability of single L-type Ca channels activated by depolarization in myocytes from rabbit mesenteric artery is unknown (Nelson *et al.* 1988). Interestingly, in isolated bovine tracheal smooth muscle cells, isoprenaline acting via β-adrenoceptors caused a 2.6-fold increase in L-type Ca current (Welling *et al.* 1991). This increase

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appeared to involve a direct G-protein link and was independent of internal cAMP levels and to the presence of the catalytic subunit of cAMP kinase in the pipette solution (Welling *et al.* 1991), unlike the situation in cardiac muscle where cAMP-dependent phosphorylation appears to account entirely for the β-adrenoceptor mediated increase in slow Ca current in heart cells (Hartzell *et al.* 1991; but cf. Yatani & Brown 1989). Dibutyryl cAMP and parathyroid hormone decreased Ca current in isolated cells from rat tail artery (Wang *et al.* 1991), whereas in A7r5 smooth muscle cells, isoprenaline and forskolin increased Ca current (Marks *et al.* 1990). Although cAMP would not seem to be involved in any modulatory action of noradrenaline on vascular smooth muscle cell Ca currents, a recent report demonstrating that the vasodilator nitroprusside decreases L-type Ca current in isolated myocytes from rabbit pulmonary artery suggests that cGMP or cGMP-dependent phosphorylation is implicated (Clapp & Gurney 1991).

In view of the primary action of noradrenaline as a vasoconstrictor, the physiological significance of a noradrenaline-induced decrease in voltage-dependent Ca current in vascular smooth muscle cells is not at all clear. But given the present diversity of observed effects, further investigation would seem necessary before a conclusive interpretation be agreed upon.

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