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NORADRENALINE MODULATION OF CALCIUM CHANNELS IN SINGLE SMOOTH MUSCLE CELLS FROM RABBIT EAR ARTERY

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SUMMARY

1. Whole-cell recordings of voltage-gated Ca^{2+} current in single smooth muscle cells from rabbit ear artery were obtained with 110 mM-Ba^{2+} as charge carrier.

2. Noradrenaline (NA, 1–20 μM) produced a sustained increase in the dihydropyridine-sensitive L-type Ca^{2+} current, ranging up to 3-fold in some cells. The dihydropyridine-resistant T-type Ca^{2+} current was not affected.

3. The time and voltage dependence of activation and inactivation of the L-type current were not significantly changed during NA modulation.

4. The NA-induced increase in L-current was enhanced in magnitude and consistency by the inclusion of 200 μM-GTP in the pipette (internal) solution.

5. The effect of NA on L-current was not abolished by pre-treatment with prazosin, phentolamine or propranolol, suggesting that it is not mediated by α- or β-adrenoceptors.

6. Phenylephrine (5 μM) was ineffective as an agonist, while adrenaline was approximately equipotent to NA. In these respects, the pharmacology of L-current modulation resembles that of 'γ'-adrenergic receptors (Hirst & Nield, 1980).

7. NA modulation of L-type Ca^{2+} channels may be particularly important in promoting sympathetic vasoconstriction in resistance vessels where Ca^{2+} stores are relatively poorly developed and where NA-evoked contractions are very sensitive to organic Ca^{2+} channel antagonists.

INTRODUCTION

Noradrenaline (NA) has powerful effects on the mechanical and electrical activity of vascular smooth muscle cells (reviewed in Vanhoutte, Verbeuren & Webb, 1981; Bolton & Large, 1986; Bulbring & Tomita, 1987). Contractions are evoked when NA is applied as an exogenous agent or when NA is co-released with ATP during sympathetic nerve stimulation (Burnstock & Kennedy, 1986). A large body of experiments in ear arteries and other vascular smooth muscle preparations suggests that NA acts through multiple mechanisms (Bolton & Large, 1986). There is little doubt that NA can promote Ca^{2+} delivery via mechanisms that do not require membrane depolarization, including release of Ca^{2+} stores or Ca^{2+} influx through

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'receptor-operated channels'; (Somlyo & Somlyo, 1968; Bolton, 1979; van Breemen, Aaronson & Loutzenhiser, 1979).

An additional possibility that has received relatively little attention is that NA enhances Ca^{2+} current through voltage-gated Ca^{2+} channels (Cohen, Janis, Taylor & Scriabine, 1986), analogous to its effect on Ca^{2+} channels in heart cells (for review, see Reuter, 1983; Tsien, Bean, Hess, Lansman, Nilius & Nowycky, 1986; Trautwein, Kameyama, Hescheler & Hofmann, 1986). Some indirect evidence consistent with this hypothesis comes from intracellular recordings that show robust action potentials as a result of NA application in whole ear artery (Droogmans, Raeymaekers & Casteels, 1977) and in cultured oviduct smooth muscle cells (Sinbach & Shain, 1980). To explore the idea that NA modulates voltage-gated Ca^{2+} channels, independent of its other actions, we have carried out whole-cell patch clamp recordings in single smooth muscle cells from rabbit ear artery. Here we present evidence that NA increases L-type Ca^{2+} channel current through interaction with an adrenergic receptor different from conventional α- and β-adrenoreceptors.

Some of these results have been presented in abstracts (Aaronson, Benham, Bolton, Hess, Lang & Tsien, 1986, Benham & Tsien, 1987*a*).

METHODS

Current measurements in single cells

Adult rabbits (1·5–2·5 kg) were killed by cervical dislocation or by intravenous pentobarbitone injection. Single smooth muscle cells from the central ear artery were dispersed as previously described (Benham & Bolton, 1986). Briefly, the ear artery was dissected free of connective tissue, cut into strips and incubated for 90 min in low-Ca^{2+} (10 μM added $CaCl_2$) modified Tyrode solution containing collagenase (Cooper Biomedical), elastase (Sigma) and bovine serum albumin. Cells were separated by trituration and stored at 4 °C until use.

The recording pipettes (1–3 MΩ) contained intracellular solution of the following composition (mM): 130 CsCl; 10 EGTA; 3 $MgCl_2$; 0·5 ATP; 5 HEPES; buffered to pH 7·2 with TEA-OH. The modified Tyrode solution used for the cell dissociation, and for tension recordings from arterial strips contained (mM): 130 NaCl; 5 KCl; 10 glucose; 10 HEPES; 1·2 $MgCl_2$; 1·5 $CaCl_2$; buffered to pH 7·3 with NaOH. The isotonic Ba^{2+} solution contained (mM): 110 $BaCl_2$; 10 glucose; 10 HEPES (buffered to pH 7·3 with TEA-OH). No correction was made for the junction potential between the pipette and 110 mM-Ba^{2+} bathing solutions, which was found to be −7 mV (pipette negative).

Drugs were applied by allowing at least 20 ml of the new solution to flow into the bath (volume ~ 1 ml). Drugs used were noradrenaline bitartrate, phenylephrine, 8-bromo-cyclic AMP, adrenaline, propranolol–HCl, guanosine triphosphate (Sigma), phentolamine mesylate (Ciba), prazosin (Pfizer), adenosine triphosphate (Boehringer) and (+) and (−) isomers of Sandoz 202 791.

Whole-cell current recordings were made at 21 °C with standard patch-clamp techniques (Hamill, Marty, Neher, Sakmann & Sigworth, 1981). Ca^{2+} channel currents were evoked by depolarizing steps of 160 ms duration every 6 s. Current records were filtered with an eight-pole Bessel filter (−3 dB at 1 kHz) and sampled at 5 kHz for analysis by a PDP-11/23 computer. Linear leak and capacitative currents were taken as the current associated with hyperpolarizing voltage steps ~ 15 mV in amplitude and have been subtracted from the whole-cell current record in all the traces shown in this paper except Fig. 1. A new leak template was measured after each drug application. The peak amplitude of leak-subtracted current records was taken as the largest value obtained by averaging over ten consecutive data points (2 ms). We saw no evidence of a lack of voltage control during depolarizing voltage steps. Klockner & Isenberg (1985) have performed experiments with two patch pipettes on urinary bladder smooth muscle cells and showed adequate voltage control. These cells were roughly twice as long as the ear artery cells used for this work and generated up to 5-fold greater Ca^{2+} currents, so we are quite confident of the space clamp in these cells. Series resistance errors were also not a problem as series resistance was usually less than 10 MΩ and whole-cell currents rarely exceeded 250 pA.

Tension measurements in arterial strips

Ear arteries, cleaned of superficial connective tissue, were threaded onto a steel needle and cut into spiral strips 5 × 1 mm. The endothelium was removed by gently rubbing the luminal surface with a blunt instrument. The arterial strips were suspended in standard 25 ml organ baths under resting tension of 400 mg and tension was recorded isometrically. High-K^+ solutions were made by

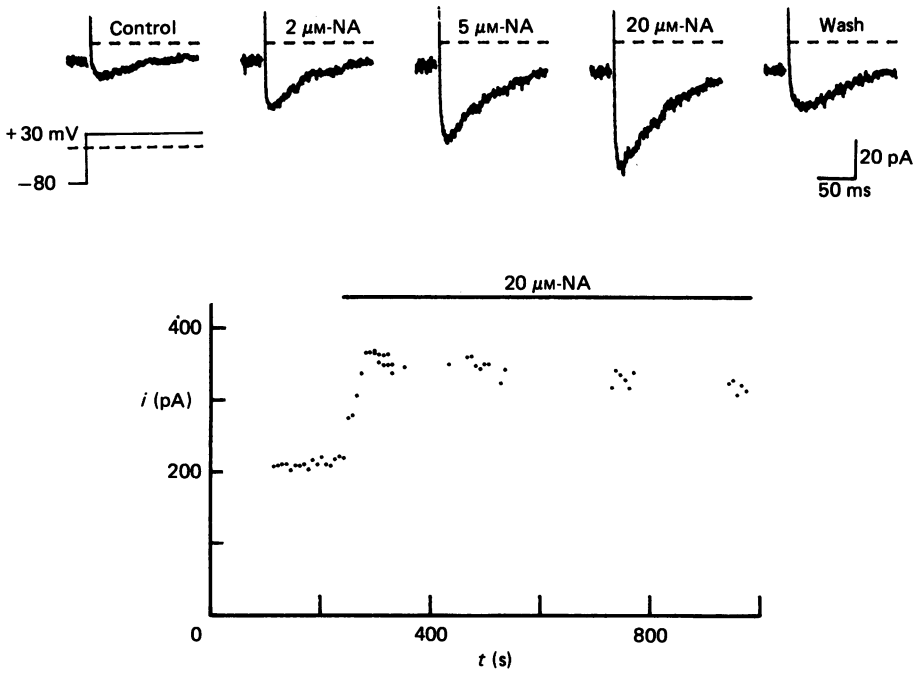

Fig. 1. Dose dependence and time course of NA effect on Ca^{2+} channel current. *A*, response to progressively higher concentrations of NA in a single ear artery cell. Inward currents (110 mM-Ba^{2+}) evoked with voltage steps from −80 to +30 mV (voltage protocol is shown under the first current record). Records have not been leak subtracted to show absolute level of the holding current. Dashed line indicates zero current level. Capacitative currents have been truncated. Records were obtained in the absence of drug and 50, 75 and 60 s after addition of 2, 5 and 20 μM-NA. *B*, plot of peak inward current against experimental time for another cell. Cell was held at −80 mV and stepped to +30 mV. Twenty micromolar NA was applied for the duration of the horizontal bar. Time zero is 240 s after starting the whole-cell recording. In subsequent figures of this type, time zero is always greater than 200 s after starting whole-cell recording.

appropriate substitution of KCl for NaCl. The bath solution was gassed with 100% O_2 and maintained at 37 °C. NA was applied in small volumes (25–150 μl) to the top of the bath to give the final concentrations shown.

RESULTS

Dose and time dependence of the noradrenaline effect

Patch clamp recordings were made from freshly isolated ear artery cells. When bathed in the standard Tyrode solution, such cells were typically ~ 100 μm long in their relaxed state, and showed prominent contractions in response to bath

application of NA or ATP. To maximize Ca^{2+} channel current and minimize interference from other ion channels, the whole-cell recordings were made with an external solution containing 110 mM-Ba^{2+} and a recording pipette containing 130 mM-Cs^{+} plus 10 mM-EGTA (Benham, Hess & Tsien, 1987). After breaking the patch and establishing the whole-cell recording mode, inward Ca^{2+} channel current often increased over $\sim$ 1 min before stabilizing, possibly as a result of increasing the intracellular ATP concentration, or by removal of inactivation through introduction of intracellular EGTA or through establishment of a holding potential (−80 mV) more negative than the resting potential.

Figure 1*A* illustrates the response of Ca^{2+} channel current in a single ear artery cell to increasing concentrations of NA. The peak inward current increases in a dose-dependent manner over the range between 2 and 20 μM-NA. This and other experiments gave us the overall impression that a maximal response was obtained at about 10 μM-NA. The increase in voltage-gated current was not associated with any sustained change in the holding current at −80 mV (Fig. 1*A*).

As Fig. 1*B* illustrates, the inward Ca^{2+} channel current increased with successive depolarizations over the course of $\sim$ 30 s, considerably slower than the time required for solution exchange. The increase was well-sustained in the continued presence of the agonist (Fig. 1*B*). Recovery following removal of NA was occasionally seen with repeated washing, but was not always easy to distinguish from run-down of Ca^{2+} channel current.

Noradrenaline increases L-type current but not T-type Ca^{2+} current

Previous experiments have established that rabbit ear artery cells display two components of Ca^{2+} channel current, carried by dihydropyridine-resistant Ca^{2+} channels that inactivate rapidly over a broad range of potentials (T-type) and dihydropyridine-sensitive Ca^{2+} channels that show little inactivation except at strong depolarizations (L-type). T-type Ca^{2+} channels are > 90% inactivated at a holding potential (HP) of −40 mV, while L-type Ca^{2+} channels are little affected by changing the holding potential from −80 to −40 mV (Bean, Sturek, Puga & Hermsmeyer, 1986; Benham *et al.* 1987).

Figure 2*A* shows records from a cell that expresses L-current but little or no T-current, judging by the almost exact agreement between currents evoked from HP = −40 and HP = −80 mV. Exposure to NA doubles the peak inward current, but the inward current remains unchanged with this change in holding potential, consistent with an increase in the magnitude of the L-current. This interpretation receives further support from experiments that rely on the pharmacological properties of L-current. Figure 2*B* illustrates results from another cell where NA nearly doubled the inward current at +30 mV. Subsequent application of the dihydropyridine Ca^{2+} channel antagonist (−)-202 791 abolished the NA-enhanced current almost completely, as expected if the enhancement were due to L-current and not T-current.

Figure 2*C* illustrates a third approach, in which L-current was first augmented by the dihydropyridine Ca^{2+} channel agonist (+)-202 791. In this case, the Ca^{2+} current was further increased by a subsequent application of NA. Since dihydropyridine Ca^{2+} agonists fully displace binding of dihydropyridine of Ca^{2+} antagonists,

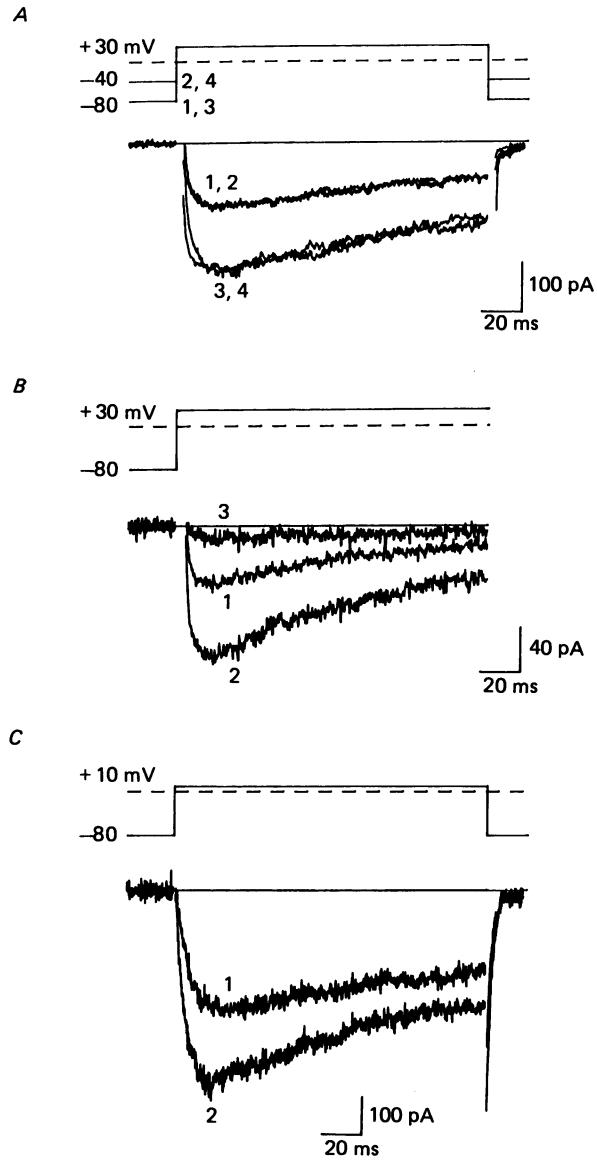

Fig. 2. NA increases L-type Ca^{2+} current. *A*, currents evoked by depolarization to +30 mV from holding potentials of −80 mV (traces 1 and 3) and −40 mV (traces 2 and 4) in a cell with L-current and little or no T-current. Records taken in the absence of drug (traces 1 and 2) and after addition of 10 μM-NA (traces 3 and 4). Note that the current evoked from the two holding potentials is similar in both cases. *B*, current records obtained from a cell in the absence of drug (trace 1), after application of 10 μM-NA (trace 2), and after addition of 5 μM-(−)-202 791 in the continued presence of 10 μM-NA (trace 3). *C*, current records obtained from a cell in the presence of 5 μM-(+)-202 791 in the bathing solution before (trace 1) and after addition of 5 μM-NA (trace 2).

presumably by binding at the same receptor sites, this experiment argues that the blocking effect of the (−) isomer (Fig. 2*B*) is not a result of competition for the NA receptor. Evidently, NA can still produce an increase in L-type Ca^{2+} channel activity over and above that achieved with dihydroyridine Ca^{2+} channel agonists alone.

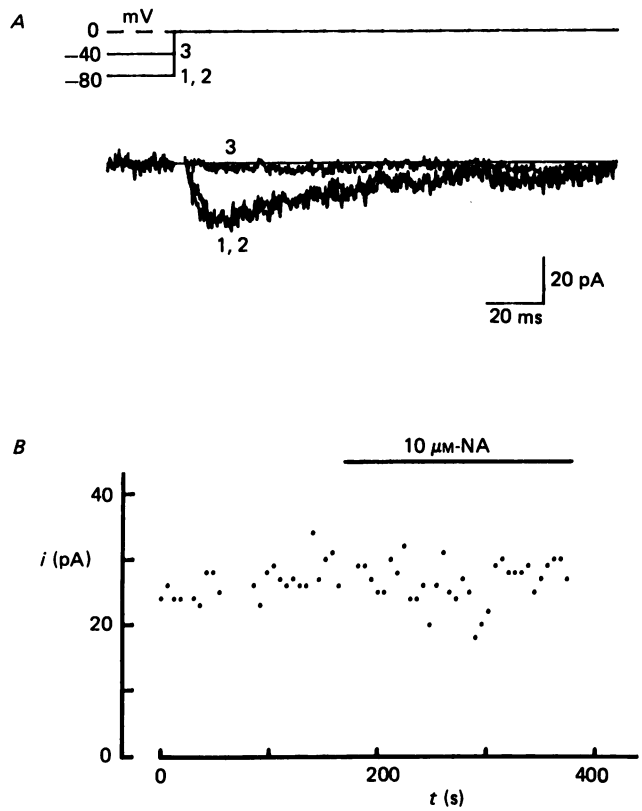

Fig. 3. NA does not increase T-type current. *A*, current records from a cell with mostly T-current. Current evoked by step depolarization from a holding potential of −80 to 0 mV in the absence of drug (trace 1), after addition of 10 μM-NA (trace 2), and after changing the holding potential to −40 mV in the presence of NA (trace 3). *B*, plot of peak current against experimental time for another cell bathed in 5 μM-(−)-202 791. Ten micromolar NA was applied for the duration of the horizontal bar.

Additional experiments were carried out to determine whether NA has additional effects on T-type Ca^{2+} channels. Figure 3*A* shows recordings from an ear artery cell with substantial T-current but little or no L-current, a pattern occasionally seen and presumably due to the lability of L-type channels (Benham *et al.* 1987). In the absence of neurotransmitter, the T-current displays a characteristically rapid decay with depolarizations from HP = −80 to 0 mV (trace 1). Exposure to NA gave no change in the T-current (trace 2), while displacement of the holding potential from −80 to −40 mV eliminated the inward current, as expected for T-type Ca^{2+} channels (Bean *et al.* 1986; Benham *et al.* 1987).

The ineffectiveness of NA in modifying T-type Ca^{2+} channels was also seen in experiments where L-currents were eliminated by dihydropyridine Ca^{2+} antagonists. Figure 3*B* describes measurements of the amplitude of T-current, with (−)-202 791 present to block L-current. Application of 10 μM-NA gave no increase in the dihydropyridine-resistant current in this example or in a total of five cells; a similar dose of NA produced clear-cut increases in the overall Ca^{2+} current in six of eight cells that had not been exposed to the dihydropyridine Ca^{2+} antagonist.

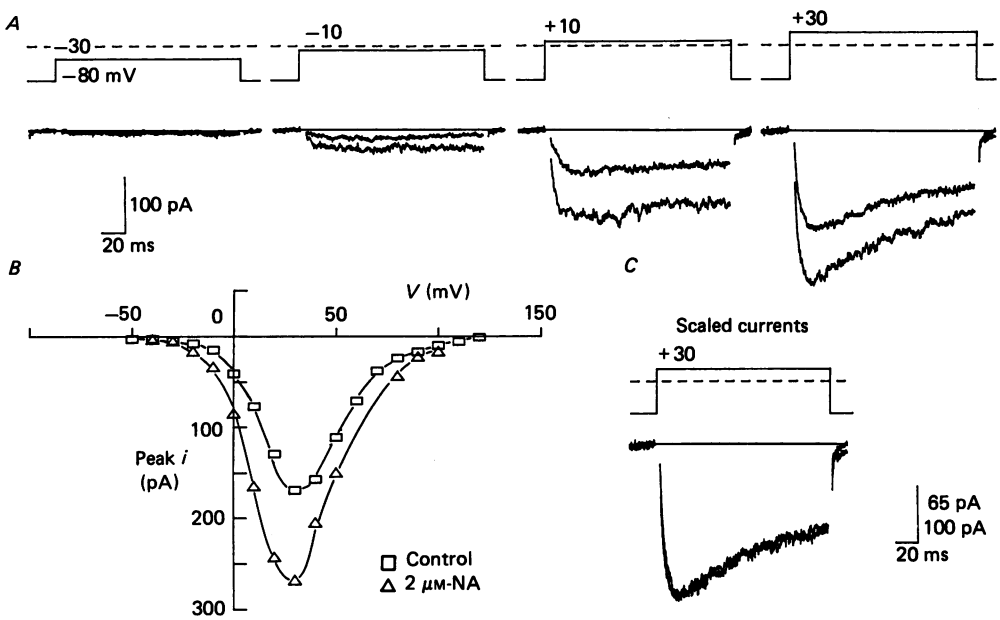

Fig. 4. Effect of NA on Ca^{2+} channel currents evoked at various test potentials. *A*, current traces recorded in a cell using voltage protocols shown above, in the absence of drug, and in the presence of 2μM-NA (larger currents) for each test potential. *B*, plot of peak current against potential. *C*, comparison of time course of currents. Current record in the absence of drug (shown in extreme right of *A*) has been scaled up to match peak current after exposure to NA.

The nature of the increase in L-type current

Activation properties. Figure 4*A* shows the effect of NA for a series of test depolarizations from HP = −80 mV. At any given test potential, the Ca^{2+} current was increased throughout the depolarizing pulse, with little change in time course. Thus, traces taken in the presence of NA closely match scaled records obtained in the absence of the neurotransmitter (Fig. 4*C*). The magnitude of the NA-induced increase is roughly 2-fold over a broad range of potentials. There is little change in the amplitude of the leak-subtracted current measured with depolarizations too weak to activate Ca^{2+} channels, or with very strong depolarizations (+100 mV), where the driving force for Ca^{2+} channel current becomes small.

To look for possible voltage dependence of the NA effect, we analysed the changes in Ca^{2+} channel conductance over the range of potentials where activation increases

most steeply (−40 to +30 mV). When we plotted Ca^{2+} channel chord conductance in the absence and presence of 5 μM-NA, there was little or no change in voltage dependence: the smooth curves fitted to the data differed only in amplitude. The lack of voltage dependence is substantiated by the analysis of collected results from five ear artery cells (Fig. 5). NA-induced changes in inward current were normalized by the n-fold increase at a potential near the peak of the current–voltage relationship (+30 mV), and then plotted against test potential. Although the data points are

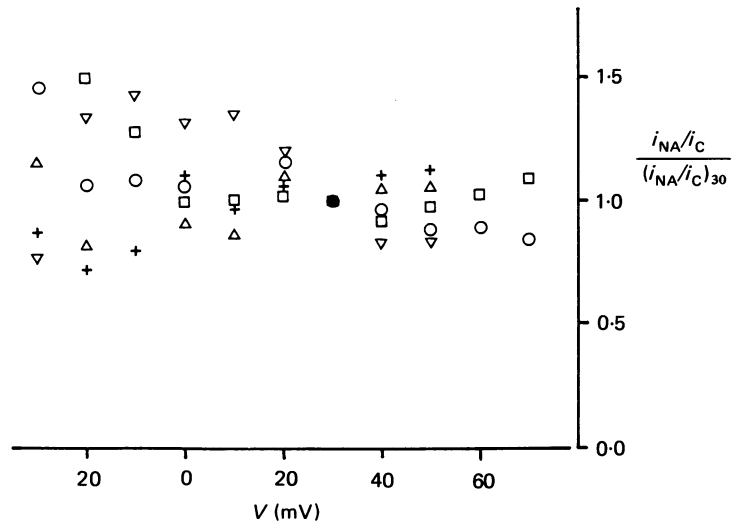

Fig. 5. Analysis of NA-evoked increase in Ca^{2+} current at different test potentials. Plot of fractional increase in current in the presence of NA (i_{NA}) normalized to the fractional increase at +30 mV in the same cell. Data from five cells. i_C, control current.

scattered at weak potentials where inward currents are small, it is clear that the NA-induced enhancement shows little or no voltage dependence overall.

Under the ionic conditions of these experiments (110 mM-external Ba^{2+}), the voltage dependence of L-type Ca^{2+} channel activation will be shifted toward positive potentials by ~ 20 mV relative to its position under physiological conditions (Benham *et al.* 1987; Fox, Nowycky & Tsien, 1987). The 7 mV junction potential brings the total shift to about 25 mV. Thus, the foot of the activation curve would lie near −55 mV. Since ear artery cells display resting potentials of −65 mV (Holman & Surprenant, 1980; Cheung & MacKay, 1986), we conclude that depolarization would be required to activate L-type Ca^{2+} channels significantly, even in the presence of NA.

Inactivation properties. L-type Ca^{2+} channels inactivate with maintained depolarizations over the range of potentials positive to −40 mV (Bean *et al.* 1986). In ear artery cells, half-inactivation of L-current occurs near −25 mV (Benham *et al.* 1987; Fig. 6*B*). Figure 6*A* shows an experiment that tests for possible changes in this voltage dependence under the influence of NA. Each panel compares current records taken with HP = −80 mV and HP = −30. The degree of inactivation with HP =

−30 mV remains unchanged with exposure to NA, despite the 2-fold increase in current.

Figure 6*B* presents data from another cell in which Ca^{2+} channel current was evoked from a series of different holding potentials. Currents were evoked by test depolarizations to +30 mV, applied 12 s after changing to a new holding potential.

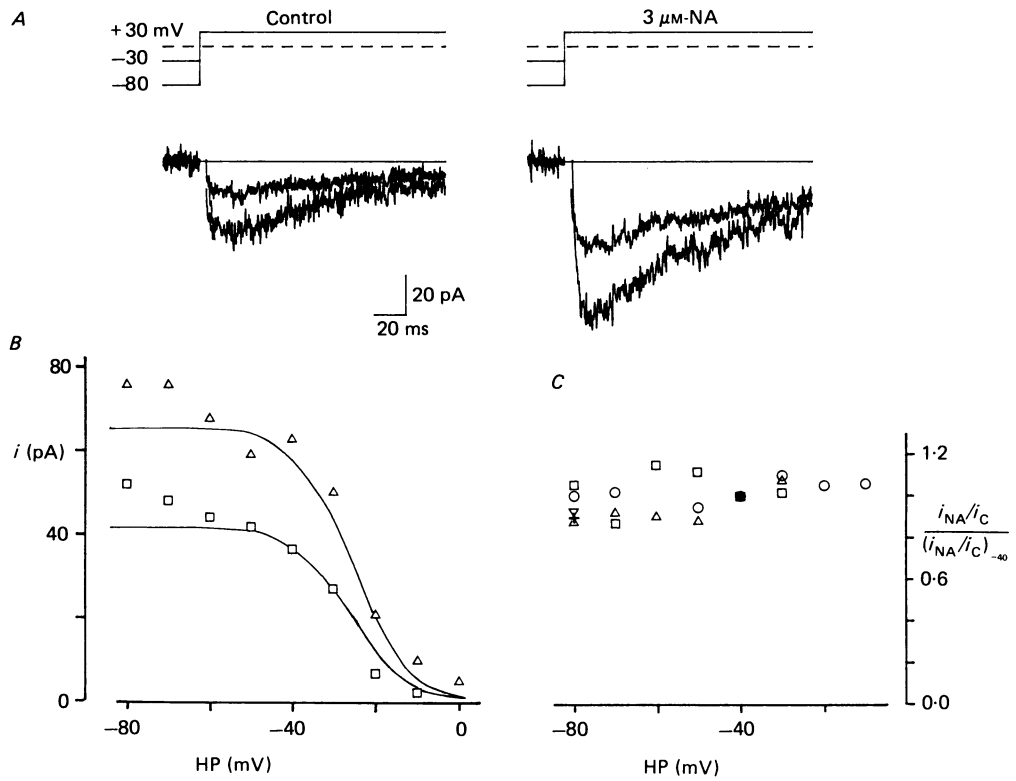

Fig. 6. Effect of holding potential on NA-evoked increase in current at +30 mV. *A*, current records evoked by voltage steps to +30 mV from holding potentials of −80 and −30 mV in the absence of drug (left panel) and in the presence of 3 μM-NA (right panel). *B*, plot of peak current against holding potential for another cell before (□) and after application of 1 μM-NA (△). Curves drawn by eye through the data points. *A* and *B*, representative records from two cells of five. *C*, plot of fractional increase in current in the presence of NA, normalized to the increase seen at HP = −40 mV in the same cell. Data from five cells including that shown in *B* (○). i_{NA}, NA current; i_C, control current.

The results show that NA increases the inward current by about 50%, regardless of the holding potential, without any clear shift in the voltage dependence of inactivation. The smooth curves show what would be expected if the currents scaled perfectly, ignoring a small amount of T-current that becomes available with strongly negative holding potentials (a minor factor in most experiments). Figure 6*C* shows data from five cells, expressed as the NA-induced enhancement of inward current at various test potentials, normalized by the enhancement at HP = −40 mV. The collected results confirm the impression that inactivation is hardly affected.

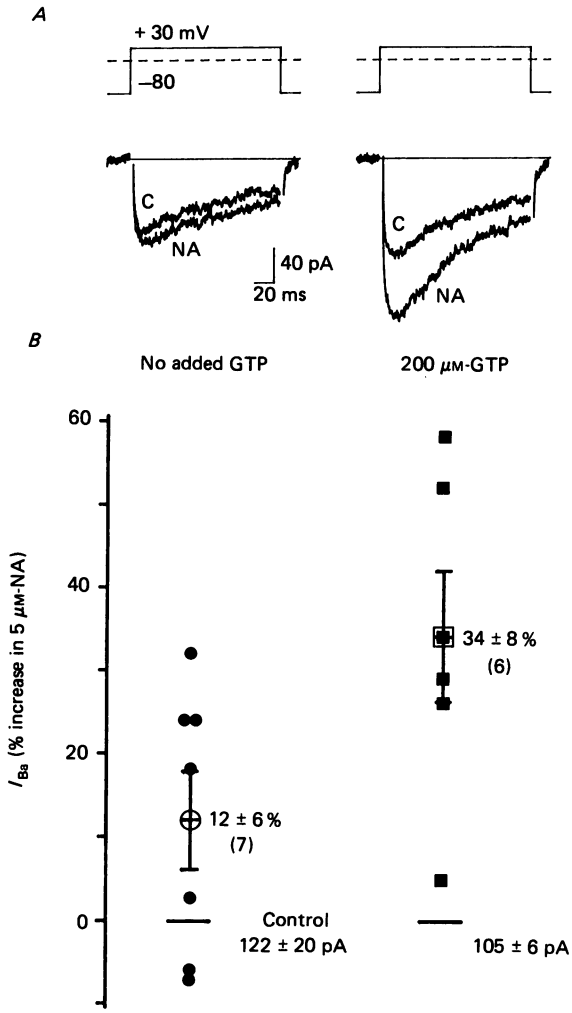

Fig. 7. GTP in the pipette solution favours NA action on Ca^{2+} channel currents. *A*, representative current records from a cell with the standard pipette solution only (left panel) and cell with the standard pipette solution plus 200 μM-GTP (right panel), in the absence (C) and in the presence of 5 μM-NA (NA). *B*, plot of increase in Ca^{2+} channel current in the presence of 5 μM-NA in seven cells with no added GTP (●) and six cells with 200 μM-GTP added to the pipette solution (■). Open symbols with error bars are mean values ± S.E.M. Mean values are significantly different ($P < 0.05$, two-tailed, unpaired *t*-test). All cells were from the same batch and recordings with the two pipette solutions were made in random order.

Variability of noradrenaline response

There was some variability in the NA responsiveness of cells, from one enzymatic dispersion to another, and from one cell to the next within a batch obtained with the same dispersion. In eight of twenty-two batches (36%), no response was seen to NA in any of a minimum of three cells in each batch. In the fourteen responsive batches, thirty-eight out of a total of sixty-two cells (61%) displayed a NA-induced increase

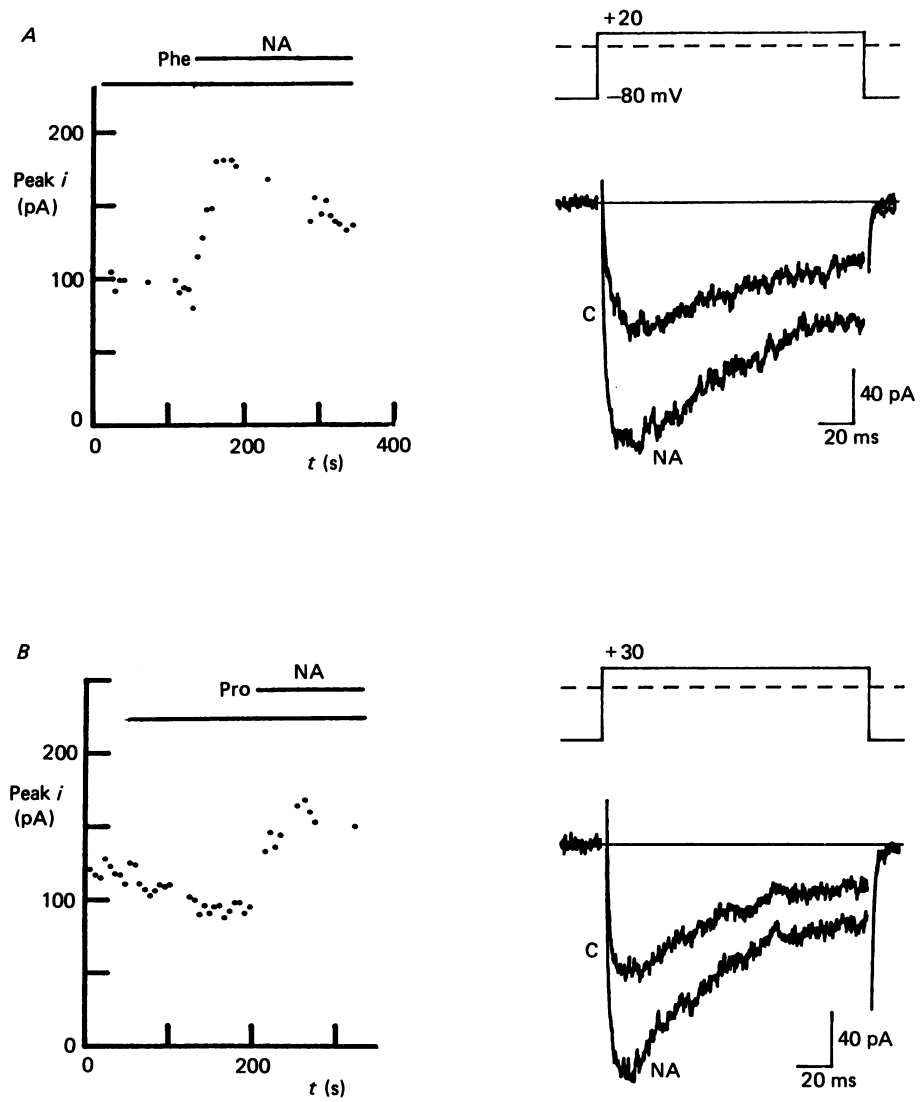

Fig. 8. Phentolamine and propranolol fail to prevent the response to a submaximal concentration of NA. *A*, plot of peak current amplitude against experimental time and representative current traces before (C) and in the presence of 5 μM-NA (NA). Five micromolar phentolamine (Phe) exposure was begun 120 s before NA application. *B*, similar data from another cell in which 1 μM-propranolol (Pro) was applied 150 s before 5 μM-NA (NA).

in Ca^{2+} channel current of greater than 20%. Responsiveness was not obviously related to the size of the Ca^{2+} channel current in the absence of the neurotransmitter (see the examples illustrated in Fig. 1).

One possible source of variability can be seen in Fig. 7, which presents collected data from cells obtained from the same ear artery. In this particular batch of cells, clear responses to NA (> 20% increase in Ca^{2+} current) were seen in only three out

of seven cells under our standard experimental conditions. Addition of GTP to the recording pipette increased the proportion of responsive cells to five out of six and significantly augmented the magnitude of the current enhancement. One possible interpretation is that the NA effect is mediated by GTP-binding proteins, and that the effectiveness of NA varies with the degree to which GTP is washed out during whole-cell recordings. Another potential source of variability may be the effect of the enzymic dispersion on adrenergic receptors.

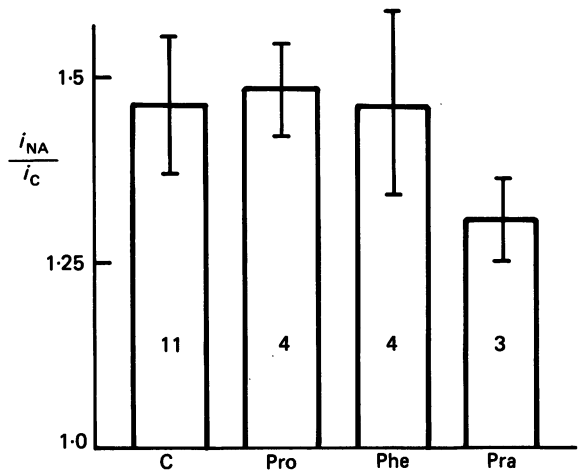

Fig. 9. Collected results showing lack of effect of adrenoceptor antagonists on fractional increases in Ca^{2+} channel current evoked by 5 μM-NA. Antagonists were propranolol (Pro, 1 μM), phentolamine (Phe, 5 μM) and prazosin (Pra, 1 μM). Number of cells indicated on each bar. Collected responses in the absence of antagonists (C) were determined in the same five batches of cells as those used for study of antagonist effects. Responses in the presence of antagonists are not significantly different from control group ($P > 0{\cdot}05$). i_{NA}, NA current; i_C, control current.

Pharmacological properties of the adrenergic response

Various pharamacological agents were tested in an effort to characterize the receptor that mediates the NA effect. As illustrated in Fig. 8*A*, the response to a submaximal dose of NA (5 μM) was not blocked by pre-exposure of cells to the α-adrenergic antagonist phentolamine (5 μM). Prazosin (1 μM), a specific α_1-blocker, was similarly ineffective (Fig. 9). Propranolol (1 μM), a blocker of β-adrenergic receptors, also failed to prevent the NA effect (Fig. 8*B*). Collected results from a series of such experiments (Fig. 9) show that the NA-induced increase was not significantly attenuated by any of the adrenergic blockers. These experiments suggest that the NA-induced enhancement does not involve either α- or β-adrenergic receptors, but may be mediated by some other form of adrenergic receptor (see Discussion).

Effects of other catecholamines were studied in another series of experiments (Fig. 10). Phenylephrine (5 μM), a known stimulator of α-adrenergic receptors, was completely ineffective in augmenting the Ca^{2+} current, nor did it prevent the

enhancement produced by a subsequent application of NA (Fig. 10*A*). Isoprenaline (1–5 μM) a β-adrenergic agonist, was also ineffective (three cells), as was 8-bromocyclic AMP (0·2 mM, four cells). On the other hand, adrenaline (5 μM) gave an increase in L-current very much like that found with NA (Fig. 10*B*); subsequent

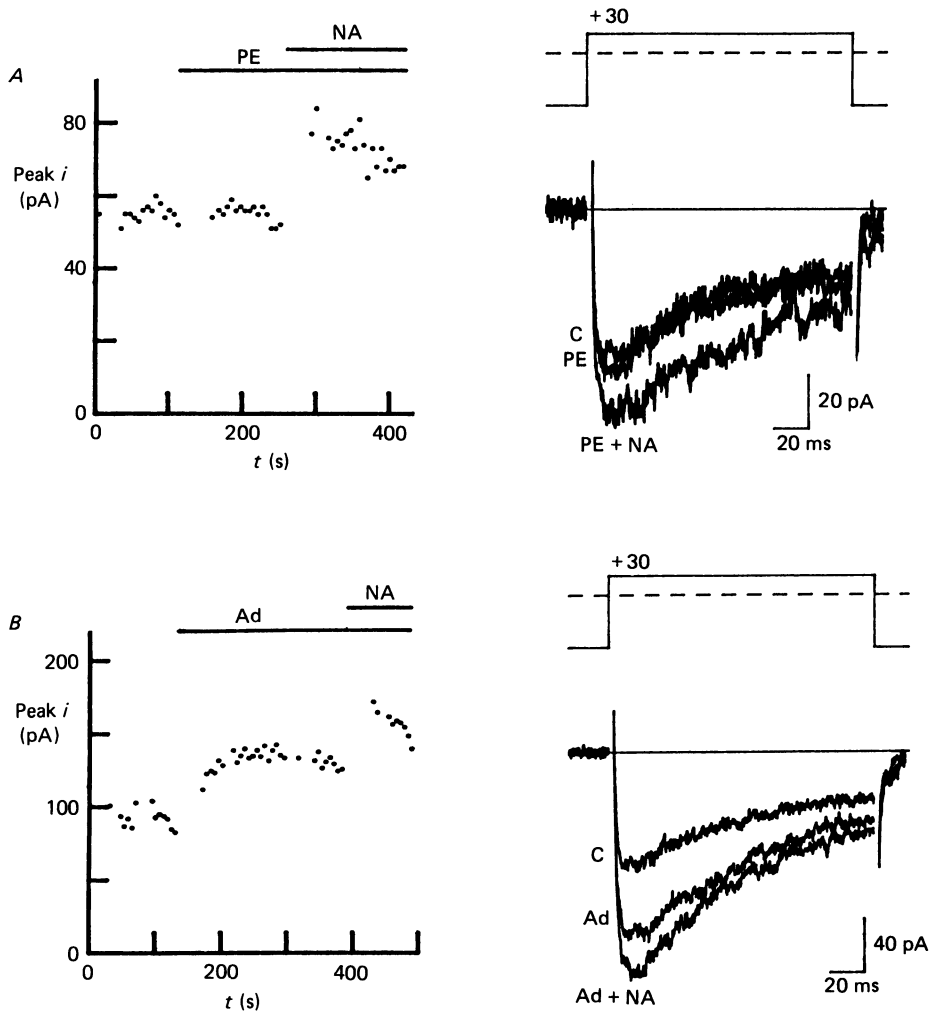

Fig. 10. Responses to NA and other adrenergic agonists displayed as in Fig. 8. *A*, 5 μM-phenylephrine (PE) applied to a cell prior to exposure to 5 μM-NA. *B*, application of 5 μM-adrenaline (Ad) followed by 5 μM-NA in another cell.

addition of NA gave a small additional effect, as expected if both agents acted through a common final mechanism.

Tension measurements in ear artery strips

The single-cell recordings demonstrated a NA-induced increase in voltage-gated Ca^{2+} current that is resistant to α- and β-blockers. Does this mechanism contribute

to the contractile response in ear artery smooth muscle? To address this question, we applied NA in the presence of phentolamine and propranolol, and compared contractile responses in normally polarized tissue where voltage-gated Ca^{2+} channels are not activated, and in K^+ depolarized tissue, in which a steady voltage-gated Ca^{2+} influx is expected.

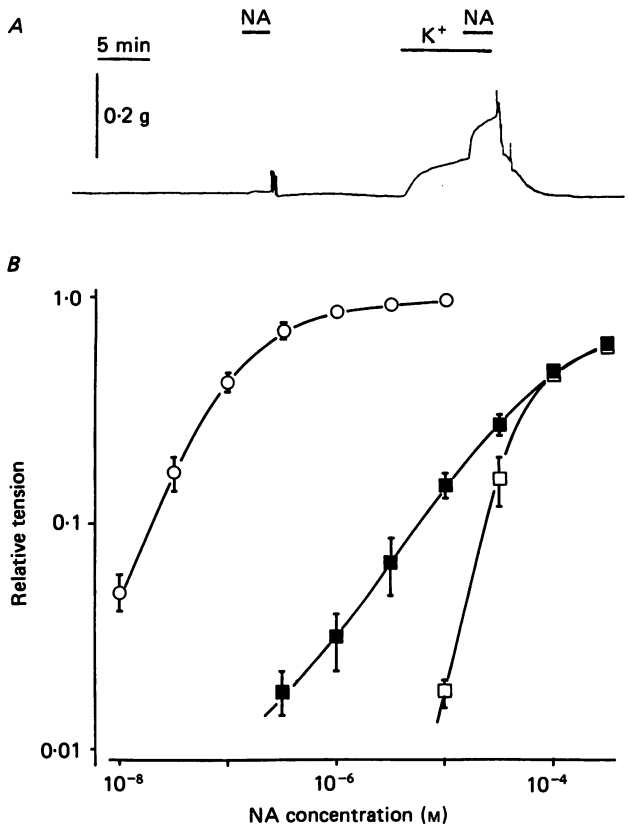

Fig. 11. α- and β-blocker-resistant contraction in arterial strips. *A*, tension record from an arterial strip in presence of 5 μM-phentolamine, 1 μM-propranolol and 3 μM-guanethidine added 20 min before first NA application shown. One micromolar NA was applied for 2 min before and in the presence of 20 mM-K^+. *B*, dose–response curves to NA in control solution (○), in the presence of 5 μM-phentolamine, 1 μM-propranolol, 3 μM-guanethidine (□) and these antagonists with the K^+ concentration increased to 32 mM (■). Tension in 5 mM-K^+ was normalized to peak tension generated by NA in 5 mM-K^+ in the absence of blockers. Similarly, in 32 mM-K^+, tension was normalized to the peak obtained in 32 mM-K^+ in the absence of blockers. Standard errors are shown as error bars when they exceed the size of the symbols ($n = 4$).

Figure 11*A* shows a force recording from a spiral ear artery strip. The preparation was bathed in Tyrode solution containing 5 μM-phentolamine, 1 μM-propranolol, as in Fig. 8, and 3 μM-guanethidine was included to reduce any effects of neuronal NA release. Application of 1 μM-NA for 2 min caused a negligible increase in tension. After wash-out of the catecholamine, the bathing solution K^+ concentration was

increased from 5 to 20 mM to depolarize the preparation. This induced an increase of tension (11% of the response to 1 μM-NA in the absence of blockers). At this point, the addition of 1 μM-NA evoked a further increase in tension similar to that induced by 20 mM-K^+ alone.

Figure 11*B* shows collected data from a series of experiments of this type. Cumulative dose–response curves were constructed by exposing spiral strips to increasing doses of NA, in the absence of adrenergic blockers (○), and in the presence of 5 μM-phentolamine (□). Phentolamine shifted the NA dose–response curve about 3 log units to the right, as expected for an α-adrenergic antagonist with pKa around 8·5. However, if the tissue was first depolarized with 32 mM-K^+ (■), an additional component of tension was seen with 10^{-7} to 10^{-5} M-NA. This is the same range of concentrations over which NA increases L-type Ca^{2+} current in the single cells (Fig. 1). Thus, the extra NA response in 32 mM-K^+ is consistent with a significant enhancement of Ca^{2+} influx through voltage-gated Ca^{2+} channels, although other interpretations cannot be excluded.

DISCUSSION

The main conclusion of this paper is that micromolar concentrations of NA increase voltage-gated Ca^{2+} channel current. The increase is mediated by an enhancement of the activity of L-type rather than T-type Ca^{2+} channels. In this respect, the NA effect in ear artery cells parallels effects of β-adrenergic agonists in heart cells (see Reuter, 1983; Tsien *et al.* 1986; Trautwein *et al.* 1986 for review), which also involves L-type and not T-type Ca^{2+} channels (Bean, 1985; cf. Mitra & Morad, 1986). The NA effect in smooth muscle shows unusual pharmacology: it is not prevented by the α-blockers phentolamine or prazosin, or mimicked by the α-agonist phenylephrine; nor is it inhibited by the β-blocker propranolol or mimicked by the β-agonist isoprenaline. Taken in combination, these results suggest involvement of an adrenergic receptor that is not an α- or β-receptor. One possible explanation invokes the so-called 'γ'-receptor proposed by Hirst & Nield (1980). The 'γ'-receptor is characterized by responsiveness to NA and adrenaline but not phenylephrine (Hirst, Nield & Silverberg, 1982), as in the results described here. However, it should be noted that maximal stimulation of L-type Ca^{2+} channels was seen at about 10 μM-NA, about 1000-fold smaller than the doses of NA used by Hirst *et al.* (1982) to stimulate 'γ'-receptors and produce maximal depolarization in the rabbit basilar artery. Further efforts in comparing these adrenergic responses would greatly benefit from new and selective adrenergic antagonists.

L-type Ca^{2+} channel modulation is distinct from other actions of noradrenaline

The pharmacology of the L-type Ca^{2+} channel modulation distinguishes it from other actions of noradrenaline in vascular smooth muscle that are prevented by phentolamine, such as release of Ca^{2+} from internal stores, or enhanced Ca^{2+} influx. This Ca^{2+} influx may be associated with a slow depolarization in ear artery that is also phentolamine and prazosin sensitive (Suzuki & Kou, 1983). It remains unclear if the slow depolarization is due to receptor-operated channels directly coupled to the α-adrenergic receptor (similar to ATP-activated Ca^{2+}-permeable channels – Benham

& Tsien, 1987*b*) or to indirectly gated cation channels controlled by intracellular Ca^{2+} release (cf. von Tscharner, Prod'hom, Baggiolini & Reuter, 1986) or inositol phosphate metabolism (Irvine & Moor, 1986; Kuno & Gardner, 1987).

As an adrenergic effect that is not mediated by α- or β-receptors, the modulation of L-type Ca^{2+} channels could easily have been overlooked in previous experiments. It could contribute to contractions and Ca^{2+} influx in smooth muscles depolarized by elevated external K^+ (Evans, Schild & Thesleff, 1958), experiments that have long been interpreted in terms of receptor-operated, voltage-insensitive Ca^{2+} channels. Our experiments with spiral strips of ear artery reveal a contractile component in elevated K^+ solutions that is consistent with modulation of L-type Ca^{2+} channels. Clearly, it would be of interest to see if this component of tension is abolished by dihydropyridines (cf. Han, Abel & Minneman, 1987, Fig. 4), but unaffected by agents that deplete Ca^{2+} stores.

In tissues that have not been depolarized with high K^+, activation of L-type Ca^{2+} channels will require membrane depolarization, and may thus depend upon an initiating depolarization mediated by stimulation of α-adrenergic receptors. Thus, the effectiveness of α-adrenergic antagonists in blocking contractions does not exclude a possible contribution of non-α-, non-β-adrenergic modulation of Ca^{2+} influx via L-type Ca^{2+} channels.

The relative importance of various adrenergic mechanisms will vary with the mode of delivery of NA. Bath application of NA will emphasize the contribution of Ca^{2+} release from internal stores as this α-adrenergic response can be seen at submicromolar NA concentrations in the absence of significant membrane depolarization (Droogmans *et al.* 1977). On the other hand, nerve stimulation will evoke a relatively high local concentration of NA, ATP release, excitatory junction potentials, and possibly action potentials. Under these circumstances, NA modulation of L-type Ca^{2+} channels may play an important role in promoting spike activity and Ca^{2+} influx. The functional balance between various sources of Ca^{2+} may also vary from tissue to tissue. Some small-resistance vessels possess relatively poorly developed Ca^{2+} stores and display NA-induced contractions with prominent sensitivity to dihydropyridine Ca^{2+} antagonists (Cauvin, Saida & van Breemen, 1984).

Droogmans, Declerck & Casteels (1987) have recently reported a decrease in Ca^{2+} current in response to NA in rabbit ear artery cells. They suggested that this was an α-response, based on agonist specificity (no antagonist effects were reported). The physiological relevance of an inhibition of Ca^{2+} current is not clear. We are not sure why we see the opposite response in the same preparation under what appear to be fairly similar conditions. It is possible that multiple mechanisms exist for modulation of L-type Ca^{2+} channels, and that minor experimental differences may favour one mechanism over another. Nevertheless, it is puzzling that we never observed the reversible decrease in Ca^{2+} channel current with NA that Droogmans *et al.* reported.

This study raises additional questions for future experiments. The pharmacological properties of the adrenergic receptor will need further study to determine the potency of agonists other than NA and adrenaline, and to find an effective blocker. Preliminary suggestions of the involvement of GTP-binding proteins as a coupling

mechanism between drug binding and channel modulation might be substantiated with experiments using pertussis toxin. Although the messenger molecule is almost certainly not cyclic AMP (as in β-adrenergic modulation of L-type Ca^{2+} channels in heart), further work is needed to test for participation of other possibilities. Finally, it will be interesting to see if the enhancement of L-type Ca^{2+} current is associated with clear-cut changes in the functional number of Ca^{2+} channels, their unitary conductance, or their pattern or probability of opening.

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Note added in proof. P. Pacaud, G. Loirand, C. Mironneau & J. Mironneau (*Pflügers Archiv.* **410**, 557–559 (1987)) have briefly reported both an increase and decrease in Ca^{2+} current in response to noradrenaline in rat portal vein. This modulation was phentolamine sensitive.

REFERENCES

AARONSON, P. I., BENHAM, C. D., BOLTON, T. B., HESS, P., LANG, R. J. & TSIEN, R. W. (1986). Two types of single channel and whole cell calcium or barium currents in single smooth muscle cells of rabbit ear artery and the effects of noradrenaline. *Journal of Physiology* **377**, 36*P*.

BEAN, B. P. (1985). Two kinds of calcium channels in canine atrial cells. Differences in kinetics, selectivity and pharmacology. *Journal of General Physiology* **86**, 1–30.

BEAN, B. P., STUREK, M., PUGA, A. & HERMSMEYER, K. (1986). Calcium channels in muscle cells isolated from rat mesenteric arteries: modulation by dihydropyridine drugs. *Circulation Research* **59**, 229–235.

BENHAM, C. D. & BOLTON, T. B. (1986). Spontaneous transient outward currents in single visceral and vascular smooth muscle cells of the rabbit. *Journal of Physiology* **381**, 385–406.

BENHAM, C. D., HESS, P. & TSIEN, R. W. (1987). Two types of calcium channels in single smooth muscle cells from rabbit ear artery studied with whole-cell and single channel recordings. *Circulation Research* **61**, suppl. 1, 110–116.

BENHAM, C. D. & TSIEN, R. W. (1987*a*). Noradrenaline increases L-type calcium current in smooth-muscle cells of rabbit ear artery independently of α- and β-adrenoceptors. *Journal of Physiology* **390**, 98*P*.

BENHAM, C. D. & TSIEN, R. W. (1987*b*). ATP receptor-operated channels permeable to calcium in arterial smooth muscle. *Nature* **328**, 275–278.

BOLTON, T. B. (1979). Mechanisms of action of transmitters and other substances on smooth muscle. *Physiological Reviews* **59**, 607–718.

BOLTON, T. B. & LARGE, W. A. (1986). Are junction potentials essential? Dual mechanism of smooth muscle cell activation by transmitter released from autonomic nerves. *Quarterly Journal of Experimental Physiology* **71**, 1–28.

BULBRING, E. & TOMITA, T. (1987). Catecholamine action on smooth muscle. *Pharmacological Reviews* **39**, 49–96.

BURNSTOCK, G. & KENNEDY, C. (1986). A dual function for adenosine 5′-triphosphate in the regulation of vascular tone. *Circulation Research* **58**, 319–330.

CAUVIN, C., SAIDA, K. & VAN BREEMEN, C. (1984). Extracellular Ca^{2+} dependence and diltiazem inhibition of contraction in rabbit conduit arteries and mesenteric resistance vessels. *Blood Vessels* **21**, 23–31.

CHEUNG, D. W. & MACKAY, M. J. (1986). The effects of Bay K 8644 and nifedipine on the neural responses of the rabbit ear artery. *British Journal of Pharmacology* **88**, 363–368.

COHEN, C. J., JANIS, R. A., TAYLOR, D. G. & SCRIABINE, A. (1986). Where do Ca^{2+} antagonists act? In *Calcium Antagonists in Cardiovascular Disease*, ed. OPIE, L. H., pp. 151–163. New York: Raven Press.

DROOGMANS, G., DECLERCK, I. & CASTEELS, R. (1987). Effect of adrenergic agonists on Ca-channel currents in single vascular smooth muscle cells. *Pflügers Archiv* **409**, 7–12.

DROOGMANS, G., RAEYMAEKERS, L. & CASTEELS, R. (1977). Electro- and pharmacomechanical coupling in the smooth muscle cells of the rabbit ear artery. *Journal of General Physiology* **70**, 129–148.

EVANS, D. H. L., SCHILD, H. O. & THESLEFF, S. (1958). Effects of drugs on depolarized plain muscle. *Journal of Physiology* **143**, 474–485.

FOX, A. P., NOWYCKY, M. C. & TSIEN, R. W. (1987). Kinetic and pharmacological properties distinguishing three types of calcium currents in chick sensory neurones. *Journal of Physiology* **394**, 149–172.

HAMILL, O. P., MARTY, A., NEHER, E., SAKMANN, B. & SIGWORTH, F. J. (1981). Improved patch-clamp techniques for high resolution current recording from cells and cell-free membrane patches. *Pflügers Archiv* **391**, 85–100.

HAN, C., ABEL, P. W. & MINNEMAN, K. P. (1987). α_1-Adrenoceptor subtypes linked to different mechanisms for increasing intracellular Ca^{2+} in smooth muscle. *Nature* **329**, 333–335.

HIRST, G. D. S. & NIELD, T. O. (1980). Evidence for two populations of excitatory receptors for noradrenaline on arteriolar smooth muscle. *Nature* **283**, 767–768.

HIRST, G. D. S., NIELD, T. O. & SILVERBERG, G. D. (1982). Noradrenaline receptors on the rat basilar artery. *Journal of Physiology* **328**, 351–360.

HOLMAN, M. E. & SURPRENANT, A. (1980). An electrophysiological analysis of the effects of noradrenaline and α-receptor antagonists on neuromuscular transmission in mammalian muscular arteries. *British Journal of Pharmacology* **71**, 651–661.

IRVINE, R. F. & MOOR, R. M. (1986). Micro-injection of inositol 1,3,4,5-tetrakisphosphate activates sea urchin eggs by a mechanism dependent on external Ca^{2+}. *Biochemical Journal* **240**, 917–920.

KLOCKNER, V. & ISENBERG, G. (1985). Calcium currents of cesium loaded isolated smooth muscle cells (urinary bladder of the guinea-pig). *Pflügers Archiv* **405**, 340–348.

KUNO, M. & GARDNER, P. (1987). Ion channels activated by inositol 1, 4, 5-triphosphate in plasma membrane of human T-lymphocytes. *Nature* **326**, 301–304.

MITRA, R. & MORAD, M. (1986). Ca^{2+} and Ca^{2+}-activated K^+ currents in mammalian gastric smooth muscle cells. *Science* **229**, 269–272.

REUTER, H. (1983). Calcium channel modulation by neurotransmitters, enzymes and drugs. *Nature* **301**, 569–574.

SINBACK, C. N. & SHAIN, W. (1980). Chemosensitivity of single smooth muscle cells to acetylcholine, noradrenaline and histamine in vitro. *Journal of Cellular Physiology* **102**, 99–112.

SOMLYO, A. P. & SOMLYO, A. V. (1968). Electromechanical and pharmacomechanical coupling in vascular smooth muscle. *Journal of Pharmacology and Experimental Therapeutics* **159**, 129–145.

SUZUKI, H. & KOU, K. (1983). Electrical components contributing to the nerve mediated contractions in the smooth muscle of the rabbit ear artery. *Japanese Journal of Physiology* **33**, 743–756.

TRAUTWEIN, W., KAMEYAMA, M., HESCHELER, J. & HOFMANN, F. (1986). Cardiac Ca channels and their transmitter modulation. *Fortschritte der Zoologie* **33**, 163–182.

TSIEN, R. W., BEAN, B. P., HESS, P., LANSMAN, J. B., NILIUS, B. & NOWYCKY, M. C. (1986). Calcium channel types in cardiac, neuronal and smooth muscle-derived cells. Differences in gating, permeation and pharmacology. In *Ion Channels in Neural Membranes*, ed. KEYNES, R. & RITCHIE, J. M., pp. 227–252. New York: Alan R. Liss, Inc.

VAN BREEMEN, C., AARONSON, P. & LOUTZENHIZSER, R. (1979). Sodium–calcium interactions in mammalian smooth muscle. *Pharmacological Reviews* **30**, 167–208.

VANHOUTTE, P. M., VERBEUREN, T. J. & WEBB, R. C. (1981). Local modulation of the adrenergic neuroeffector interaction in the blood vessel wall. *Physiological Reviews* **61**, 151–247.

VON TSCHARNER, V., PROD'HOM, B., BAGGIOLINI, M. & REUTER, H. (1986). Ion channels in human neutrophils activated by a rise in free cytosolic calcium concentration. *Nature* **324**, 369–372.