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Mechanism of action of α-adrenoceptor activation in single cells freshly dissociated from the rabbit portal vein

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1 The action of noradrenaline was studied in freshly dispersed cells of the rabbit portal vein using microelectrode techniques.

2 In normal physiological salt solution, the ionophoretic application of noradrenaline evoked an α-adrenoceptor-mediated depolarization and sometimes a β-adrenoceptor-mediated hyperpolarization. Experiments were carried out in the presence of propranolol to study the membrane mechanism associated with α-adrenoceptor activation.

3 In the current clamp mode of recording, the equilibrium potential of the noradrenaline-evoked depolarization was −1.9 mV. The depolarization was brought about by an increase in membrane conductance.

4 Under voltage clamp conditions, noradrenaline produced an inward current with a reversal potential of -7 ± 3 mV (mean ± s.e. mean).

5 The relationship between the noradrenaline-induced inward current and clamp potential was non-linear. Depolarization enhanced the conductance elicited by noradrenaline.

6 Evidence is presented which suggests that an additional conductance mechanism (probably an increase in potassium conductance) is also evoked by α-adrenoceptor stimulation in dispersed cells of rabbit portal vein.

Introduction

It is well known that many veins are innervated by the sympathetic nervous system. Stimulation of these motor nerves or the addition of noradrenaline produce vasoconstriction. In electrophysiological studies it has been shown that repetitive nerve stimulation produces an excitatory junction potential (e.j.p.) which has a time to peak of several seconds in the rabbit portal vein (Holman *et al.*, 1968) and in the guinea-pig mesenteric vein (Suzuki, 1981). In these tissues, application of noradrenaline evokes depolarization and this response and the e.j.p. are blocked by α-adrenoceptor antagonists (Holman *et al.*, 1968; Suzuki, 1981). In some preparations the α-adrenoceptor-mediated e.j.p. is preceded by a rapid e.j.p. which is resistant to α-receptor antagonists (dog mesenteric vein, Suzuki, 1984; rat saphenous vein, Cheung, 1985). In addition it has been shown that noradrenaline depolarizes the guinea-pig portal vein (Golenhofen *et al.*, 1973) and the canine saphenous vein (Matthews *et al.*, 1984).

Although noradrenaline depolarizes most veins, there have been very few published studies on the membrane mechanism underlying α-receptor activation in venous smooth muscle. Using radioactive tracer techniques in rat portal vein, Wahlström (1973) showed that stimulation of α-receptors increased the membrane permeability to chloride ions. In contrast, Suzuki (1981) suggested that in the guinea-pig mesenteric vein the depolarization to noradrenaline occurred as a consequence of a decrease in potassium conductance. There have been no published studies concerning α-receptor activation in freshly dispersed cells of venous smooth muscle. In the present experiments we have studied the membrane mechanism associated with α-receptor activation in freshly dispersed cells of the rabbit portal vein, using both current and voltage clamp techniques. With microelectrode techniques in isolated

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smooth muscle cells it is possible to study agonist-induced responses and to analyse the membrane mechanism associated with drug receptor activation (Byrne & Large, 1987c). It is possible not only to measure accurately reversal potentials and conductance mechanisms of drug-induced responses, but also to study the voltage dependence of the underlying conductance change. In the present study, we showed that α-adrenoceptor activation produces a voltage-dependent increase in membrane conductance which generates an inward current that drives the membrane potential to close to 0 mV.

Methods

Rabbits (2–2.5 kg) of either sex were killed by an overdose of i.v. sodium pentobarbitone. The hepatic portal vein was removed, dissected free of connective tissue and cut open to form a strip. A small piece of tissue approximately 8 × 8 mm square was incubated for 10 min at 37°C in a modified physiological salt solution containing low calcium (10 μM), after which the solution was replaced with low Ca^{2+} solution containing bovine albumin (3 mg ml^{-1}), papain (5 mg ml^{-1}) and dithiothreitol (2.5 μM). The tissue was incubated in the enzyme solution for 20 min after which it was washed with approximately 10 ml low Ca^{2+} solution at 37°C. Single cells were obtained by trituration of the tissue through a wide-bore pipette in low Ca^{2+} solution at room temperature (20–24°C). The resulting cell suspension was centrifuged at 1000 r.p.m. for 1 min and the pellet resuspended in 0.7 mM Ca^{2+} solution. Cells were stored on cover-slips at 4°C and were used on the same day.

A single microelectrode was employed for recording membrane potential and current-passing with either the bridge or switching voltage clamp circuits of an Axoclamp-2A microelectrode clamp amplifier (Axon Instruments Inc.). When the voltage clamp mode of recording was used the sampling rate was between 0.6 and 2.0 kHz. Current records from the voltage clamp experiments were low pass filtered (EF 3 Barr and Stroud) at 500 Hz, otherwise data recording and data illustration were as described previously (Large, 1982). Microelectrodes were filled with 1.0 M KCl and had resistances of 60–150 MΩ. Noradrenaline and isoprenaline were applied by ionophoresis from pipettes filled with a 0.2 M solution and having resistances of 150–300 MΩ. The ionophoretic electrode was placed within 5 μm of the cell. Antagonists were added to the bathing solution. The composition of the normal physiological salt solution used throughout was (mM): Na^+ 131, K^+ 6, Mg^{2+} 1.2, Ca^{2+} 1.5, Cl^{-1} 137, glucose 11, HEPES 10, buffered to pH 7.2 with NaOH and gassed with O_2. Experiments were carried out at room temperature (20–24°C).

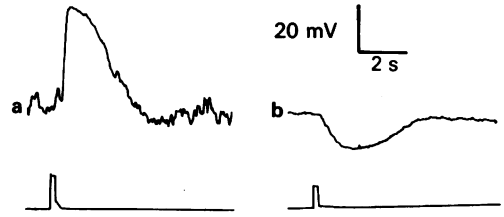

Figure 1 Responses to noradrenaline in two isolated cells of the rabbit portal vein using current clamp. Membrane potential (E_m): −57 mV for (a); and −40 mV for (b). (a) and (b) were recorded in normal physiological salt solution. Parameters of ionophoresis (lower records): 10 nA for 500 ms (a and b).

Drugs used: bovine albumin, dithiothreitol (both from Sigma), isoprenaline sulphate (Burroughs Wellcome) noradrenaline bitartrate, papain type IV (both from Sigma), phentolamine mesylate (Ciba Geigy), (±)-propranolol hydrochloride (I.C.I.).

The values in the text are the mean ± s.e. mean.

Results

Responses to noradrenaline under current clamp

In freshly dispersed cells of the rabbit portal vein the value of the resting membrane potential (E_m) was usually between −20 and −40 mV compared to a mean value of about −40 mV in whole tissue preparations (Somlyo *et al.*, 1969). In part the lower value of E_m in isolated cells may be due to membrane leakage incurred by microelectrode impalement. The input resistance of cells impaled with microelectrodes was 529 ± 67 MΩ (n = 15). With patch pipettes the input resistance was in excess of 1 GΩ (Byrne & Large, unpublished). A reduced membrane resistance (and E_m) due to microelectrode impalement was also observed in freshly dispersed cells of the rat anococcygeus muscle (Byrne & Large, 1987c). However, as with the anococcygeus muscle, the microelectrode-induced membrane leakage did not appear to affect unfavourably membrane responses to agonist drugs. The membrane potential was set usually between −40 and −60 mV by passing a small retaining (inward) current.

The most common response to ionophoretically-applied noradrenaline was depolarization (Figure 1a). The amplitude of this response could be increased by increasing the ionophoretic charge (range, 10 nA for 20–2000 ms) and the largest depolarization observed was 46 mV at a resting potential of −59 mV. There was a delay between application

Table 1 Time-course of the depolarization or inward current evoked by noradrenaline

A *Depolarization studied with current clamp*			
Membrane potential (mV)	*Amplitude* (mV)	*Latency** (ms)	*Rise time†* (ms)
−52.4 ± 3.6	17.2 ± 3.2	917 ± 62	1257 ± 92
B *Inward current studied with voltage clamp*			
Clamp potential (mV)	*Amplitude* (pA)	*Latency** (ms)	*Rise time†* (ms)
−48.3 ± 2.9	67 ± 9.3	587 ± 43	1000 ± 92

* The latency was measured from the start of the ionophoretic pulse to the beginning of the response.
† The rise time was measured from the onset to the peak of the response.

of noradrenaline and the onset of the response. The mean latency of the depolarization was 917 ms and the total time to peak was over 2 s (Table 1A) even when short (< 100 ms) ionophoretic pulses were used.

In a few cells noradrenaline evoked hyperpolarization (Figure 1b) that had a similar time-course to the evoked depolarization (compare Figure 1a and b). Ionophoretic application of isoprenaline also produced hyperpolarization, a finding which suggests that β-adrenoceptors are present in rabbit portal vein cells. These data confirm the results of Holman *et al.* (1968) who demonstrated β-adrenoceptor-mediated hyperpolarization in whole tissue preparations of rabbit portal vein. In order to study the mechanism underlying α-adrenoceptor activation, propranolol (10^{-6} M) was added to the bathing solution for the remainder of the experiments.

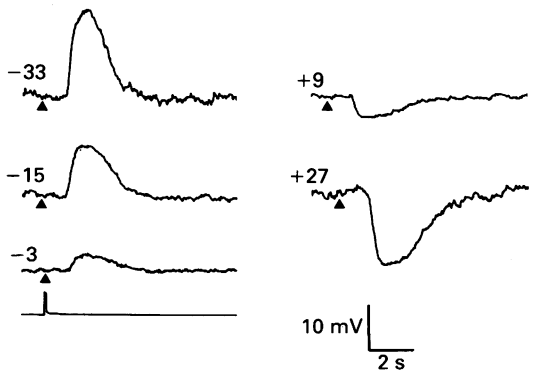

Figure 2 Effect of membrane potential on the noradrenaline-evoked response using current clamp. Values of holding potential in mV are given beside each trace. Ionophoretic pulse: 10 nA for 100 ms, at arrowheads.

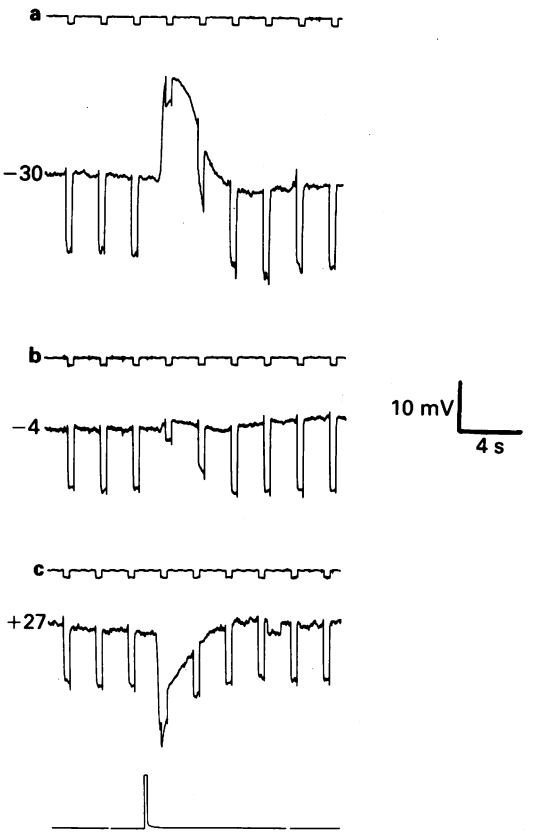

Figure 3 Effect of noradrenaline on input resistance under current clamp. Hyperpolarizing current pulses (upper trace) of 50 pA in amplitude and 400 ms in duration were passed through the recording electrode every 2.2 s. Values of holding potential in mV are beside each trace. Ionophoretic pulse (bottom record): 10 nA for 200 ms.

In many cells no responses were observed to noradrenaline. Also, in responding cells the amplitude of the depolarization often declined on repeated administration. In the experiments to be described only cells which gave reproducible depolarizations were used.

No depolarizations to noradrenaline were observed in the presence of 10^{-6} M phentolamine.

Conductance mechanism underlying noradrenaline-induced depolarization

Figure 2 illustrates the relationship between membrane potential and the noradrenaline-induced response. Conditioning depolarization reduced the amplitude of the response and the depolarization

reversed to hyperpolarization at between −3 and +9 mV. The interpolated reversal potential was +3 mV. In 6 cells the mean reversal potential was −1.9 ± 2.8 mV.

Figure 3 shows the results of an experiment in which the input resistance was measured before and during the action of noradrenaline. Input resistance was estimated from the voltage response to small hyperpolarizing current pulses. The membrane resistance was greatly decreased during the depolarization evoked by noradrenaline (Figure 3a). When the membrane potential was set close to the reversal potential, noradrenaline reduced the membrane resistance even though there was little actual change in membrane potential (Figure 3b). Finally, at positive membrane potentials the input resistance was decreased during the hyperpolarization evoked by noradrenaline (Figure 3c).

These data suggest that the depolarization evoked by noradrenaline was produced by an increase in membrane conductance. Further experiments to substantiate this hypothesis were carried out with the voltage clamp technique, which also provided information concerning the voltage dependence of the conductance change induced by noradrenaline.

Voltage clamp analysis of the noradrenaline-evoked responses

Figure 4a shows a depolarization of 25 mV amplitude to noradrenaline recorded in current clamp at −60 mV. Under voltage clamp conditions at the same holding potential, noradrenaline evoked an inward current of 120 pA (Figure 4b). In a different cell it can be seen that increasing the ionophoretic pulse produced responses of greater amplitude (Figure 5a and b) and the largest response recorded was 730 pA at a holding potential of −62 mV. Some characteristics of the inward currents are shown in Table 1B. The latency of the inward currents appear to be smaller than the latency of the depolarizations (587 ms vs 917 ms). However, it is likely that this difference is due to the fact that the ambient temperature increased from about 20 to 24°C between carrying out the current and voltage clamp experiments. Previously it has been shown that the latency of α-receptor-mediated depolarizations in the mouse anococcygeus muscle is highly sensitive to changes in temperature (Large, 1982). Moreover, it can be seen from Figure 4 that the time course of the depolarization and the underlying inward current are similar when recorded from the same cell.

Biphasic currents were sometimes observed when using voltage clamp recording. Figure 5c shows 1 cell at a holding potential of −49 mV; the inward current elicited by noradrenaline was preceded by a small outward current. When the holding potential

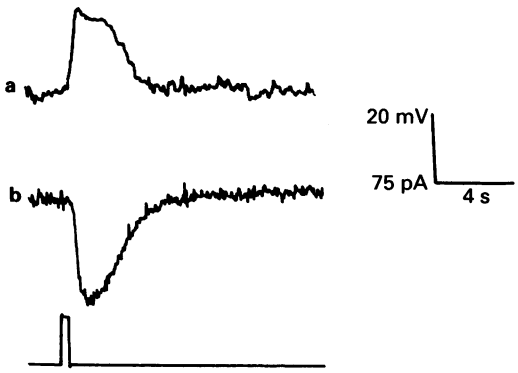

Figure 4 Depolarization (a) and inward current (b) recorded in the same cell. The response to noradrenaline (10 nA for 500 ms) was recorded first in current clamp (a) and then in voltage clamp (b). Note the similar time course of the depolarization and inward current.

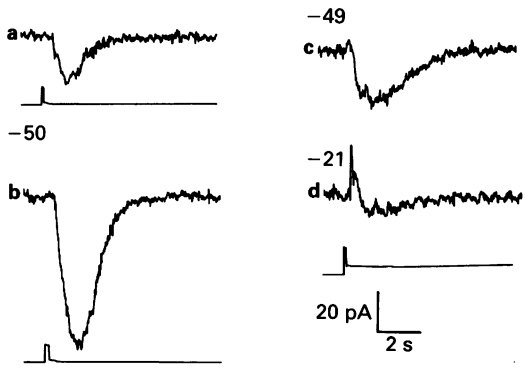

Figure 5 Different types of response to noradrenaline in 2 cells using voltage clamp; (a and b) reveal a 'dose'-effect relationship to noradrenaline in a cell held at −50 mV. Increasing the ionophoretic pulse of 10 nA from 100 ms (a) to 200 ms (b) produced a larger inward current. (c and d) were recorded from another cell and show responses consisting of the two components to noradrenaline at holding potentials of −49 mV (c) and −21 mV (d). Ionophoretic pulse for (c and d) was 10 nA for 100 ms. It should be noted that 10^{-6} M propranolol was present in each case.

was set at −21 mV, the outward current was increased in amplitude while the inward current was reduced (Figure 5d). This is evidence that at least two ionic mechanisms are associated with α-receptor activation.

Voltage-dependence of the noradrenaline-induced current

Figure 6 shows the influence of the holding potential on the response to noradrenaline in voltage clamp.

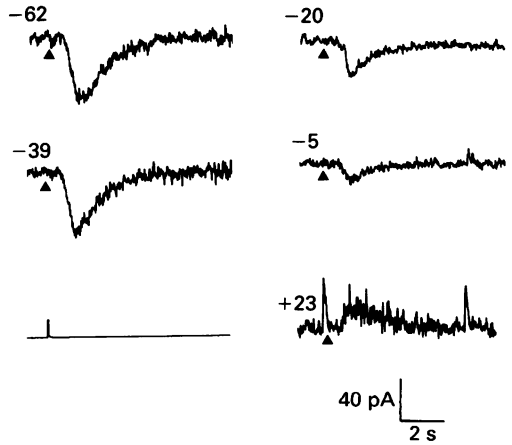

Figure 6 Influence of clamp potential on the noradrenaline-induced response recorded using voltage clamp. Clamp potential in mV is beside each record. Ionophoretic pulse: 10 nA for 50 ms, at arrow-heads. Note the spontaneous outward (upward deflexion) currents at +23 mV.

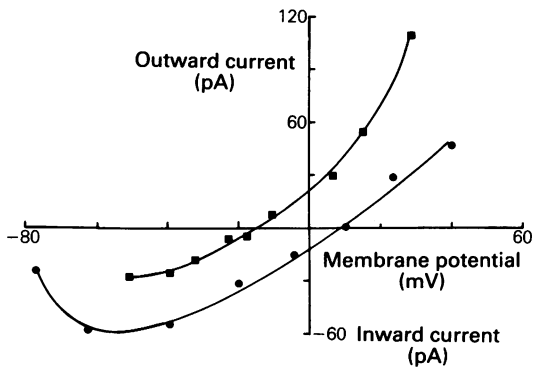

Figure 7 Amplitude of current to noradrenaline as a function of clamp potential. (●) Represent the same cell as illustrated in Figure 6. (■) Show the current-voltage relationship from another cell.

The noradrenaline-induced inward current was reduced at depolarized clamp potentials and reversed to an outward current at between −5 and +23 mV. A more complete illustration of the influence of clamp potential on the noradrenaline-induced response is shown in Figure 7. The reversal potential of the cell illustrated in Figure 6 (solid circles in Figure 7) was +9 mV. In 6 cells the mean reversal potential of the noradrenaline-evoked current was -7.3 ± 3.2 mV.

Figure 7 also shows that the relationship between the amplitude of the noradrenaline-induced current and clamp potential is non-linear. In the cell illustrated in Figure 6 (circles in Figure 7) this non-linearity is most apparent at potentials more negative than −40 mV. At −62 mV the current was only 3 pA greater than the response at −39 mV and at −75 mV the amplitude of the inward current was markedly reduced (Figure 7). The current-voltage relationship of another cell is also shown by the squares in Figure 7 and it can be seen that the non-linearity is highly pronounced at positive membrane potentials.

It is possible to calculate the chord conductance (g) using the equation:

$$g = \frac{I}{(E_c - E_r)}$$

where I is the noradrenaline-induced current at the clamp potential E_c and E_r is the reversal potential estimated by interpolation in each experiment. In 6 cells at a mean clamp potential of -51.0 ± 3.5 mV, g was 1.61 ± 0.37 nS. In the same cells g was 5.40 ± 2.92 nS at a clamp potential of $+27 \pm 10.0$ mV. Thus the ratio $g_{+27} : g_{-51}$ is 3.35. These data show that the conductance increase to noradrenaline is enhanced by depolarization.

The results so far suggest that the major membrane response to α-adrenoceptor activation is to increase membrane conductance to some ion(s) which drives the membrane potential to about 0 mV. However, in a few cells there appears to be an additional ionic mechanism (e.g. Figure 5c and d). Further evidence for this notion was obtained from voltage jump experiments. Figure 8a shows an experiment in which the holding potential was held at −50 mV and stepped to −40 mV for 500 ms every 1.2 s. During the inward current to noradrenaline at −50 mV, the current steps were increased in amplitude (indicating an increase in membrane conductance) and the extrapolated reversal potential was −15 mV. (This value is estimated assuming that there is a linear relationship between the agonist-induced response and clamp potential. Although, as discussed above, this assumption is not entirely valid, the degree of non-linearity between about −40 and −10 mV was not excessive. Thus, the rough estimates obtained were sufficient to illustrate the differences in the reversal potentials in Figure 8a and b). The value is in good agreement with the value of −16 mV (same cell as solid squares in Figure 7) estimated by interpolation. The record in Figure 8a represents the thirteenth response from that particular cell. Figure 8b illustrates the first response to noradrenaline in another cell; in this experiment the holding potential was −50 mV and the membrane potential was stepped to −32 mV every 2.2 s. During the response to noradrenaline the current was

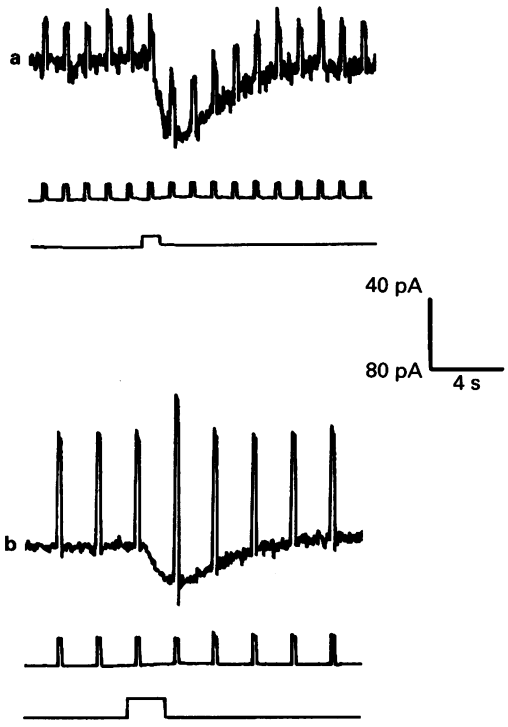

Figure 8 Voltage jump analysis of the action of noradrenaline in two cells. The membrane potential (middle records) was voltage clamped at −50 mV and stepped for 500 ms to −40 mV every 1.2 s in (a) and to −32 mV every 2.2 s in (b). The upper record in each set of traces is the membrane current. Ionophoretic pulse: 10 nA for 1 s (a) and 2 s (b). Vertical calibration bar represents 40 pA for (a) and 80 pA for (b). See text for further details.

inward at −50 mV but outward at the test potential of −32 mV; the interpolated reversal potential was −41 mV. During subsequent responses to noradrenaline in this cell the current was inward at both −50 and −32 mV with an extrapolated reversal potential of about −10 mV. Thus it seems that noradrenaline increases potassium conductance in addition to the membrane conductance increase responsible for the inward current. The increase in potassium conductance is most apparent (for some unknown reason) during the first application of noradrenaline.

Discussion

In dispersed cells of the rabbit portal vein the depolarization to α-adrenoceptor activation is mediated by an increase in membrane conductance. The increase in conductance generates an inward current which drives the membrane potential towards about 0 mV. The resting membrane potential of whole tissue preparations of portal vein is about −40 mV (Somlyo *et al.*, 1969), which is in the normal range of voltages (about −40 to −50 mV) for opening voltage-dependent calcium channels (see Bolton & Large, 1986). Thus it might be expected that any noradrenaline-induced depolarization would open calcium channels. In whole tissue preparations of rabbit portal vein, the noradrenaline-induced depolarization increased the frequency of spike activity which caused contraction (Holman *et al.*, 1968).

There are a number of similarities between the depolarizations to α-adrenoceptor activation seen in isolated cells of rabbit portal vein and the depolarizations observed to α-adrenoceptor stimulation in dispersed cells of the guinea-pig pulmonary artery (Byrne & Large, 1987a) and the rat anococcygeus muscle (Byrne & Large, 1987b, c). Firstly, there is a characteristic latency of 0.5–1.0 s between the ionophoretic application of noradrenaline and the onset of response. This long delay is not seen when ATP is applied to isolated smooth muscle cells (Benham *et al.*, 1987). It is thought that the latency represents the time taken for intracellular mediators to link α-adrenoceptor binding to membrane channel opening. Secondly, in all three tissues the depolarization is produced by an inward current which occurs as a consequence of an increase in membrane conductance. In portal vein and the rat anococcygeus muscle, the reversal potential of the noradrenaline-induced depolarization is close to 0 mV. In the rat anococcygeus muscle it has been shown that the depolarization to noradrenaline is mediated by an increase in membrane conductance to chloride ions (Byrne & Large, 1987b). Therefore, it is tempting to speculate that the same ionic mechanism is associated with α-adrenoceptor activation in rabbit portal vein smooth muscle. However, it should be emphasized that a non-selective increase in membrane cation conductance could also give a reversal potential of about 0 mV. Therefore additional experiments with patch pipettes are required to identify the ions carrying the inward current in the rabbit portal vein. Thirdly, the increase in membrane conductance produced by noradrenaline is highly voltage-dependent. The direction of the voltage-dependence is such that in whole tissue, at a resting potential of about −40 mV, depolarization evoked by noradrenaline would enhance the underlying increase in membrane conductance which, in turn, would produce an even greater degree of depolarization. This regenerative property of the conductance mechanism associated with α-adrenoceptor activation might be expected to counterbalance, at least to some extent, the depolarization-induced increase in potassium con-

ductance which seems to limit the extent of the noradrenaline-evoked depolarization in vascular smooth muscle (see Bolton & Large, 1986). It is also interesting that hyperpolarizing the membrane to values more negative than about −60 mV decreases the amplitude of the noradrenaline-evoked inward current. This characteristic produces a skewed U-shaped current-voltage plot which has also been observed with muscarinic receptor activation in isolated cells of rabbit jejunum (Benham *et al.*, 1985). These authors also demonstrated that increasing the extracellular concentration of potassium shifted to the right the whole current-voltage curve (Figure 2C, Benham *et al.*, 1985). Under these conditions and without reliable measurements of membrane resistance, it would be possible to conclude falsely that an agonist-evoked depolarization was due to a decrease in potassium conductance rather than an increase in membrane conductance to other ions. This fact illustrates the need for reliable measurements of membrane resistance and also to establish actual reversal of the membrane response, in order to obtain an accurate estimate of the equilibrium potential of the underlying conductance mechanism. Both of these aims may be difficult to attain using whole tissue preparations and highlights the usefulness of isolated cells for analysing agonist-induced membrane mechanisms in smooth muscle. Fourthly, in the three tissues there is good evidence that noradrenaline evokes an increase in potassium conductance. This mechanism is usually most obvious during the early stages of the noradrenaline-induced response (e.g. Figure 5d) and is subsequently overridden by the inward current mechanism. It has been argued previously that the potassium conductance increase associated with α-adrenoceptor activation is produced by an increase in intracellular calcium concentration (i.e. $gK_{Ca^{2+}}$, Byrne & Large, 1987a,b,c).

It has been postulated that the noradrenaline-induced depolarization in whole tissue preparations is mediated by the suppression of a potassium conductance in guinea-pig mesenteric vein (Suzuki, 1981) and pulmonary artery (Suzuki, 1986). In contrast, only an increase in membrane conductance to α-adrenoceptor activation has been observed in isolated cells of rabbit portal vein (present work), guinea-pig pulmonary artery (Byrne & Large, 1987a) and rat anococcygeus muscle (Byrne & Large, 1987b). Previously (Byrne & Large, 1987a) it was argued that this discrepancy might represent difficulties in studying agonist-induced membrane mechanisms in whole tissue syncytial preparations as discussed above. Another explanation was that different mechanisms might be activated depending on whether noradrenaline is bath applied (e.g. Suzuki, 1981; 1986) or ionophoresed (our experiments). For whole tissues, evidence has been presented that there are two mechanisms of depolarization of different time courses associated with α-adrenoceptor activation in the rat anococcygeus muscle (Byrne & Large, 1985; Bolton & Large, 1986) and similar results have been obtained in the guinea-pig pulmonary artery and mesenteric vein (Large, unpublished). It was argued in the rat anococcygeus muscle that the faster of the two depolarizations was due to an increase in membrane conductance, possibly to chloride ions (Byrne & Large, 1985). Subsequently it was shown in isolated cells of the anococcygeus muscle that noradrenaline does increase the membrane conductance to chloride ions (Byrne & Large, 1987b). However, in isolated cells of all the tissues studied, we have been unable to record any response that corresponds to the second slower depolarization to noradrenaline which is observed in whole tissue. It is possible that for technical reasons it will be difficult to record the slow depolarization to noradrenaline in freshly dissociated smooth muscle cells.

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