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The action of noradrenaline on single smooth muscle cells freshly dispersed from the guinea-pig pulmonary artery

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1 Responses to ionophoretically-applied noradrenaline were investigated with micro-electrodes in whole tissue preparations and with patch pipettes in isolated cells dispersed from the guinea-pig pulmonary artery.

2 In whole tissue and dispersed cells noradrenaline evoked monophasic depolarizations which had a similar time course.

3 In dispersed cells the amplitude of electronic potentials was reduced during the noradrenaline-evoked depolarization. Under voltage clamp noradrenaline elicited an inward current, which persisted in 18 mM external potassium with the membrane potential set at the potassium equilibrium potential.

4 In voltage clamp experiments the amplitude of current steps to hyperpolarizing voltage jumps was increased during the noradrenaline-induced inward current. These data suggest that the depolarization to noradrenaline in the guinea-pig pulmonary artery is mediated by an increase in membrane conductance.

Introduction

Stimulation of α-adrenoceptors in vascular smooth muscle cells produces contraction. There is evidence that noradrenaline can produce vasoconstriction by a process independent of depolarization (pharmacomechanical coupling — see Bolton, 1979), though in some preparations α-adrenoceptor activation in vascular smooth muscle is associated with membrane depolarization, especially with high concentrations of noradrenaline (see review by Bolton & Large, 1986). However, there are conflicting results concerning the mechanism underlying the electrophysiological response. For example, using the partition chamber technique for applying current pulses and recording membrane potential with micro-electrodes, it has been found that noradrenaline (or adrenaline) increases membrane conductance in the rabbit carotid artery (Mekata & Niu, 1972), pulmonary artery (Casteels *et al.*, 1977) and the guinea-pig mesenteric artery (Kuriyama & Makita, 1983; Bolton *et al.*, 1984). In contrast a decrease in conductance to noradrenaline has been observed in the guinea-pig ear artery (Kajiwara *et al.*, 1981), mesenteric vein (Suzuki, 1981), mesenteric jejunal artery (Karashima, 1981) and pulmonary artery (Suzuki, 1986). This discrepancy in the results concerning the membrane mechanism of noradrenaline in vascular smooth muscle may be due to the different tissues used. Alternatively, it is possible that the study of receptor-operated membrane mechanisms in smooth muscle is made difficult by the syncytial nature of whole tissue preparations. For example, receptor-mediated changes in membrane resistance may be obscured (or magnified) by changes in the electrical coupling between cells. In the present study we have investigated the nature of the conductance change in isolated cells freshly dispersed from the guinea-pig pulmonary artery using patch pipettes. We used the pulmonary artery as it is one of the few arterial preparations in which all the membrane responses to nerve stimulation are blocked by α-adrenoceptor antagonists (Suzuki, 1983; Bolton & Large, 1986).

In early experiments responses to noradrenaline were studied in whole tissue preparations for comparison with the depolarizations in freshly dispersed cells.

A brief account of these results has been published (Byrne & Large, 1986).

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Methods

Guinea-pigs of either sex were stunned and bled. The main pulmonary artery was dissected out and a small piece, approximately 5 mm long × 3 mm diameter, was set up for intracellular recording as described previously for the mouse anococcygeus muscle (Large, 1982). Membrane potentials were recorded using micro-electrodes filled with 0.5 M KCl which had resistances of 100–200 MΩ. Micro-electrodes were inserted from the adventitial side. The normal Krebs solution contained (mM): NaCl 119, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, glucose 11 and was bubbled with 5% CO_2 and 95% O_2.

Single cells were obtained by incubating small pieces (5 × 2 mm) of artery for three successive periods of 45, 30 and 20 min at 37°C in a modified physiological salt solution containing low (10 μM) Ca^{2+}, 2 mg ml^{-1} bovine albumin and various concentrations of collagenase (Worthington) and elastase (Porcine pancreas type 1, Sigma). The concentrations of collagenase and elastase were respectively: 0.45 mg ml^{-1} and 9.0 u ml^{-1}; 0.25 mg ml^{-1} and 5.0 u ml^{-1}; 0.15 mg ml^{-1} and 3 u ml^{-1} in the three consecutive incubation periods. Between the second and third incubation periods the tissue pieces were agitated in the second incubation solution by passing them in and out of a wide bore pipette repeatedly until a cloudy suspension was formed (2–3 min). This suspension was centrifuged at 1000 r.p.m. for 1 min after which the supernatant was discarded and the pellet resuspended in the third enzyme solution. Subsequently the suspension of cells obtained was pipetted onto cover slips at room temperature and the cells allowed to stick for 5–10 min. Enzyme solution was removed by washing the cover slips with physiological salt solution containing 1.5 mM Ca^{2+} and the cells were stored at 4°C before their use on the same day.

Whole-cell membrane currents were measured using standard patch-clamp techniques (Hamill *et al.*, 1981) using patch pipettes with resistances of 2–5 MΩ. The composition of the normal external physiological solution used throughout was (mM): Na^+ 131, Cl^- 126.7, K^+ 5.9, Ca^{2+} 1.5, Mg^{2+} 1.2, $H_2PO_4^-$ 1.2, SO_4^{2-} 1.2, and that of the pipette solution (mM): Na^+ 8, Cl^- 126, K^+ 127, Mg^{2+} 1.2, $H_2PO_4^-$ 1.0, SO_4^{2-} 1.2, ATP 1, EGTA 0.77. In addition, both solutions contained HEPES (10 mM) and glucose (11 mM) and were buffered to pH 7.2 with NaOH and gassed with O_2. High external K^+ (18 mM) solution was obtained by adding KCl.

Experiments on whole tissues and single cells were carried out at room temperature (20–23°C). Whole cell recordings were low pass filtered (EF3 Barr and Stroud) at 200 Hz, otherwise data recording and illustration was carried out as described previously (Large, 1982).

Noradrenaline was applied by ionophoresis from micropipettes filled with 0.1–0.5 M noradrenaline as described previously (Byrne & Large, 1984). In the experiments with isolated cells, the ionophoretic electrode was placed within 5 μm of the cell. The values given in the text are the mean ± s.e.mean.

Drugs used were: (−)-noradrenaline bitartrate (Sigma) and phentolamine mesylate (Ciba).

Results

Comparison of responses in isolated cells and whole tissue preparations

In whole tissue preparations the resting membrane potential of the guinea-pig pulmonary artery, measured with micro-electrodes, was −50.5 ± 0.7 mV ($n = 49$). The ionophoretic application of noradrenaline produced a monophasic depolarization which was characterized by a lag period between the start of the ionophoretic pulse and the onset of depolarization (Figure 1a and b). The latency of the response was 1.90 ± 0.13 s and the rise time was 2.15 ± 0.14 s. Figure 1 also illustrates that the amplitude of the depolarizations could be increased by applying a larger ionophoretic pulse and amplitudes of up to 25 mV were recorded. The depolarization was blocked when the tissue was pretreated with phentolamine (10^{-7}–10^{-6} M) and therefore we can conclude the response is mediated by α-adrenoceptors.

Responses to noradrenaline were recorded with patch pipettes from freshly dispersed cells which had input resistances of 0.7 to 2.0 GΩ. Using the current clamp mode of recording the membrane potential was set at about −50 mV by passing a small amount of inward current. Figure 1 (c and d) illustrates depolarizations to noradrenaline in freshly dispersed cells recorded with a patch pipette. The amplitude of the responses was dependent on the ionophoretic charge and responses of up to 38 mV were measured. Sometimes the noradrenaline-induced depolarization triggered a spike (e.g. Figure 2a). In isolated cells the latency and the rise time of the depolarizations were, respectively, 0.82 ± 0.13 s and 2.27 ± 0.56 s. Depolarizations to noradrenaline were not recorded in every cell tested and the amplitude of the responses usually declined on repeated administration of noradrenaline. Electrical responses due to α-adrenoceptor stimulation desensitize in the rat anococcygeus muscle (Large, 1983) but not to the same extent as seen in the present experiments. There is no obvious explanation for the transient nature of the responses in isolated cells but this phenomenon does occur with other agonist-induced responses in isolated cells (Marty *et al.*, 1984; Benham *et al.*, 1987). The transient nature of the response may be due to the use of the

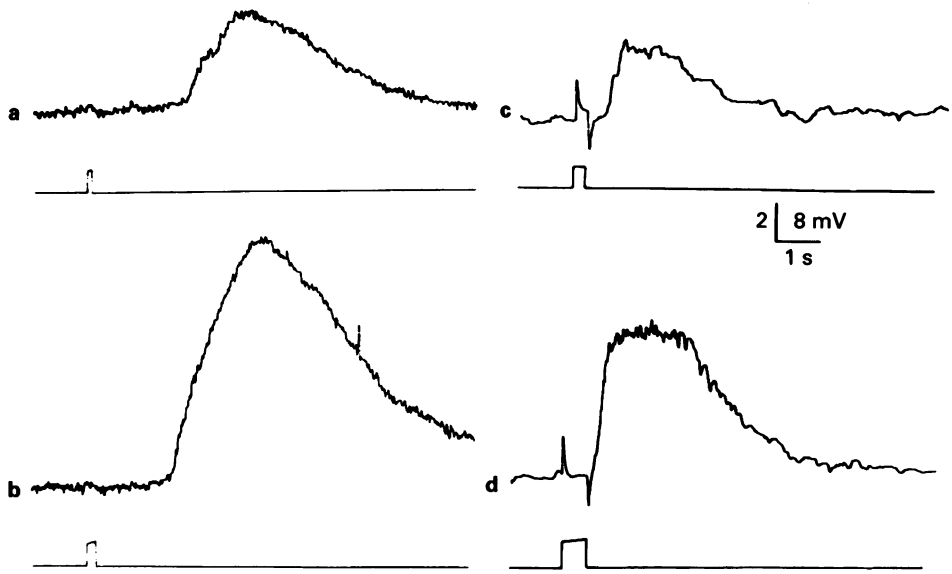

Figure 1 Comparison of depolarizations to noradrenaline in whole tissue (a and b) and a freshly dispersed cell (c and d). The resting membrane potential of the cell in whole tissue measured with a micro-electrode was − 52 mV. The potential of the freshly dispersed cell was set at − 51 mV by passing a small amount of inward current. The lower trace represents the ionophoretic pulse: 25 nA for 100 ms (a), 200 ms (b and c) and 500 ms (d).

whole cell patch recording method causing loss of diffusible materials from the cytosol. Despite the shorter latency of the depolarization in isolated cells it seemed that the enzymic digestion procedure had not greatly compromised the functional state of the α-adrenoceptor.

Nature of conductance change produced by noradrenaline

It can be seen from Figure 2a that the amplitude of the electrotonic potentials evoked by hyperpolarizing current pulses was greatly reduced during the depolarization evoked by noradrenaline. The membrane resistance had returned to normal after the membrane potential had declined to its control level (right hand record Figure 2a). This result indicates that the depolarization to noradrenaline may be mediated by an increase in membrane conductance. However, it is possible that the increased conductance resulted from the depolarization per se rather than reflect the mechanism of α-receptor activation. Thus further experiments were carried out using voltage clamp mode of recording. Figure 2b illustrates the depolarization due to the application of noradrenaline. Using voltage clamp at a holding potential of − 50 mV in the same cell it was observed (Figure 2c) that the same pulse of noradrenaline produces an inward current of about 50 pA. The time course of the inward current was similar to that of the noradrenaline-induced depolarization. The latency and time to peak of the inward current were respectively, 0.82 ± 0.14 s and 1.54 ± 00.22 s ($n = 18$).

Since the potassium equilibrium potential (E_K) with these external and pipette solutions is about − 77 mV, it is possible that the excitatory response to noradrenaline might be due to a decrease in potassium conductance. We tested this possibility in experiments where the extracellular potassium concentration was increased to 18 mM. Under these conditions E_K is approximately − 49 mV and in cells held at a membrane potential of − 49 mV by the application of a steady current, noradrenaline evoked depolarization (current clamp) or inward currents of up to 100 pA (voltage clamp).

Further evidence that noradrenaline produces an increase in conductance was obtained from voltage clamp experiments in which voltage jumps were applied before and during the action of noradrenaline. In Figure 3b a 200 ms ionophoretic pulse of noradrenaline evoked an inward current of about 80 pA in amplitude. Using 10 mV hyperpolarizing voltage steps it can be seen that near the peak of the noradrenaline-induced inward current the membrane conductance had increased about three fold (compare Figure 3a and b). Figure 3 (d) and (e) are the same records as (a) and

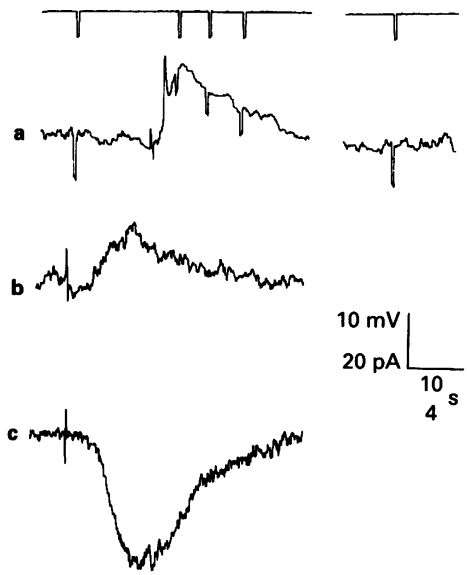

Figure 2 Responses to noradrenaline in an isolated cell recorded in current and voltage clamp. (a) The membrane potential (lower trace) was −51 mV and hyperpolarizing current pulses (upper trace 500 ms, 15 pA) were applied. Ionophoretic pulse, 25 nA for 500 ms. The gap in the trace represents about 40 s. (b) Current clamp and (c) voltage clamp were recorded from another cell, membrane potential of −50 mV. Ionophoretic pulse, 25 nA, 100 ms. Note that time course of the depolarization (b) and inward current (c) are similar.

(b) but on a higher gain and faster sweep speed. During the voltage jump in the presence of noradrenaline the current trace sags suggesting that the conductance increase induced by noradrenaline may be voltage-dependent. This observation merits further study. Also during the action of noradrenaline the trace becomes 'noisier' (Figure 3d and e). This result is consistent with the notion that noradrenaline opens membrane channels.

Discussion

Using the present dispersal procedures it was extremely difficult to obtain viable single cells. Consequently the present study does not provide any quantitative information concerning the relationship between the amplitude of the noradrenaline-induced current and membrane potential. Despite the deficiency in quantitative data we feel that the experiments yielded strong qualitative evidence that the depolarization to noradrenaline results from an increase in membrane conductance. There are three arguments for this conclusion. (1) In isolated cells of the guinea-pig pulmonary artery the amplitude of the electrotonic potential is reduced during the noradrenaline-induced depolarization. (2) With the membrane potential set at the potassium equilibrium potential noradrenaline evokes depolarization and inward current. (3) Under voltage clamp, voltage jumps produce larger current steps during the inward current to noradrenaline than in normal physiological solution.

It is interesting that Suzuki (1986) provided evidence that noradrenaline decreases membrane conductance in whole tissue preparations of the guinea-pig pulmonary artery. This discrepancy may reflect the difficulties in recording changes of membrane resistance using syncytial preparations as mentioned in the Introduction. Alternatively the difference in results may be explained by the two different methods of application of noradrenaline. Suzuki (1986) applied noradrenaline in the bathing solution whereas the technique of ionophoresis was used in the present experiments.

In dispersed cells of the rabbit ear artery noradrenaline evokes a calcium-mediated potassium conductance (GK_{Ca}) increase and an additional mechanism generates an inward current by an ionic conductance increase (Benham, Bolton, Byrne & Large, unpublished observations). In the present study noradrenaline did not elicit an outward current. Therefore, it appears that the mechanisms linked to α-adrenoceptors in the guinea-pig pulmonary and rabbit ear artery may be different. However, it may be that noradrenaline does activate GK_{Ca} in the pulmonary artery but that the expected outward K^+ current is masked by the inward current. In whole tissue preparations bathed in low chloride solution noradrenaline evokes hyperpolarization (Large, unpublished), which suggests that α-receptor stimulation does lead to an increase in potassium conductance in the pulmonary artery. It is interesting that in the rabbit pulmonary artery noradrenaline increases the efflux of ^{42}K, ^{36}Cl and ^{22}Na (Casteels *et al.*, 1977; Smith & Jones, 1985).

The distinct latency between the application of noradrenaline and the onset of the response appears to be a characteristic of the excitatory α-adrenoceptor mechanism. This latency has been observed in the mouse and rat anococcygeus muscle (Large, 1982; Byrne & Large, 1984) and in dispersed cells of the rabbit ear artery (Benham, Bolton, Byrne & Large, unpublished observations). In the present experiments the ionophoretic electrode was placed within 5 μm of the cell membrane and therefore it is extremely unlikely that diffusion could account for the latency of the response.

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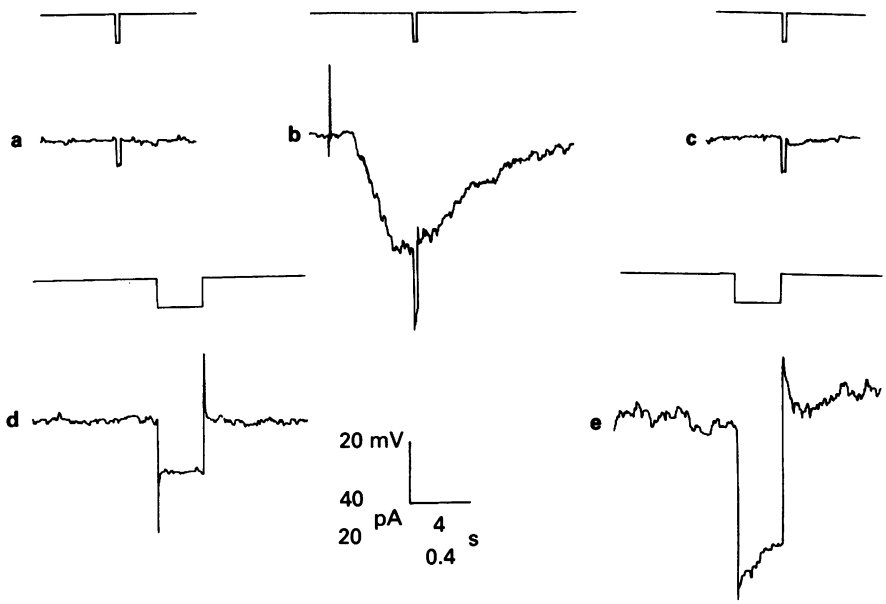

Figure 3 Voltage steps applied to an isolated cell in the presence and absence of noradrenaline. Traces (a) and (c) were recorded 10–20 s before and after, respectively, the ionophoretic pulse of noradrenaline (25 nA for 200 ms). Holding potential −50 mV, 10 mV hyperpolarizing steps were applied for 300 ms. (d and e) The same traces as (a) and (b) on a higher gain and faster time base. Upper traces are the clamp potential and lower traces are the membrane current. Calibration bars: 40 pA and 4 s in (a–c) and 20 pA and 0.4 s in (d) and (e). Note the increased 'noise' in (e) compared to (d) and the outward relaxation during the voltage jump.

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