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# Evidence for two mechanisms of depolarization associated with $\alpha_1$-adrenoceptor activation in the rat anococcygeus muscle

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**1** Membrane potential responses in the rat isolated anococcygeus to bath-applied noradrenaline and field stimulation have been investigated by use of intracellular microelectrode and combined extracellular electrical and mechanical (sucrose gap) recording techniques. Intracellular recordings were made usually from tissues immobilized with hypertonic Krebs solution.

**2** Bath-application of noradrenaline produced depolarizations which consisted of two components; an initial 'fast' phase which peaked within 1–2 s and which was followed by a 'slow' sustained depolarization. Both components were concentration-dependent.

**3** Noradrenaline could also evoke oscillations in membrane potential which, unlike the 'fast' component of depolarization, were prevented by conditioning hyperpolarization of the membrane and were evoked by direct membrane depolarization with externally applied current pulses. Thus, the oscillations are voltage-dependent phenomena.

**4** Replacement of the external NaCl of the Krebs solution with an equimolar amount of Na benzenesulphonate abolished the noradrenaline-evoked 'fast' depolarization while the 'slow' phase was unaffected. This suggests that two mechanisms of depolarization are activated in this muscle by the bath-application of noradrenaline. The adrenergic excitatory junction potential was also abolished in Na benzenesulphonate.

**5** Prazosin reduced both the 'fast' and 'slow' components of depolarization produced by noradrenaline indicating their mediation by $\alpha_1$-adrenoceptors.

**6** The membrane potential (−29 mV) at the maximum amplitude of the 'fast' depolarization was similar to the equilibrium potential (−27 mV) for the depolarization evoked by ionophoretically applied noradrenaline and which was obtained by extrapolation from the relationship between amplitude of the ionophoretic response and membrane potential displacement in the partition chamber. These results suggest that the 'fast' depolarization and the ionophoretic response are due to an increased membrane conductance, possibly to chloride.

## Introduction

The major postsynaptic electrical response to field stimulation of the rat isolated anococcygeus muscle is an excitatory junction potential (e.j.p.) which is blocked by low concentrations of phentolamine and prazosin (Creed *et al.*, 1975; Byrne & Large, 1984) and thus the e.j.p. is likely to be produced by the action of noradrenaline on $\alpha_1$-adrenoceptors. The ionophoretic application of noradrenaline onto the mouse and rat anococcygeus produces a monophasic depolarization and since the kinetics and pharmacology of this depolarization and the e.j.p. were similar, it was suggested that the noradrenaline ejected from the ionophoretic microelectrode activated those receptors which are stimulated by noradrenaline released from intrinsic nerves (Large, 1982; Byrne & Large, 1984). The ease with which the junctional $\alpha$-adrenoceptors could be activated by ionophoresis of noradrenaline suggests that the anococcygeus muscle is a suitable tissue for the study of excitatory junctional $\alpha_1$-adrenoceptor-mediated mechanisms in smooth muscle.

It has also been demonstrated that in normal Krebs solution the depolarization produced by the iono-

phoretic application of noradrenaline onto the rat anococcygeus muscle is associated with a small contraction. When NaCl of the bathing solution was replaced with Na benzenesulphonate the depolarization to ionophoretic noradrenaline was abolished but contraction could still be observed (Large 1984). In organ bath studies it was found that the equilibrium contractile response to bath-applied noradrenaline was unaffected by substitution of chloride with benzenesulphonate, although the time course of the contraction was prolonged in low chloride solution. It was concluded that noradrenaline can elicit contraction by two distinct mechanisms, one associated with depolarization and another mechanism which is independent of a change in membrane potential. However, in this latter study (Large, 1984) noradrenaline was in contact with the tissue for 2–3 min in the contraction studies whereas in the electrophysiological experiments the ionophoretic pulse width was rarely in excess of 200 ms. We have therefore investigated the membrane responses to prolonged bath application of noradrenaline in tissues that have been immobilized with hypertonic sucrose Krebs solution. The main finding is that noradrenaline applied by bath application evoked a biphasic depolarization and the two components can be differentiated in terms of kinetics and susceptibility to chloride substitution. It is suggested that noradrenaline can produce depolarization by two receptor-operated mechanisms.

A preliminary account of this work has been published (Byrne & Large, 1985).

## Methods

The isolated anococcygeus of the rat was set up either for intracellular recording of membrane potential (Large, 1982; 1984) or for extracellular electrical recording by use of a single sucrose gap (Bülbring & Tomita, 1969). In the intracellular studies, the muscle was mounted either in a partition chamber (Abe & Tomita, 1968) or in a bath that contained two Ag/AgCl electrodes for field stimulation placed on either side of the preparation. The baths (volume approximately 0.5 ml) were perfused continuously at a rate of 1–3 ml $min^{-1}$ with Krebs solution or hypertonic Krebs solution containing sucrose (400 mM) which immobilised the tissue. Membrane potentials were recorded with intracellular microelectrodes filled with K acetate (4M) and with resistances of 80–150 MΩ. During experiments in which current was applied to the tissue by external plate electrodes the changes in membrane potential were recorded within 1.0 mm of the leading plate.

For extracellular recording of electrical activity, thin strips (0.5–1.0 mm) of muscle were mounted in a single sucrose gap similar to that described by Bülbring & Tomita (1969). Changes in membrane potential were measured via an agar bridge across the sucrose channel; tension was measured isometrically using a Grass transducer (FTO3C).

The normal Krebs solution used throughout contained (mM): NaCl 119, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $KH_2PO_4$ 1.2, $NaHCO_3$ 25 and glucose 11 and was bubbled with 5% $CO_2$–95% $O_2$. Reduction in $[Cl]_o$ was by substitution of NaCl with an equimolar amount of Na benzenesulphonate (purum, non recrystallised; Fluka Chemicals). Noradrenaline and antagonists were added by dissolving them in the Krebs solution (isotonic or hypertonic) with which the tissue was superfused. Ionophoretic applications were made by means of micropipettes, similar to those used to record membrane potential, filled with 0.5M noradrenaline (Large, 1982; 1983). All experiments were carried out at room temperature (20–24°C) to make the present conditions comparable with those used in earlier studies (Large, 1982; 1983; 1984). It has been shown previously that the resting membrane potential is unaltered by lowering the temperature from 34°C to room temperature (cf. Creed *et al.*, 1975; Large, 1984). Moreover the depolarizing responses to noradrenaline are qualitatively the same at the lower temperature although the kinetics are slower (Large, 1982).

Some rats were pretreated with 6-hydroxydopamine in order to remove the sympathetic innervation. The dose regimen was: $2 \times 50$ mg $kg^{-1}$ i.p. on day 1; $2 \times 100$ mg $kg^{-1}$ on day 4 and the animals were killed on days 5 and 6. This schedule has been shown to produce an effective sympathectomy in the rat (Gibson & Gillespie, 1973) and mouse (Gibson & Wedmore, 1981). In the present study, sympathetic denervation was confirmed by the absence of an adrenergically-mediated e.j.p. in response to field stimulation.

Drugs used were: noradrenaline bitartrate (Sigma); prazosin hydrochloride (Pfizer) and 6-hydroxydopamine hydrochloride (Sigma).

## Results

### *Responses to exogenously applied noradrenaline in normal and hypertonic Krebs solution*

In normal Krebs solution, the application of noradrenaline by bath perfusion ($10^{-7}$–$10^{-6}$M) evoked a fast depolarization which peaked within 1–2 s. This was accompanied invariably by a powerful contraction which dislodged the microelectrode and prevented continuous recording of the membrane potential. In order to facilitate continuous recording from single cells during repeated applications of noradrenaline it was necessary to abolish the contractions without eliminating the electrical response to

noradrenaline. Hypertonic Krebs solution containing sucrose (300 mM) has been used to immobilize other smooth muscle preparations (Ohashi, 1970; Bolton, 1976). In the present study, all of the intracellular recordings obtained to the bath-application of noradrenaline were obtained in Krebs solution containing sucrose (400 mM).

In hypertonic Krebs solution, the resting membrane potentials (Em) though stable were slghtly depolarized; the mean value, Em = $-48.3 \pm 0.8$ mV (mean ± s.e.mean, $n = 61$) compared with a mean value of $-59.5 \pm 1.2$ mV recorded in normal isotonic solution (Byrne & Large, 1984). Stable recordings of Em were usually possible after some 1.5 to 2 h in the presence of sucrose after which the electrical responses to noradrenaline became consistent. Noradrenaline ($10^{-7}$–$10^{-5}$M) evoked depolarizations which consisted of two components. The first, a 'fast' component peaked within 1–2 s and was similar to that recorded in normal isotonic Krebs solution; the second slow phase of depolarization was well maintained throughout the period of perfusion with noradrenaline (Figure 1a–d). The presence of two components, each with different kinetics, was unexpected and suggested that they represented the activation of two distinct mechanisms (see later). Both components of de-

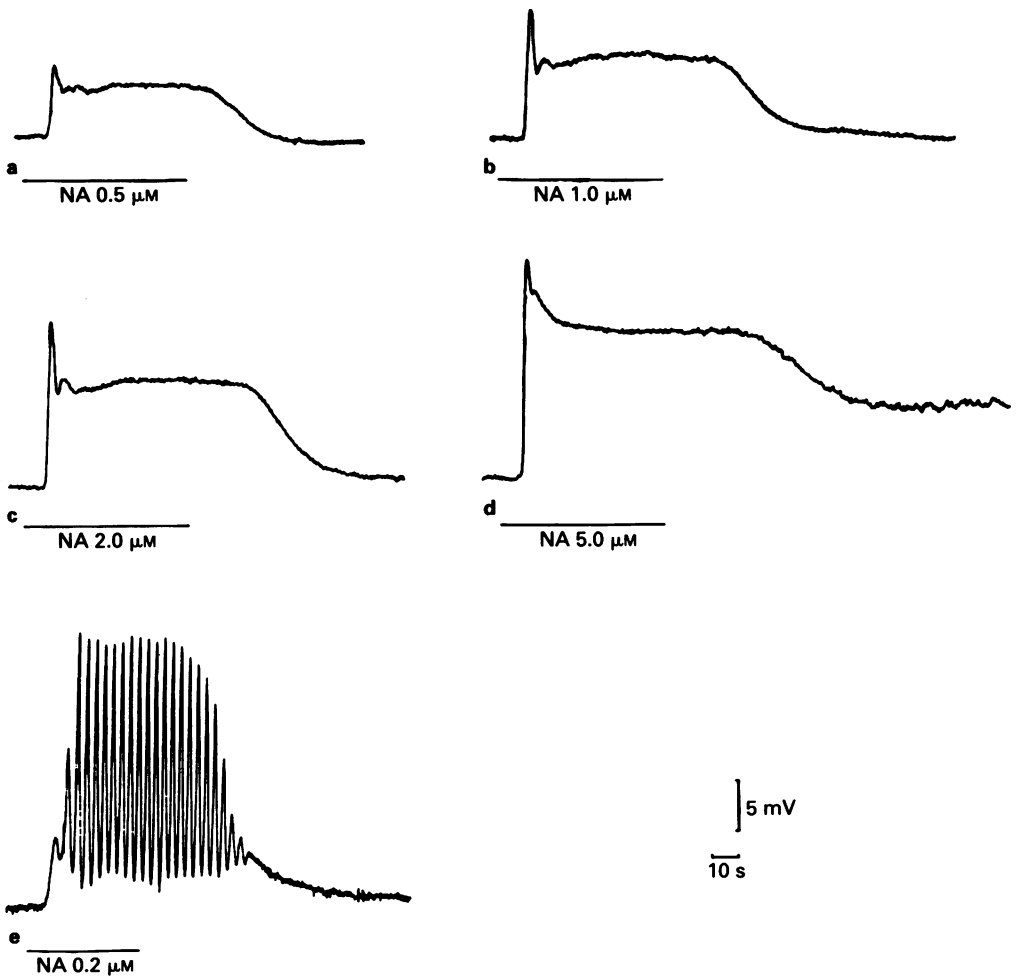


**Figure 1** Effects of bath-perfusion with noradrenaline (NA for the periods indicated by the bars): (a–d) shows the concentration-dependence of the 'fast' and 'slow' phase of depolarization recorded from one cell (Em = − 48 mV). In (e), recorded from another cell (Em = − 39 mV), the depolarization evoked by noradrenaline triggered off oscillations in membrane potential. Each recording was obtained in Krebs solution made hypertonic with sucrose (400 mM) to immobilize the muscle.

polarization were concentration-dependent and could each attain an amplitude of some 30 mV (Figure 1a–d). The 'fast' and the 'slow' phase were not abolished by pretreatment of the animals with 6-hydroxydopamine and thus neither component was due to the release of transmitter from adrenergic nerves. Although in Figure 1 the 'slow' component was smaller than the 'fast' over the whole concentration-range for noradrenaline this was not invariably so. The ratio of the amplitudes of each component varied and often the 'slow' phase was larger than the 'fast' (see Figure 6a). Thus the 'slow' component was not due merely to a decline in the initial 'fast' depolarisation.

The ability to record the 'fast' component was, unlike the 'slow' component, highly dependent on the rate of bath perfusion. With low perfusion rates ($<1$ ml min$^{-1}$) and, thus slow equilibration of noradrenaline, the 'fast' depolarization was often not observed although the 'slow' phase was invariably produced (Figure 2a). When the bath perfusion rate was increased (up to 1.25 ml min$^{-1}$; Figure 2b) and

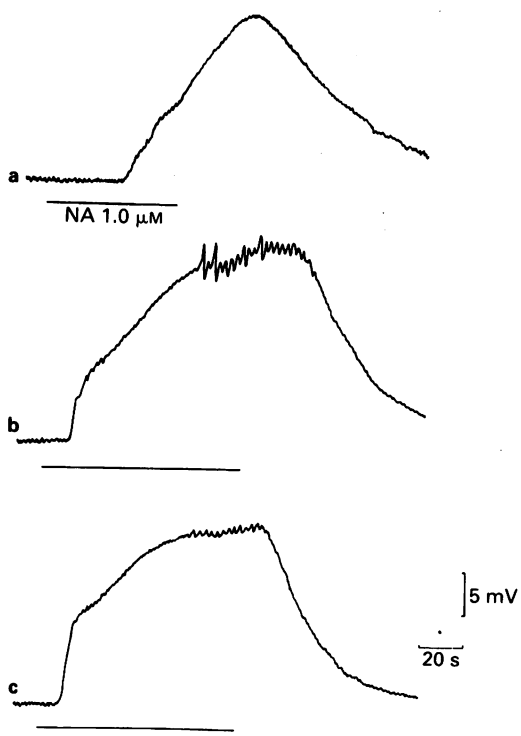


**Figure 2** Effects of bath perfusion rate on the depolarization produced by noradrenaline. The flow rate of the Krebs solution, which contained noradrenaline (NA) for the periods indicated by the bars was 1.0 ml min$^{-1}$ in (a), 1.25 ml min$^{-1}$ in (b) and 1.5 ml min$^{-1}$ in (c). These records are all from one cell, Em = − 50 mV.

noradrenaline re-applied the 'fast' phase of depolarization emerged causing the overall response to become distinctly biphasic. The amplitude of the 'fast' depolarization was enhanced when the flow rate was increased further (to 1.5 ml min$^{-1}$; Figure 2c). The apparent absence of the 'fast' depolarization at low perfusion rates is probably because of two effects caused by slow drug equilibration; first, the asynchronous activation of receptors, and secondly, desensitization of the receptors mediating the fast depolarization. Densensitization to ionophoretically applied noradrenaline readily occurs in the mouse anococcygeus (Large, 1983).

Low concentrations ($10^{-7}$ to $2 \times 10^{-7}$M) of noradrenaline sometimes evoked a 'slow' depolarization without any obvious preceding 'fast' phase though the latter did emerge when the concentration of noradrenaline was increased. The threshold concentration of noradrenaline required to evoke the 'fast' and 'slow' depolarizations in hypertonic Krebs solution was approximately ten times that required for contraction in isotonic Krebs. Moreover, the sensitivity to ionophoresed noradrenaline was also reduced in hypertonic Krebs solution (unpublished data). These effects were unaccompanied by any apparent change in the pharmacological characteristics of the receptors as low concentrations of prazosin ($10^{-8}$M) reduced or abolished both components of the depolarization to noradrenaline (see Figure 6a and b). From Figure 6a and c the dose-ratio of noradrenaline in the presence of $10^{-8}$M prazosin was about 5 and by use of the Schild equation an estimate of the dissociation constant of prazosin can be obtained which was approximately $2 \times 10^{-9}$M and is similar to that obtained for $\alpha_1$-adrenoceptors on the mouse anococcygeus by use of mechanical recording techniques (Gibson & Yu, 1983).

It was impossible, because of impalement instability, to confirm the presence of a slow phase of depolarization in response to noradrenaline in normal isotonic Krebs solution by intracellular recording techniques. Accordingly, the sucrose gap technique was employed since this allows electrical recordings to be made extracellularly from tissue bathed in isotonic Krebs solution. The presence of both the 'fast' and 'slow' components of depolarization to noradrenaline was confirmed by this technique which suggests that biphasic response can also be recorded in isotonic solution. Thus, we believe that qualitatively the response recorded to noradrenaline in both hypertonic and isotonic Krebs solution are the same.

*Effects of membrane potential displacement on the responses to noradrenaline*

The similarity in time course between the initial 'fast' phase of depolarization induced by noradrenaline and

the oscillations in membrane potential (e.g. Figure 1a and e) suggested that the former response may be a voltage-dependent phenomenon evoked as a result of the 'slow' depolarization reaching a threshold potential. Accordingly, the effects of membrane potential displacement on the responses to noradrenaline was investigated. As Figure 3 shows, noradrenaline added at the resting Em (− 49 mV; Figure 3a) evoked a 'fast' depolarization followed by large oscillations. However, when reapplied during conditioning hyperpolarization of the membrane to a steady value of − 59 mV, noradrenaline evoked a 'fast' depolarization but without oscillations (Figure 3b). On cessation of the externally applied current, Em returned to − 52 mV and the oscillations in response to noradrenaline were restored (Figure 3c) indicating that the tissue did not deteriorate during the application of relatively small inward currents for prolonged periods.

At a resting Em of − 51 mV (Figure 3d) noradrenaline evoked a 'fast' depolarization followed by the 'slow' component, but, when reapplied during conditioning depolarization of only some 2 mV (giving an Em value of − 49 mV) oscillations were produced (Figure 3e). Clearly it is possible to distinguish between the 'fast' depolarization, which appears to be a direct receptor-mediated event, and the oscillations which were activated only when the membrane was sufficiently depolarized by noradrenaline. In a few experiments in which the partition chamber was used depolarizing pulses applied in the absence of noradrenaline produced oscillations in membrane potential. Since these membrane responses had a threshold potential between − 40 and − 50 mV and were recorded in tissues taken from rats pretreated with 6-hydroxydopamine we assume that these oscillations represent an active membrane response.

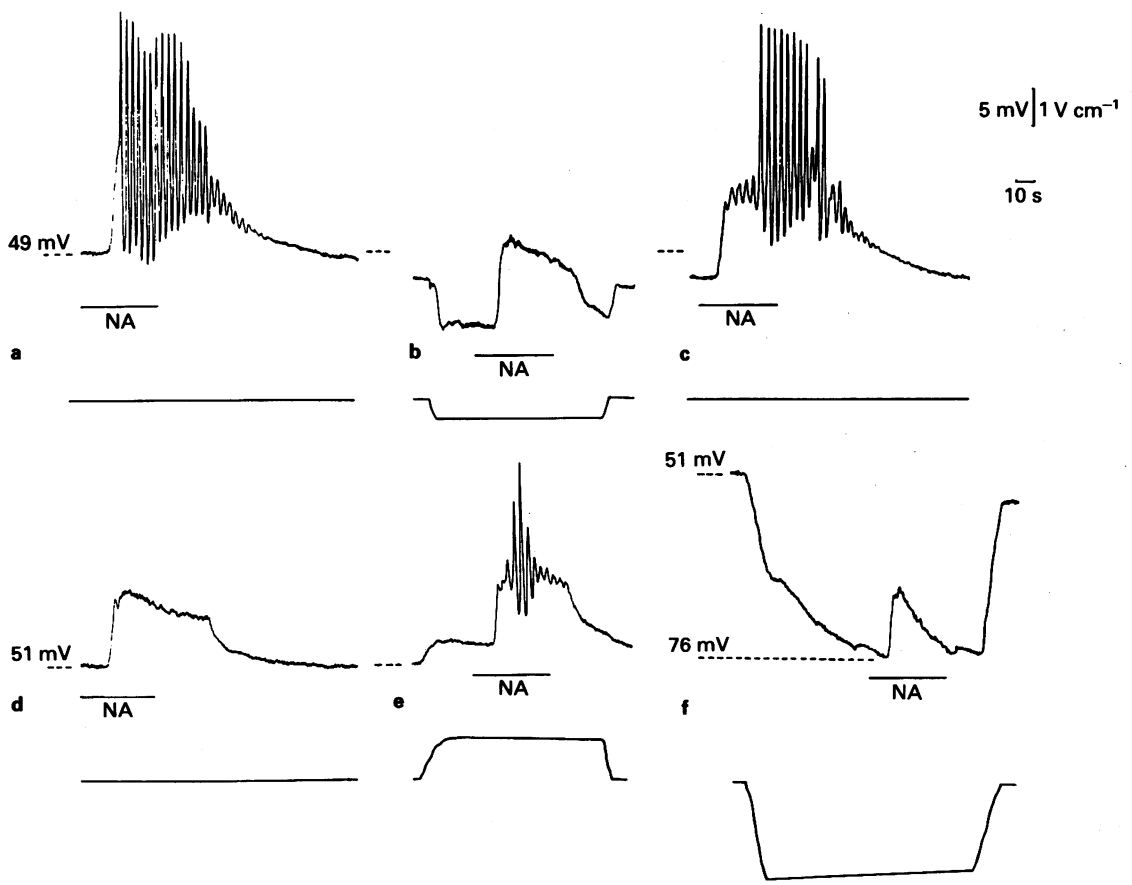


**Figure 3** Effects of membrane potential (upper trace) displacement by outward and inward current (intensity V cm$^{-1}$; lower trace) on the response to noradrenaline (NA 0.5 μM for the times indicated by bars). The broken lines represent in (a) to (c), − 49 mV; (d) to (e), − 51 mV and (f), as indicated. These records are from one cell in hypertonic Krebs solution.

Interestingly, the slow phase of depolarization in response to noradrenaline was poorly maintained during large displacements of the membrane potential from −48 to approximately −70 mV by inward current (Figure 3f). However, as this was often followed by a reduction in the slow depolarization at the restored resting Em, the effect was not investigated further.

*Effects of reducing the extracellular $Cl^-$ on the depolarization produced by noradrenaline and field stimulation*

In a previous study, Large (1984) showed that the depolarization evoked by the ionophoretic application of noradrenaline was abolished by the replacement of the external NaCl with an equivalent amount (119 mM) of Na benzenesulphonate. Thus, the effects of this substitution on the response to the bath application of noradrenaline ($10^{-6}$–$10^{-5}$M) and the adrenergically mediated e.j.p. were investigated. Since e.j.ps were abolished in hypertonic Krebs solution, the effects of Na benzenesulphonate on them was investigated in isotonic Krebs. In normal Krebs solution, e.j.ps of up to 30 mV were evoked in response to field stimulation at 10 Hz (2 pulses; Figure 4a). These responses were similar to those described previously by Creed *et al.* (1975) and Byrne & Large (1984), and in the mouse anococcygeus by Large (1982). Replacement of NaCl with Na benzenesulphonate abolished the e.j.ps after some 20 min (Figure 4b) although upon visual observation, reduced contractile responses to field stimulation were seen. In some cells, a small hyperpolarization accompanied the contraction evoked by field stimulation (Figure 4b). The e.j.ps did not recover when the number of pulses applied was increased (up to 5 at 10 Hz). Abolition of the e.j.p. was not prevented by an increased concentration (5 mM) of $Ca^{2+}$ in the modified Krebs solution and was not therefore due to $Ca^{2+}$-chelation by benzenesulphonate. Readmission of normal Krebs solution (119 mM) NaCl) restored the e.j.ps to control values.

When the NaCl of the bathing solution was replaced with Na benzenesulphonate the initial 'fast' depolarization produced by bath-applied noradrenaline was abolished while the 'slow' response was unaffected (Figure 5a and c). The effect on the 'fast' depolarization is more apparent when the responses are observed at a fast time-base (Figure 5b and d). Abolition of the 'fast' but not the 'slow' depolarizaton by sodium benzenesulphonate was confirmed in isotonic low $Cl^-$ solution by using the sucrose gap method and thus appeared to be unconnected with the use of hypertonic Krebs solution to immobilize the tissue. The 'fast' depolarization was not restored in the presence of sodium benzenesulphonate by increasing the concentration of noradrenaline applied (up to $5 \times 10^{-5}$M).

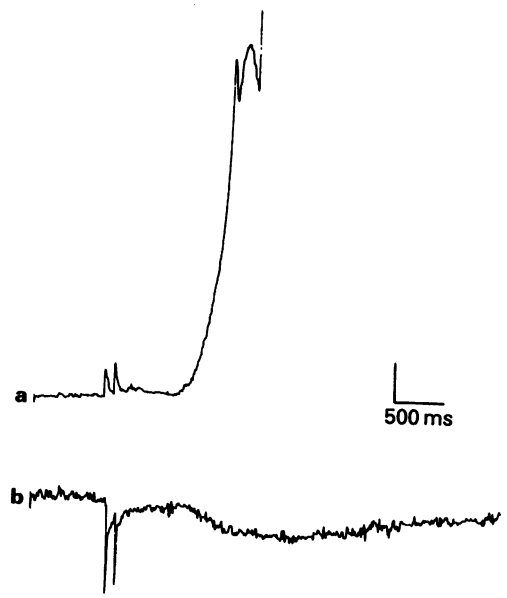


**Figure 4** The effect of chloride replacement by benzenesulphonate on the e.j.p.: (a) control; (b) low chloride. The vertical calibration is 4 mV for (a) and 2 mV for (b). In (a) the tissue was field stimulated with 2 pulses (at 10 Hz) in normal Krebs (note the active response triggered by the upward stroke of the e.j.p.). In (b) the tissue was stimulated (2 pulses at 10 Hz) after benzenesulphonate substitution and in this solution $CaCl_2$ concentration was increased to 5.0 mM to overcome the calcium chelating action of benzenesulphonate. Note the small nerve-evoked hyperpolarization in (b), illustrating that transmitter was released.

The effects of sodium benzenesulphonate on the fast depolarization were reversed when NaCl was readmitted (Figure 5e and f).

*Effects of prazosin on the depolarization evoked by noradrenaline*

The selective $\alpha_2$-adrenoceptor antagonist prazosin ($10^{-8}$–$10^{-7}$M) reduced or abolished both phases of depolarization in response to noradrenaline ($10^{-7}$–$10^{-6}$M; Figure 6a and 6b) indicating their mediation via $\alpha_1$-adrenoceptors. However, although the blocking effect of prazosin on the slow depolarization was overcome completely by increasing the concentration of noradrenaline there was no accompanying recovery of the 'fast' phase (compare Figure 6a and c). Following the removal of prazosin, the 'slow' depolarization recovered more quickly than the 'fast' phase (Figure 6d). These data suggest that both components of depolarization to bath-applied noradrenaline are mediated by $\alpha_1$-adrenoceptors.

*Comparison of the limiting potential for the 'fast' depolarization to bath applied noradrenaline with the equilibrium potential of the ionophoretic response*

The rapid time course of the 'fast' noradrenaline-induced depolarization suggests that this response is due to an increase in membrane conductance. If this is so then the amplitude of the noradrenaline-induced response ($\Delta V$) will be governed by the equation (Ginsborg, 1973):

$$\Delta V = \frac{G_T}{G_T + G_m} (e - E) \qquad (1)$$

where E and $G_m$ are the membrane potential and non-junctional conductance respectively and $G_T$ is the noradrenaline-induced conductance increase and e is the equilibrium potential for the response. If $G_T > G_m$ this equation predicts that the amplitude of the noradrenaline-induced depolarization will be related linearly to the driving force (e − E) and moreover the membrane potential in the presence of noradrenaline will be close to the equilibrium potential. This condition of $G_T >> G_m$ is likely to be met when maximal concentrations of noradrenaline are used and in the present experiments we have calculated the most depolarized value of the membrane potential produced during the 'fast' response to noradrenaline (concentrations of $5 \times 10^{-6}$M and above were maximal and records where active responses were triggered were not used). The value of the limiting potential was $-29.2 \pm 1.2$ mV (mean ± s.e.mean of 11 experiments). Further evidence that noradrenaline increases membrane conductance was obtained in experiments where the influence of the membrane potential on the ionophoretically-induced depolarizations

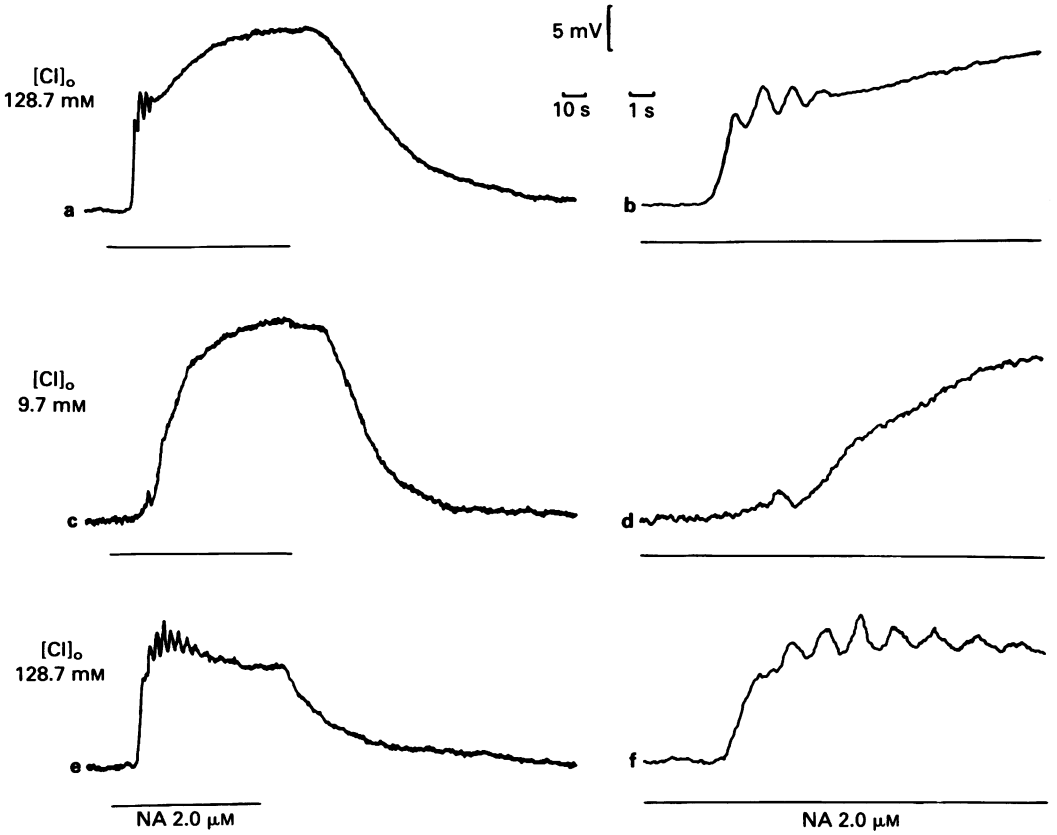


**Figure 5** Effects of replacing NaCl with Na benzenesulphonate ($[Cl]_o$ = 9.7 mM) on the 'fast' and 'slow' phases of depolarization produced by noradrenaline (NA $2 \times 10^{-6}$M for the periods indicated by bars). (a), (c) and (e) show both phases of depolarization recorded at slow time base (calibration 10 s). In (b), (d) and (f), the initial phase of each response in (a), (c) and (e) are shown at a faster time base (calibration 1 s) to show the abolition of the fast depolarization in sodium benzenesulphonate (for 34 min). Recordings were made in hypertonic solution: (a–d) are from one cell, Em = − 55 mV; (e–f) from another cell, Em = − 52 mV after 30 min wash.

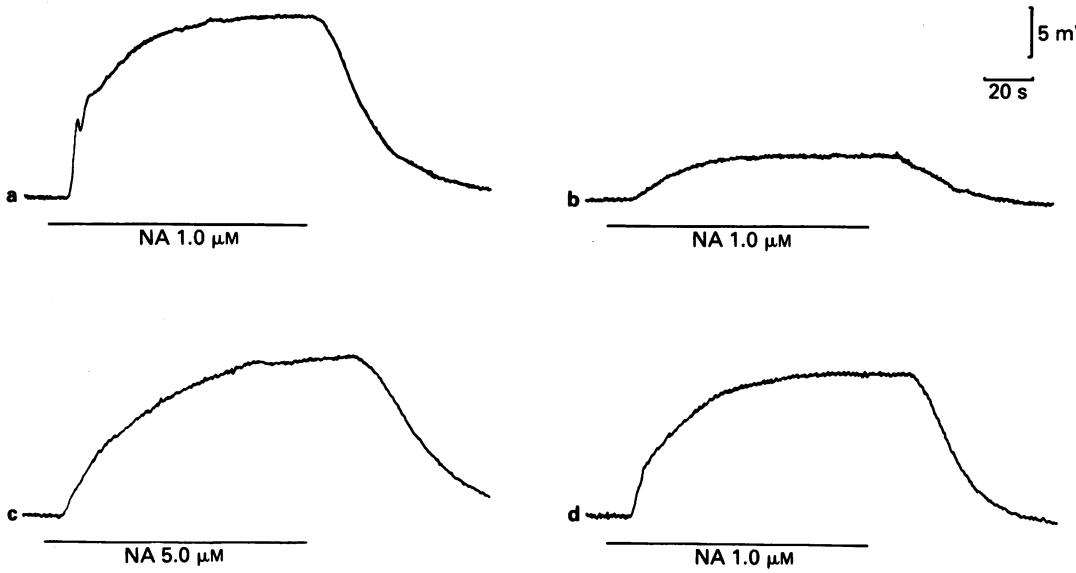


**Figure 6** Effects of prazosin on the 'fast' and 'slow' phases of depolarization produced by noradrenaline (NA for periods indicated by bars). (a) Control; prazosin (10 nM) was present for 10 min in (b) and 25 min in (c). In (d) the response obtained after 60 min wash is shown. These recordings are all from one cell, Em = −49 mV, in hypertonic Krebs solution.

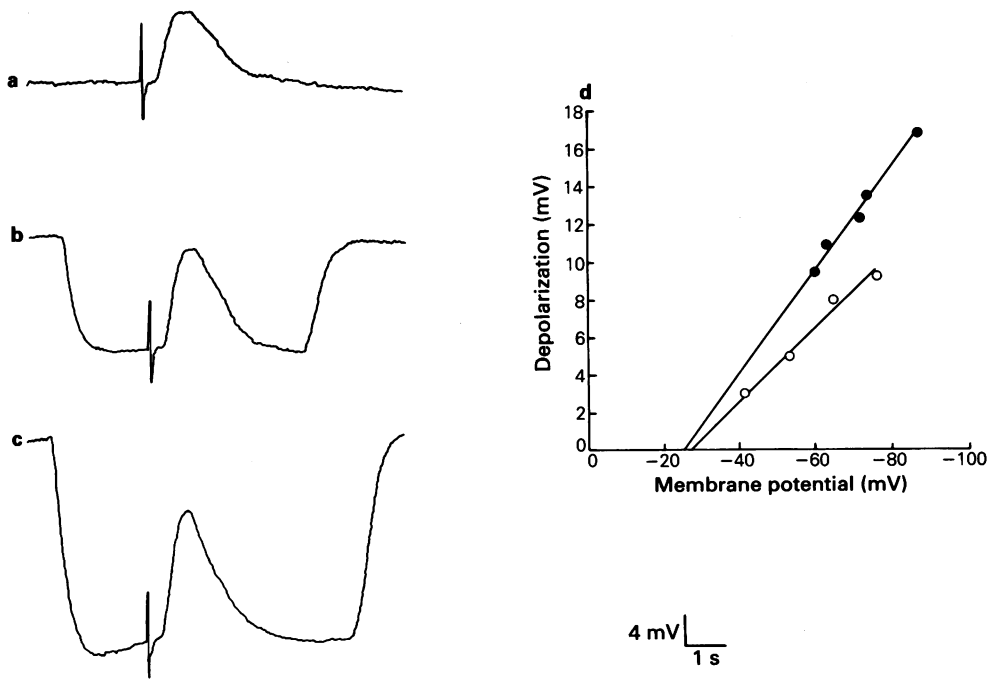


**Figure 7** Effect of membrane potential displacement on the depolarization produced by ionophoretically-applied noradrenaline. The resting membrane potential was −60 mV (a) and the same charge (0.5 nC) of noradrenaline was ionophoresed after the membrane potential was displaced to −74 mV and −87 mV in (b) and (c) respectively. (d) Relationship between membrane potential and amplitude of the depolarization for the cell shown in (a–c) (●) and in another cell where the resting membrane potential was −42 mV (○).

were examined. In these studies the membrane potential was displaced by use of a partition chamber and noradrenaline was applied by ionophoresis within 100 $\mu$m of the recording electrode which was itself placed within 100 $\mu$m of the leading plate which was used to change the membrane potential. One experiment is illustrated in Figure 7 and it can be seen that hyperpolarization increases the amplitude of the noradrenaline-induced depolarization. The resting membrane potential is $-60$ mV in Figure 7a and is displaced to $-74$ mV in b and $-87$ mV in c. A fuller analysis of the relationship between the amplitude of the depolarization and the membrane between the amplitude of the depolarization and the membrane potential is shown in Figure 7d (●). There appears to be a linear relationship between the membrane potential (albeit in a rather narrow range) and the amplitude of the depolarization and the straight line extrapolates to a value of about $-25$ mV. However, the extent of the extrapolation is rather large but in another cell where the resting membrane potential was somewhat lower ($-42$ mV) the extrapolated equilibrium potential was $-27$ mV (○ in Figure 7d). In nine experiments the extrapolated equilibrium potential for the ionophoretically-induced depolarization was $-27.1 \pm 1.6$ mV (mean ± s.e.mean) which is in good agreement with the value of $-29$ mV for the limiting potential of the 'fast' depolarization produced by bath-applied noradrenaline. It was not possible to observe how the noradrenaline-induced responses varied with depolarization of the membrane because sometimes passive depolarization caused contraction and invariably depolarization plus noradrenaline evoked contraction and loss of impalement. However, because of the extent of the extrapolation there are obvious uncertainties concerning the true value of the equilibrium potential of the depolarization produced by the ionophoretic application of noradrenaline.

## Discussion

The results of these experiments provide evidence that two mechanisms of depolarization, which differ in kinetics, are activated by the interaction between noradrenaline and the adrenoceptor in the rat isolated anococcygeus muscle. The most striking evidence for this proposition is that replacement of the external NaCl with Na benzenesulphonate abolishes the 'fast' component of depolarization while the 'slow' response is unaffected.

The interpretation of the data depends strongly on whether the noradrenaline responses are affected by the hypertonic solution used in the present experiments. The cells were slightly depolarized in hypertonic solution (by about 10 mV) compared with the value in normal Krebs but the mean value ($-48$ mV) is only slightly outside the range ($-51$ to $-75$ mV) reported by Creed *et al.* (1975) in normal Krebs solution. Slightly higher concentrations of noradrenaline were necessary to evoke depolarization in hypertonic solution as was found in the rabbit carotid artery by Mekata & Niu (1972) but these authors demonstrated that the qualitative nature of the noradrenaline-evoked depolarization was not altered in hypertonic sucrose solution. Also in the present experiments the equilibrium dissociation constant of prazosin was about $2 \times 10^{-9}$M which is in the range of published data. Thus we feel that in hypertonic solution the qualitative characteristics of the $\alpha_1$-adrenoceptor in the rat anococcygeus are not altered importantly and conclude that noradrenaline can activate two membrane conductance mechanisms in this tissue. In this respect the 'fast' phase produced by bath application is similar to the depolarization produced by ionophoresis of noradrenaline and since the e.j.p. also is completely eliminated in the presence of benzenesulphonate, one explanation is that the 'fast' and ionophoretically-induced depolarization represent the activation of the junctional adrenoceptor and the 'slow' mechanism represents stimulation of extra-junctional receptors. However in normal Krebs solution, depolarizations to noradrenaline can be recorded readily when brief ionophoretic pulses are applied at any point on the surface of the anococcygeus muscle (Large, 1982) which suggests that these receptors are distributed homogeneously over the muscle surface. It might have been expected that as in guinea-pig submucous arterioles (Hirst & Neild, 1981) there would be many more occasions when the ionophoretic application would not depolarize the muscle because the electrode was placed at an extra-junctional site. Although not reported previously, field stimulation of the anococcygeus at room temperature sometimes produces a slow depolarization (Bolton & Large, 1985), in addition to the e.j.p., which may represent activation of the 'slow' mechanism observed in the present study. In the rat tail artery, field stimulation evokes an e.j.p. followed by a slow depolarization (Cheung, 1982) although in the rat tail the e.j.p. (but not the slow response) is resistant to $\alpha$-adrenoceptor antagonists and therefore differs from the anococcygeus in which the e.j.p. is blocked by phentolamine. However, it is possible that in the anococcygeus muscle that both 'fast' and 'slow' adrenoceptor mechanisms may be activated by neurally released noradrenaline.

Both phases of depolarization produced by bath-application of noradrenaline were abolished or markedly reduced by $10^{-8}$M prazosin which suggests that the receptor(s) mediating both components of depolarization are $\alpha_1$-adrenoceptors. However, prazosin did seem to be more potent against the 'fast' response. Previously it was suggested (Large, 1983) that there

may be sub-types of the $\alpha_1$-adrenoceptor in the mouse anococcygeus as ionophoresis of naphazoline (a drug related to imidazoline) sometimes depolarized the mouse anococcygeus but on many occasions contraction but not depolarization (unlike noradrenaline) was produced. The depolarization produced by naphazoline (and noradrenaline) and the contraction were antagonized by low concentrations of prazosin. From pharmacological studies it has been suggested that there are two sub-types of $\alpha_1$-adrenoceptor in the mouse and rat anococcygeus (McGrath, 1982; Coates *et al.*, 1982; Gibson & Yu, 1983; Coates & Weetman, 1984). The present data provide further support for the existence of two sub-types of $\alpha_1$-adrenoceptor although further experiments with selective agonists and antagonists are required to substantiate this point.

There was good agreement between the most depolarized value of the membrane potential reached by the 'fast' phase to bath applied noradrenaline (mean value of $-29$ mV) and the extrapolated equilibrium potential ($-27$ mV) of the ionophoretically-induced depolarization. This is evidence that the two responses represent the activation of the same mechanism. The experiments in which the influence of membrane potential on the depolarization to ionophoretically-applied noradrenaline was studied suggest strongly that the response is due to an increase in membrane conductance with an equilibrium potential between $-20$ and $-30$ mV. Since large depolarizations were recorded close to the potassium equilibrium potential (about $-80$ mV) the depolarizations are unlikely to be mediated by a potassium conductance decrease. It is interesting that the chloride equilibrium potential in the guinea-pig vas deferens is $-24$ mV (Aickin & Brading, 1982) and it is possible that the depolarization produced by ionophoresis of noradrenaline and the 'fast' response to bath application of noradrenaline is mediated by an increase in chloride permeability. Recently some doubt was expressed concerning this ionic mechanism (Large, 1984) because the ionophoretic responses to noradrenaline were reduced or abolished in low chloride solutions when the chloride equilibrium potential is at more positive membrane potentials than in normal solution. However, it has been shown the probability of opening of certain chloride channels is dependent on the intracellular and extracellular concentrations of chloride (Chesnoy-Marchais, 1983) and thus the depolarizing responses may be expected to decline in low chloride solutions (which decreases intracellular chloride; Aickin & Brading, 1982) irrespective of the increase in chloride driving force.

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## References


ABE, Y. & TOMITA, T. (1968). Cable properties of smooth muscle. *J. Physiol.*, **196**, 87–100.

AICKIN, C.C. & BRADING, A.F. (1982). Measurement of intracellular chloride in guinea-pig vas deferens by ion analysis, $^{36}$Chloride efflux and micro-electrodes. *J. Physiol.*, **326**, 139–154.

BOLTON, T.B. (1976). On the latency and form of the membrane responses of smooth muscle to the iontophoretic application of acetylcholine or carbachol. *Proc. R. Soc. B.*, **194**, 99–119.

BOLTON, T.B. & LARGE, W.A. (1985). Are junction potentials essential? Dual mechanism of smooth muscle cell activation by transmitter released from autonomic nerves. *Q. J. Physiol.*, (in press).

BÜLBRING, E. & TOMITA, T. (1969). Increase of membrane conductance by adrenaline in the smooth muscle of guinea-pig taenia coli. *Proc. R. Soc. B.*, **172**, 89–102.

BYRNE, N.G. & LARGE, W.A. (1984). Comparison of the biphasic excitatory junction potential with membrane responses to adenosine triphosphate and noradrenaline in the rat anococcygeus muscle. *Br. J. Pharmac.*, **83**, 751–758.

BYRNE, N.G. & LARGE, W.A. (1985). Membrane potential and mechanical responses evoked by bath-applied noradrenaline in the isolated rat anococcygeus muscle. *J. Physiol.*, **360**, 60P.

CHESNOY-MARCHAIS, D. (1983). Characterization of a chloride conductance activated by hyperpolarization in Aplysia neurones. *J. Physiol.*, **342**, 277–308.

CHEUNG, D.W. (1982). Two components in the cellular response of rat tail arteries to nerve stimulation. *J. Physiol.*, **328**, 461–468.

COATES, J., JAHN, U. & WEETMAN, D.F. (1982). The existence of a new subtype of α-adrenoceptor on the rat anococcygeus is revealed by Sgd 101/75 and phenoxybenzamine. *Br. J. Pharmac.*, **75**, 549–552.

COATES, J. & WEETMAN, D.F. (1984). Occurrence of $\alpha_{1s}$-adrenoceptors in the mouse but not in the rabbit isolated anococcygeus preparations. *Br. J. Pharmac.*, **78**, 117–122.

CREED, K.E., GILLESPIE, J.S. & MUIR, T.C. (1975). The electrical basis of excitation and inhibition in the rat anococcygeus muscle. *J. Physiol.*, **245**, 33–47.

GIBSON, A. & GILLESPIE, J.S. (1973). Effect of immunosympathectomy and 6-hydroxydopamine on the responses of the rat anococcygeus to nerve stimulation and to some drugs. *Br. J. Pharmac.*, **47**, 261–267.

GIBSON, A. & WEDMORE, C.V. (1981). Responses of the isolated anococcygeus muscle of the mouse to drugs and to field stimulation. *J. autonom. Pharmac.*, **1**, 225–233.

GIBSON, A. & YU, O. (1983). Pharmacology of postsynaptic α-adrenoceptors in the mouse anococcygeus muscle. *J.*

*autonom. Pharmac.*, **3**, 1–6.

GINSBORG, B.L. (1973). Electrical changes in the membrane in junctional transmission. *Biochim. biophys. Acta.*, **300**, 289–317.

HIRST, G.D.S. & NEILD, T.O. (1981). Localization of specialized noradrenaline receptors at neuromuscular junctions on arterioles of the guinea-pig. *J. Physiol.*, **313**, 343–350.

LARGE, W.A. (1982). Membrane potential responses of the mouse anococcygeus muscle to ionophoretically applied noradrenaline. *J. Physiol.*, **326**, 385–400.

LARGE, W.A. (1983). Membrane potential responses to ionophoretically applied α-adrenoceptor agonists in the mouse anococcygeus muscle. *Br. J. Pharmac.*, **79**, 233–243.

LARGE, W.A. (1984). The effect of chloride removal on the responses of the isolated rat anococcygeus muscle to $\alpha_1$-adrenoceptor stimulation. *J. Physiol.*, **352**, 17–29.

MEKATA, F. & NIU, H. (1972). Biophysical effects of adrenaline on the smooth muscle of the rabbit common carotid artery. *J. gen. Physiol.*, **59**, 92–102.

McGRATH, J.C. (1982). Evidence for more than one type of postjunctional adrenoceptor. *Biochem. Pharmac.*, **31**, 267–484.

OHASHI, H. (1970). An estimate of the proportion of the resting membrane conductance of the smooth muscle of the guinea-pig taenia coli attributable to chloride. *J. Physiol.*, **210**, 405–419.

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