Br. J. Pharmac. (1983), **79**, 233–243

Membrane potential responses to ionophoretically applied α-adrenoceptor agonists in the mouse anococcygeus muscle

W. A. Large

Department of Pharmacology, Chelsea College, University of London, Manresa Road, London SW3 6LX

1 Noradrenaline, phenylephrine, naphazoline and oxymetazoline were applied by ionophoresis to the mouse anococcygeus muscle and the membrane potential was recorded with an intracellular microelectrode.

2 The ionophoretic application of noradrenaline and phenylephrine produced dose-related depolarizations in 96% of the cells tested; in contrast, naphazoline and oxymetazoline depolarized only 62% of the cells although contraction was always seen.

3 The depolarizations produced by all four drugs had similar characteristics in shape and time course except that the latency of responses induced by the imidazoline-related drugs was significantly longer than the value obtained with the phenylethanolamines. This discrepancy was not due to the difference in susceptibility to neuronal uptake of the two groups of drugs.

4 The time to peak depolarization for naphazoline and oxymetazoline was longer than that for noradrenaline and phenylephrine but was not sufficient to account for the considerably slower contraction produced by the former drugs.

5 At room temperature the sensitivity of the mouse anococcygeus to ionophoretically applied naphazoline and oxymetazoline was significantly lower than that to noradrenaline and phenylephrine but at 35°C the sensitivity was similar for all drugs.

6 These results suggest that there might be two subclasses of α_1-adrenoceptor in the mouse anococcygeus; stimulation of one type leads to depolarization and contraction and activation of the other class produces contraction with no change in membrane potential.

Introduction

The mouse anococcygeus muscle is innervated by the sympathetic nervous system and stimulation of the motor nerves or addition of noradrenaline causes contraction of this tissue (Gibson & Wedmore, 1981). Both these responses can be blocked with α-adrenoceptor antagonists and since the receptors mediating contraction are about 100 times more sensitive to prazosin than to yohimbine they can be classified as α_1-adrenoceptors (Gibson, 1981). An interesting result of these experiments was that the time course of the contractions produced by imidazoline-related drugs was 3–5 times longer than the contractions induced by the phenylethanolamines (Gibson, 1981), even though the responses appeared to be mediated via the same α-adrenoceptor. It seemed unlikely that this difference in time course could be accounted for in terms of different rates of free diffusion of the two groups of drugs and thus experiments were carried out to test the possibility that a difference in the kinetics of interaction between the various agonists and the α-adrenoceptor could explain the difference in the contraction time course.

For these studies, membrane potential changes produced by ionophoresis of drugs were recorded because of the better temporal resolution of this technique compared to other methods of drug application. Previously it has been shown that brief ionophoretic pulses of noradrenaline onto the mouse anococcygeus muscle produce charge-dependent depolarizations (Large, 1982). The total duration of these responses is 1–2 s at room temperature and there is an unusually long latency (several hundred milliseconds) between the start of the ionophoretic pulse and the onset of depolarization. It was suggested that the slow time course reflected, at least to some extent, a property of the noradrenaline-α-adrenoceptor interaction (rather than drug diffusion)

because (1) the time course of the ionophoretic responses was similar to that of the excitatory junction potential and (2) the latency and rise time of the depolarizations were very sensitive to changes in temperature of the bathing solution. Consequently I was hopeful that this type of experiment would reveal any differences in the kinetics of the interaction of various agonists with the α-adrenoceptor.

A preliminary account of some of this work has already been published (Large, 1981).

Methods

Most of the experimental details have been described elsewhere (Large, 1982). The mouse isolated anococcygeus was carefully stripped of connective tissue with the aid of a dissection microscope (Wild M5A) before setting up. The tissue was pinned onto a bed (about 1 mm deep) of Sylgard resin (Dow-Corning) attached to a microscope slide which was mounted on the modified stage of a Zeiss Nomarski microscope. Muscles were superfused continuously with Krebs solution at room temperature (20–22°C) or at 33–36°C. The lower temperature was used because the slower time course allowed more accurate measurement of the responses. 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 and glucose 11, and was bubbled with 5% CO_2–95% O_2.

Membrane potentials were measured with intracellular glass micro-electrodes filled with 4M-K-acetate and had resistances of 100–200 MΩ. Drugs were applied by ionophoresis from similar electrodes filled with the appropriate drug solution (always at 0.5 M concentration) and these micropipettes had resistances of 300–500 MΩ. These drugs were ionophoresed as cations using a constant current pump similar to that described by Dreyer & Peper (1974). In ionophoretic experiments it is usual to express the sensitivity of the tissue to applied stimulants in mV change in membrane potential (in this case depolarization) per nC charge (charge = current × time) passed through the ionophoretic pipette. This practice is used in these experiments but caution must be used when this criterion is used for comparing agonists as will be discussed later.

The membrane potential was recorded simultaneously on a chart recorder (Kipp and Zonen BD41)

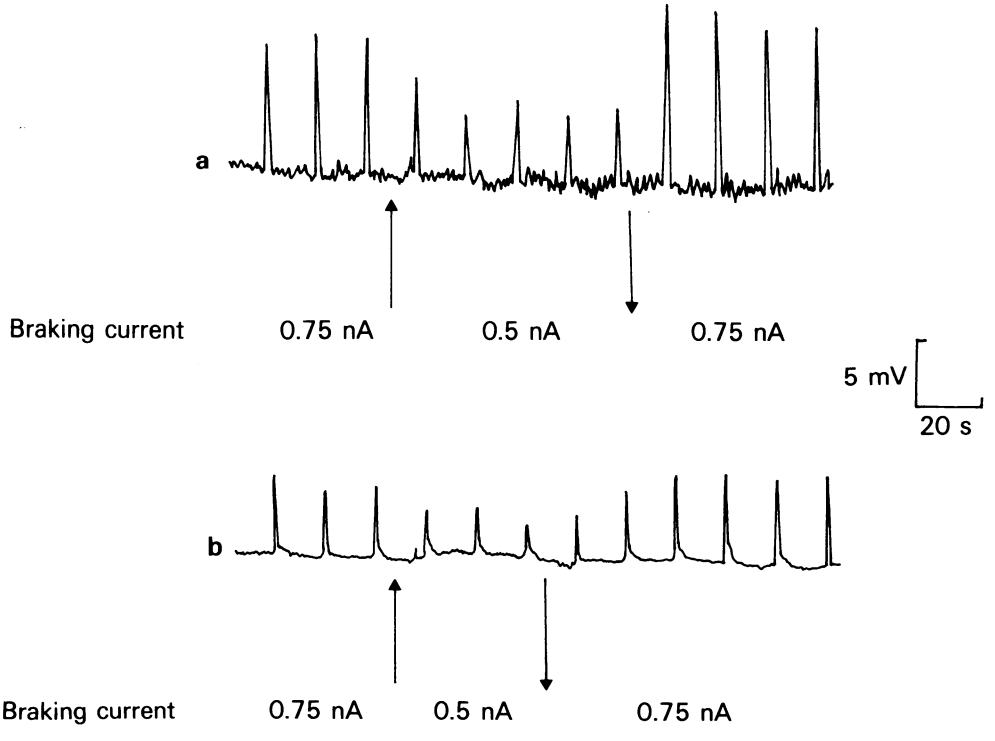

Figure 1 Effect of varying the braking current on the depolarizations produced by the ionophoretic application of noradrenaline in two cells. Between the two arrows in each record the braking current was reduced from 0.75 to 0.5 nA while the ejecting current was maintained at a constant value. In the lower trace there is a small but discernible depolarization of about 1 mV when the retaining current was reduced.

and on an FM tape recorder (Racal Store 4D); subsequently data were captured using a transient recorder (Datalab DL 901) and transferred to paper by an X–Y plotter (Bryans). The Figures shown are tracings made from these hard copies.

The following drugs were used: noradrenaline bitartrate; phenylephrine hydrochloride; naphazoline hydrochloride; oxymetazoline hydrochloride; phentolamine mesylate; prazosin hydrochloride; cocaine hydrochloride and 6-hydroxydopamine hydrochloride.

Results

Difficulties in ionophoresing α-adrenoceptor agonists

When comparing the time course of the responses produced by the ionophoresis of two drugs it would be advantageous if the drugs could be expelled from either barrel of a double-barrelled micropipette to ensure that the drugs are released at the same distance from the receptors. However, this was not possible in the present experiments because it was not possible to produce double-barrelled electrodes (separated by a solid glass spacer rod to prevent inter-barrel coupling) of sufficiently high resistance. It was noticed in the course of experiments, when noradrenaline electrodes were broken accidentally to a resistance of 100–200 MΩ that much smaller depolarizations to ionophoretic application of noradrenaline were obtained even when the retaining current was increased. This suggested that sufficient noradrenaline leaked from a broken electrode to cause receptor desensitization and this possibility was investigated. Figure 1 shows an experiment in which a constant ionophoretic pulse of noradrenaline was applied evey 20 s (from a micropipette of 350 MΩ resistance) with a retaining current of 0.75 nA; at the first arrow the retaining current was reduced to 0.5 nA and the responses were reduced to about half the control value. When the 'braking' current was increased to 0.75 nA at the second arrow the depolarizations achieved their former amplitude. The same result was obtained when a second noradrenaline micropipette was used to apply a steady conditioning background concentration of noradrenaline (in this case the retaining current of the first electrode was kept constant). Moreover it was observed that if noradrenaline was applied more frequently than about 0.2 Hz, the amplitude of the depolarizations declined and the reduction became greater as the frequency of the application increased. Qualitatively these results resemble those found with acetylcholine at the skeletal neuromuscular junction originally described by Katz & Thesleff (1957) and which have been attributed to receptor desensitization. Consequently only ionophoretic electrodes with resistances greater than 300 MΩ were used in the present experiments and it was not possible to make a double-barrelled assembly in which the resistance of each barrel exceeded 300 MΩ so single barrelled ionophoretic micropipettes were used. Since separate electrodes were used for the various agonists it was necessary to adopt some criteria to accept or reject the responses obtained. Firstly, the ionophoretic electrode was touching the tissue surface which was detected by monitoring the voltage applied to the tip of the ionophoretic electrode. Secondly for each impalement with the recording electrode, responses were obtained after placing the ionophoretic electrode in various positions (always within 100 μm of the recording electrode) and only depolarizations from the most sensitive 'spots' (which also had the fastest time course) were measured. Usually for any impalement the sensitivity to any agonist varied by no more than 10–20% but differences in sensitivity between impalements could be marked (3–5 fold differences) especially for the imidazoline-related drugs. The problem was heightened when different muscles were compared as muscle sensitivities to noradrenaline between 8–100 mV/nC were obtained. Thus to overcome these difficulties agonists were compared to noradrenaline, when possible on the same cells but always on the same muscle. Ionophoresis of the imidazoline-related drugs was difficult and the electrodes blocked very easily but of the drugs tested, most success was obtained with naphazoline and so this compound was studied extensively. A few results were obtained with oxymetazoline for comparison. In contrast, ionophoresis of the phenylethanolamines is much easier and the two drugs used were noradrenaline and phenylephrine.

Responses induced by ionophoretic application of agonists

The ionophoretic application of noradrenaline and phenylephrine produced depolarization in most of the areas tested over the entire muscle surface but it was more difficult to depolarize cells with ionophoretic application of naphazoline and oxymetazoline. On occasions when depolarizations were not observed it is possible that the drug was not expelled from the microelectrode, for example because the micropipette had blocked or because the drug molecules did not carry part of the ionophoretic current with that electrode. Consequently ionophoresis of a drug was deemed successful when either a depolarization was recorded and/or a localized contraction could be observed. The results from one particular survey are shown in Table 1. At room temperature, norad-

Table 1 Ability of α-adrenoceptor agonists to depolarize and/or contract the mouse anococcygeus muscle using ionophoresis

	20–22°C		33–35°C	
Drugs	*Number of cells depolarized*	*Number of cells contracted but not depolarized*	*Number of cells depolarized*	*Number of cells contracted but not depolarized*
Noradrenaline	59	4	38	0
Naphazoline	34	18	19	9
Phenylephrine	18	1	15	1
Oxymetazoline	16	15	—	—

renaline and phenylephrine depolarized 94% and 95% respectively and at 33–35°C depolarized 100% and 94% of the cells tested. However, with naphazoline and oxymetazoline there were many more occasions when ionophoretic application produced contraction with no change in membrane potential as naphazoline depolarized only 65% of the cells tested at room temperature and 68% at 33–35°C. The proportion of depolarized cells (52%) was even smaller when oxymetazoline was ionophoresed.

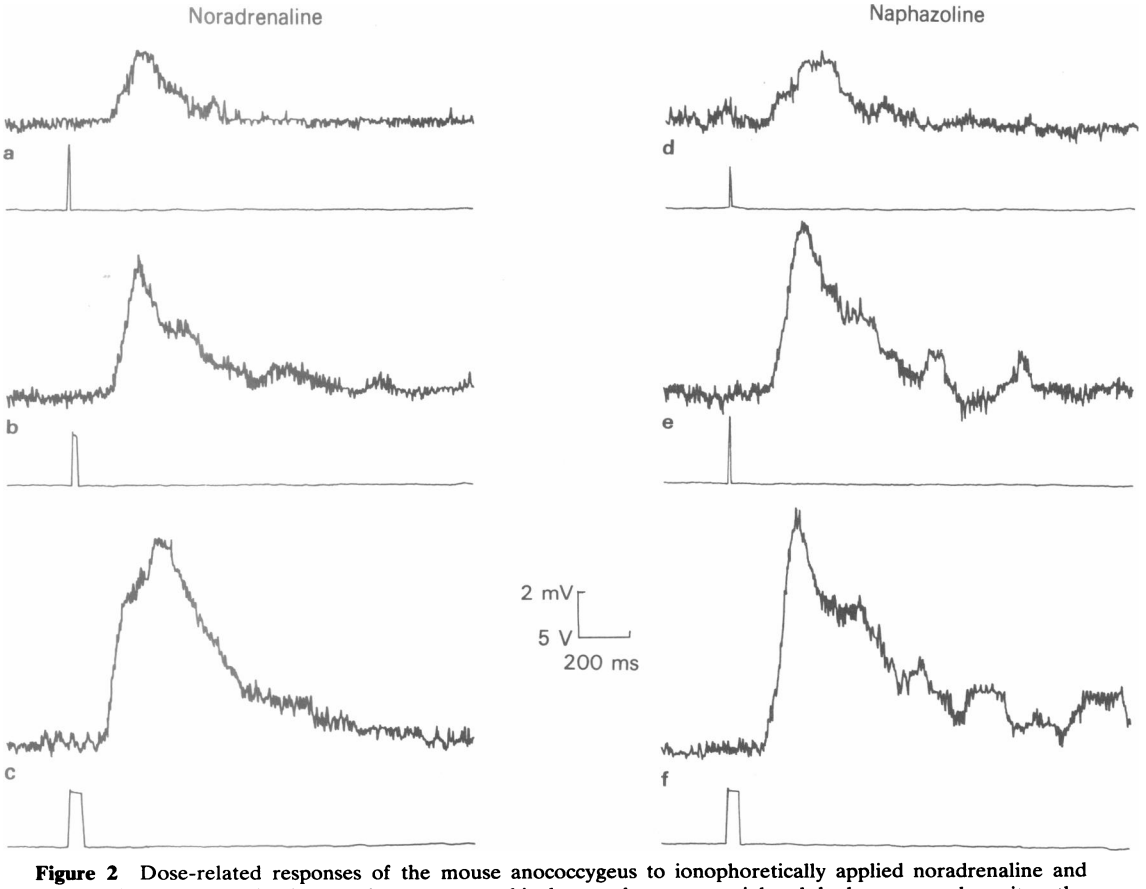

Figure 2 Dose-related responses of the mouse anococcygeus to ionophoretically applied noradrenaline and naphazoline. In each pair of traces the upper record is the membrane potential and the lower record monitors the voltage applied to the ionophoretic electrode. The ionophoretic pulses appear triangular because they are brief compared to the total sweep time and are limited by the frequency response of the X–Y recorder. All records from the same cell, Em = −58 mV; temperature, 34°C.

Comparison of noradrenaline and naphazoline

Some of the characteristics of the noradrenaline-induced depolarizations have been reported previously (Large, 1982) and new data are included for comparison with the values obtained with the other adrenoceptor agonist drugs.

Ionophoresis of noradrenaline and naphazoline usually produced monophasic depolarizations as shown in Figure 2. As published previously (Large, 1982) increasing the ionophoretic charge invariably increased the amplitude of the noradrenaline-induced responses (Figure 2 a–c) but it was more difficult to obtain a dose-response relationship with naphazoline. It is probable that this is related to the difficulty of ionophoresing naphazoline as a dose-response relationship similar to that of noradrenaline could be obtained on a few occasions (Figure 2 d–f).

In a few cells ionophoresis of those agonists produced more complex responses as shown in Figure 3. These responses were polyphasic and had a much longer time course. It can be seen from Figure 3 that the time to depolarization was the same for the monophasic responses but the decay phase was prolonged and contained many peaks. These complex responses were observed more often at 35°C than at room temperature but the significance of these depolarizations have not been investigated further and were not included in the analysis of the time course of the depolarizations.

Concern has been expressed that in ionophoretic experiments current passed through the drug-filled electrode might stimulate intrinsic nerves so that depolarizations observed are caused by transmitter released from the neurones rather than the drug from the micro-electrode (Holman, 1981). Previously it was demonstrated that in muscles in which the noradrenergic nerves had been destroyed by 6-hydroxydopamine pretreatment, noradrenaline produced similar responses to those observed in innervated tissue (Large, 1982) but as the naphazoline-

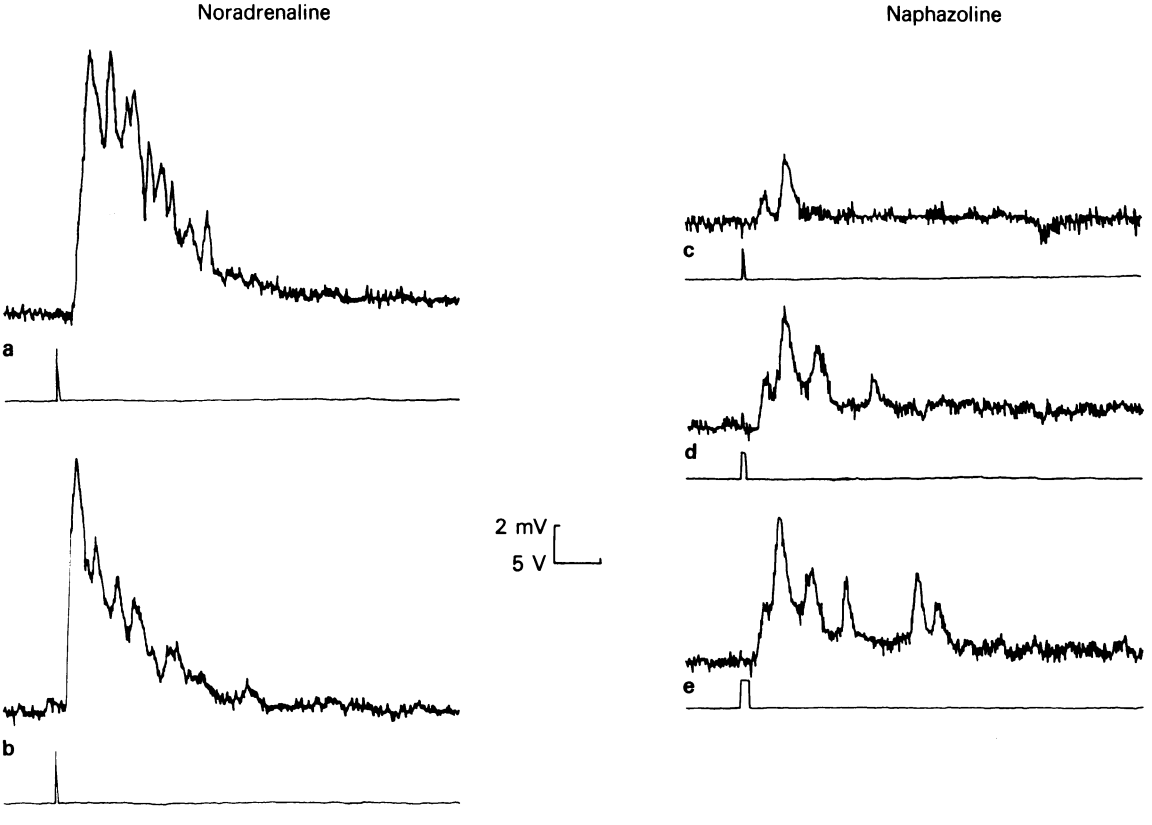

Figure 3 Multiphasic depolarizations produced by ionophoresis of noradrenaline and naphazoline (a) and (b) from one cell and (c) (d) and (e) from a different cell. Temperature, 33°C. Horizontal bar represents 1 s for (a) and (b) and 500 ms for (c) (d) and (e). In records (c), (d) and (e) the ionophoretic pulse was increased which caused an increase in the number of dispersed peaks, compare with Figure 2.

Table 2 Characteristics of the depolarizations produced by the ionophoretic application of noradrenaline and naphazoline at various temperatures

	Amplitude (mV)	*Latency* (ms)	*Rise time* (ms)	*Total time to peak* (ms)	*Half-decay time* (ms)	*Sensitivity* (mV/nC)
A (33–35°C)						
Noradrenaline ($n=31$)	4.93 ± 0.34	123 ± 8.9	186 ± 8.8	309 ± 10	275 ± 56	32.1 ± 6.6
Naphazoline ($n=33$)	5.05 ± 0.66	156 ± 7.6*	211 ± 14	367 ± 19*	325 ± 35	26.4 ± 6.8
B (20–22°C)						
Noradrenaline ($n=26$)	5.37 ± 0.47	482 ± 24	507 ± 36	989 ± 36	543 ± 57	22.1 ± 4.0
Naphazoline ($n=28$)	4.63 ± 0.45	728 ± 33**	540 ± 48	1268 ± 61**	618 ± 65	6.2 ± 1.9**

The figures are the mean ± s.e.mean and n represents the number of cells.
The means were compared using Students' t test. Statistically different from noradrenaline responses (*$P<0.01$; **$P<0.001$).

induced responses closely resembled those of noradrenaline it seemed worthwhile to apply naphazoline to denervated muscle. In two muscles denervated by pretreatment with 6-hydroxydopamine the sensitivity to naphazoline was 22.3 ± 3.6 mV/nC ($n=10$) which was similar to the sensitivity of 26 mV/nC (Table 2) found in innervated tissue. This result shows that the depolarizations produced by the ionophoretic application of naphazoline were caused by naphazoline released from the electrode rather than noradrenaline released from the intrinsic nerves.

It can be seen from Figure 2 that some characteristics of the time course, as well as the shape, of the noradrenaline- and naphazoline-induced depolarizations were similar and the results of several experi-

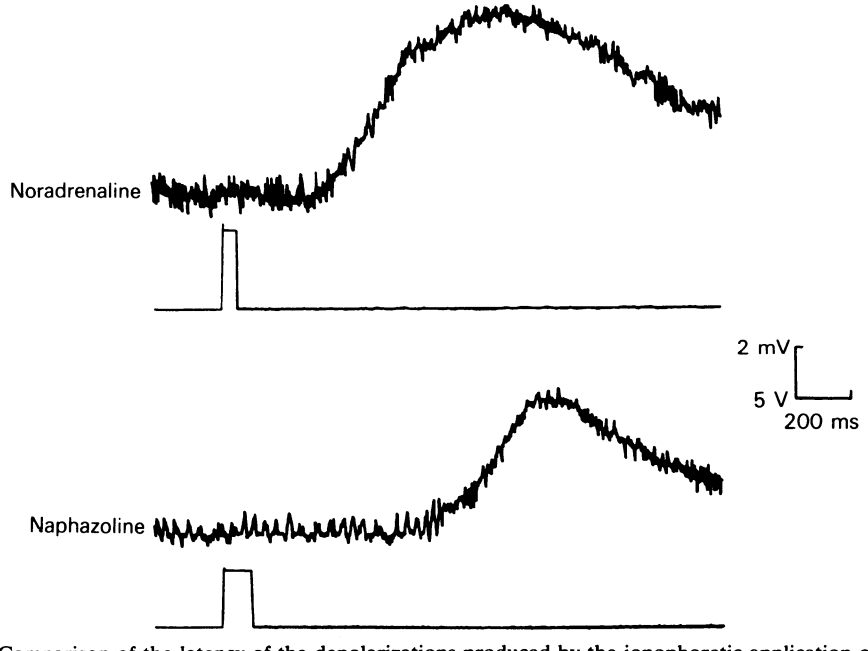

Figure 4 Comparison of the latency of the depolarizations produced by the ionophoretic application of noradrenaline. Temperature, 21°C. Note the much longer delay of the onset of the response produced by naphazoline compared to noradrenaline.

ments are summarized in Table 2. At 33–35°C (Table 2A) the rise time (onset of depolarization to peak of response) and half-decay time of the depolarizations produced by the ionophoretic application of naphazoline were similar to the values obtained with noradrenaline. In contrast the latency of the naphazoline-induced responses was significantly longer, the mean was 156 ms compared to 123 ms for noradrenaline. The sensitivity of the anococcygeus was the same for both drugs at 33–35°C. In these experiments cocaine was not included in the Krebs solution as it has been shown that cocaine does not alter the amplitude, latency, or the rise time of the depolarizations produced by the ionophoretic application of noradrenaline (Large, 1982).

Previously it has been demonstrated that the latency and rise time of the depolarizations produced by the ionophoretic application of noradrenaline are highly sensitive to changes in the temperature of the bathing solution and thus it was of interest to see if this is the case also for naphazoline. Experiments carried out at room temperature (20–22°C) show that the responses induced by naphazoline are slowed down considerably by lowering the temperature and moreover the difference in the latency becomes more marked than at 33–35°C, the mean latency for naphazoline is 728 ms compared to 482 ms for noradrenaline, and this observation is clearly illustrated in Figure 4. The amplitude of the naphazoline-induced depolarization is a little smaller than the noradrenaline response and the latency of depolarizations is inversely related to the amplitude (Large, 1982) but the difference in the amplitudes shown in Figure 4 is not sufficiently large to account for the difference in latencies (see Figure 6, Large 1982). Since noradrenaline, unlike naphazoline, is removed from its site of action by neuronal uptake it is possible that uptake might account for the discrepancy in latencies for the two. However, the total time peak for noradrenaline-induced depolarizations was not altered by 2 μM cocaine, the time to peak in normal Krebs at 35°C was 340 ± 19.4 ms ($n = 10$) and in cocaine the mean

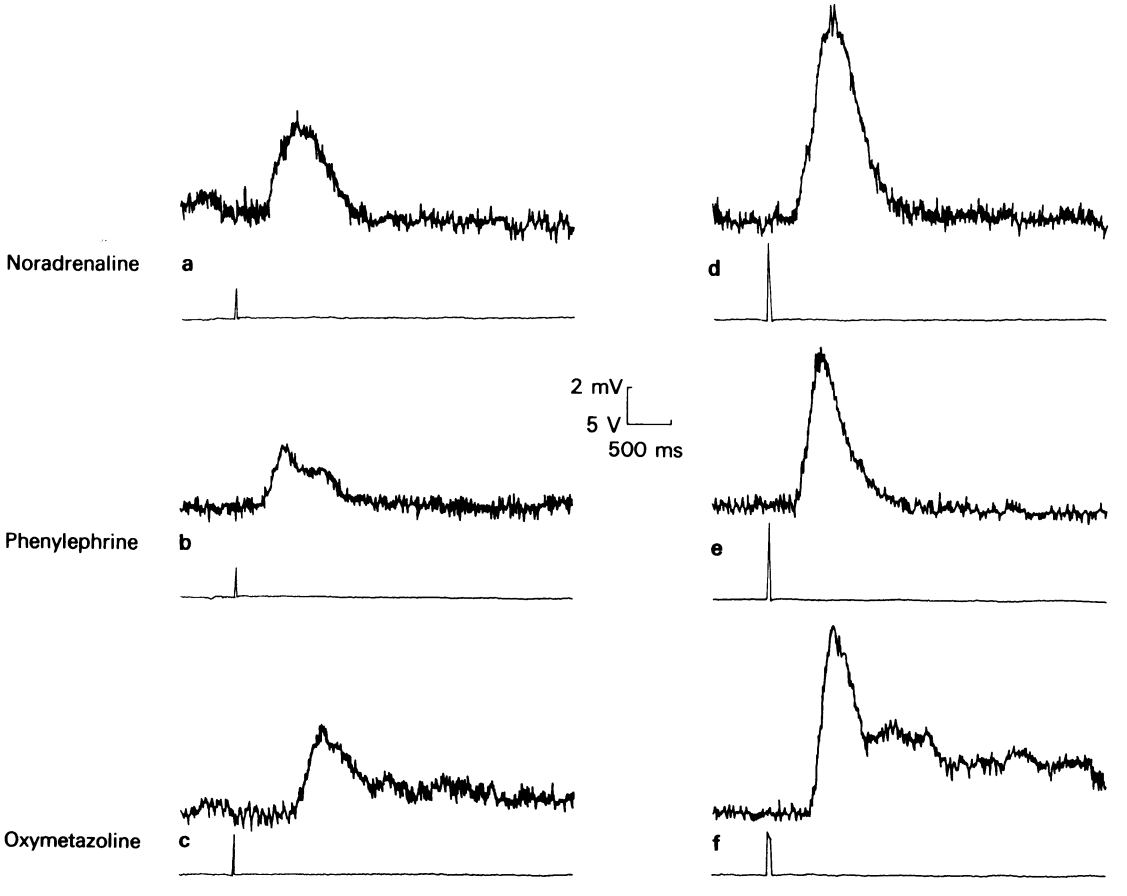

Figure 5 Dose-related depolarizations produced by ionophoresis of noradrenaline, phenylephrine and oxymetazoline. Temperature, 22°C. Responses were obtained from different cells.

was 359 ± 22.4 ms ($n = 11$) and so neuronal uptake cannot account for the difference in the latency of the depolarizations produced by noradrenaline and naphazoline.

An interesting result is that the muscle sensitivity to naphazoline is significantly lower than the sensitivity to noradrenaline (Table 2B) at 20–22°C although at 33–35°C the values are similar. It seems unlikely that this is due to a difference in drug diffusion or a difficulty in ionophoresing naphazoline as these factors are not expected to be very sensitive to temperature changes.

The depolarizations induced by the ionophoretic application of noradrenaline are mediated by α_1-adrenoceptors as the responses are antagonized by prazosin and phentolamine (the equilibrium constants are about 1 nM and 10 nM respectively) so it was of interest to see if the depolarizations evoked by naphazoline were also mediated by α_1-adrenoceptors. These studies were not totally satisfactory because it was very difficult to obtain a 'dose-response' relationship with the imidazolines. Although quantitative experiments were not carried out with naphazoline it should be noted that the depolarizations produced by the ionophoretic application of naphazoline were antagonized by prazosin. For example, in one muscle naphazoline depolarized 9 of 12 cells tested in normal Krebs and the mean sensitivity of the responding cells was 2.10 ± 0.53 mV/nC. After 10^{-8} M prazosin was added to the perfusion fluid 0/6 cells responded to naphazoline but after washing out the α-adrenoceptor antagonist 4 of 7 cells were depolarized and had a mean sensitivity of 2.09 ± 0.80 mV/nC. In contrast naphazoline-induced depolarizations were unaffected by 10^{-6} M yohimbine. These results show that the depolarizations are mediated via α_1-adrenoceptors.

Responses to ionophoretically applied phenylephrine and oxymetazoline

Depolarizations produced by phenylephrine and oxymetazoline are shown in Figure 5 and the results are summarized in Tables 3 and 4. The characteristics of the phenylephrine-induced responses were similar to those of noradrenaline in that the depolarizations were normally monophasic, and the latency, rise time, half-decay time and the muscle sensitivity were not significantly different from the value obtained with noradrenaline at either 33–35°C or at room temperature (Table 3).

In contrast, the characteristics of the oxymetazoline-induced responses resembled those of naphazoline rather than noradrenaline. The pertinent features were an increased latency and a reduced muscle sensitivity when compared to noradrenaline (Figure 5 and Table 4). The responses produced by the ionophoretic application of both phenylephrine and oxymetazoline were antagonized by prazosin (10^{-8} M) and phentolamine (5×10^{-8} M) and so the depolarizations appear to be mediated via the same α_1-adrenoceptor as noradrenaline.

Discussion

In the mouse anococcygeus the ionophoretic application of the adrenoceptor agonists noradrenaline, phenylephrine, naphazoline and oxymetazoline produced depolarization of the muscle cells which appears to be mediated via α-adrenoceptors. However, these drugs can be subdivided into two groups according to three main observations. First, noradrenaline and phenylephrine readily depolarize the muscle (and cause contraction) whereas naphazoline

Table 3 Characteristics of the depolarizations produced by the ionophoretic application of noradrenaline and phenylephrine at various temperatures

	Amplitude (mV)	*Latency* (ms)	*Rise-time* (ms)	*Total time to peak* (ms)	*Half-decay time* (ms)	*Sensitivity* (mV/nC)
A (33–35°C)						
Noradrenaline ($n = 10$)	5.90 ± 0.57	141 ± 15	182 ± 28	323 ± 33	238 ± 39	47.6 ± 9.5
Phenylephrine ($n = 15$)	7.00 ± 0.83	159 ± 13	193 ± 21	352 ± 24	258 ± 24	52.5 ± 11.1
B (20–23°C)						
Noradrenaline ($n = 18$)	7.19 ± 0.79	387 ± 18	417 ± 29	804 ± 30	449 ± 38	22.7 ± 3.7
Phenylephrine ($n = 18$)	7.70 ± 1.05	412 ± 30	460 ± 28	872 ± 47	412 ± 33	29.8 ± 5.1

Table 4 Characteristics of the depolarizations produced by the ionophoretic application of noradrenaline and oxymetazoline at room temperature (22–24°C)

	Amplitude (mV)	*Latency* (ms)	*Rise time* (ms)	*Total time to peak* (ms)	*Half-decay time* (ms)	*Sensitivity* (mV/nC)
Noradrenaline ($n = 11$)	5.41 ± 0.94	375 ± 24	352 ± 49	727 ± 43	273 ± 27	57 ± 4.2
Oxymetazoline ($n = 16$)	3.30 ± 0.57	688 ± 40*	295 ± 28	983 ± 42*	275 ± 30	15 ± 3.6*

Statistically different from noradrenaline responses ($P < 0.001$)

and oxymetazoline on many more occasions can initiate muscle contraction in the absence of any change in membrane potential. Second, the latency of the depolarizations produced by the ionophoretic application of noradrenaline and phenylephrine is much smaller than the latency of the responses produced by naphazoline and oxymetazoline. Third, at room temperature a given ionophoretic charge applied to a noradrenaline-filled electrode produces a greater depolarization of the membrane potential than the same ionophoretic pulse passed through a micropipette filled with an imidazoline; at 35°C this difference in sensitivity to the various agonists does not exist.

It is worth considering to what extent these discrepancies can be attributed to technical problems. In the rat anococcygeus the noradrenaline-induced depolarization is accompanied by a fall in membrane resistance (Creed, 1975; Large, unpublished observation) which suggests that the action of noradrenaline is to increase the membrane permeability to some ion. In this case the depolarization detected with an intracellular micro-electrode will depend on the membrane resistance and thus it is possible that the lack of depolarization following an ionophoretic pulse of drug is due to a low membrane resistance, for example caused by cellular damage produced during impalement with the recording electrode. In this case it would be expected that there would be a similar proportion of cells to respond with contraction unaccompanied by depolarization for all the drugs used. Clearly this was not the case as can be seen from Table 1 and if the results at all temperatures of noradrenaline and phenylephrine are pooled, then only 6 of a total of 136 cells contracted with no change in membrane potential. In contrast, with ionophoretic application of naphazoline and oxymetazoline contraction was not associated with depolarization in 42 of 111 cells. Contraction and depolarization might occur independently of each other or another explanation is that different mechanisms of action are initiated by each of the two classes of α-adrenoceptor agonists, one depolarization-dependent and the other not. The latter is considered more probable and the conclusion thus follows that there are two subtypes of α_1-adrenoceptor which appear not to be readily distinguishable in their affinities for prazosin. In the framework of this theory noradrenaline and phenylephrine would have a greater affinity than naphazoline and oxymetazoline for the depolarizing receptor. It is possible that the 'searching' procedure biased the results but the striking observation was that ionophoretically-applied noradrenaline depolarized the smooth muscle cells on nearly all occasions even at those positions which were not selected for analysis. This is in direct contrast to the results with the imidazoline-related drugs which failed to produce depolarization on about 40% of the sites tested using the same rigourous 'searching' procedure. It is interesting to compare the present results with the experiments of Hirst & Neild (1980a). They found that ionophoresis of noradrenaline onto guinea-pig arterioles caused depolarization at 5–10% of the areas tested but at the great majority of sites tested contraction was observed which was not accompanied by a membrane potential change. Thus there appears to be a difference in the distribution of adrenoceptors in guinea-pig arterioles and the mouse anococcygeus muscle as in the present experiments the ionophoretic application of noradrenaline produced depolarization at over 90% of the sites examined. These results suggest that the 'depolarizing receptors' are fairly evenly distributed over the entire surface of the mouse anococcygeus muscle whereas in guinea-pig arterioles the depolarizing receptors are concentrated at discrete areas in the muscle surface. The noradrenaline-induced depolarizations in guinea-pig arterioles are not blocked by phentolamine and Hirst & Neild (1980b) suggested the receptor mediating depolarization in guinea-pig arterioles is called the γ-adrenoceptor and subsequently produced evidence that the γ-adrenoceptor is confined to the subsynaptic membrane whereas the 'non-depolarizing receptor' is situated in the extrasynaptic region (Hirst & Neild, 1981). The adrenoceptors in the mouse anococcygeus and guinea-pig arterioles are similar with respect to the physiological consequences of

adrenoceptor stimulation but the recognition sites must be different in the two tissues because of the discrepancy in susceptibility to α-adrenoceptor antagonists.

From organ bath experiments where contractions have been studied it seems that the α-receptor population of the mouse anococcygeus is not homogenous because the K_D values of the antagonists, phentolamine and prazosin, vary depending on whether noradrenaline or naphazoline is used as the agonist (Gibson & Yu, personal communication). In the rat anococcygeus Coates, Jahn & Weetman (1982) found that the compound Sgd 101/75 (a derivative of clonidine and a full agonist in this tissue) was antagonized to a greater extent than noradrenaline by phenoxybenzamine and postulated the existence of a new subtype of α_1-receptor which they termed the α_{1s}-adrenoceptor. Also using the rat anococcygeus, McGrath (1982) observed that the dose-response curve obtained with the phenylethanolamines has a shoulder whereas the non-phenylethanolamines produce a monophasic dose-response curve and speculated that this was due to the presence of two types of adrenoceptor in the tissue which he called α_{1a} and α_{1b}. Thus the results of the present experiments are consistent with the hypothesis that stimulation of one subtype produces depolarization whereas activation of the second class leads to contraction not associated with a change in membrane potential.

An interesting result was the change in sensitivity to ionophoretically applied naphazoline. At room temperature the muscle was less sensitive to naphazoline (and oxymetazoline) than to noradrenaline but at 35°C the two drugs were equieffective in their ability to depolarize the muscle. It was more difficult to ionophorese naphazoline but it is not obvious how warming up the bathing solution could increase the amount of naphazoline delivered by a given ionophoretic charge. Technical difficulties cannot be discounted, but if there are subclasses of adrenoceptor in this muscle it is possible that the receptors are in equilibrium which can be shifted one way or another by a change in temperature.

The initial purpose of the present experiments was to ascertain whether the difference in the time to peak contraction produced by noradrenaline and phenylephrine compared to naphazoline and oxymetazoline could be accounted for in terms of a difference in the rate of membrane depolarization produced by the various drugs. The time to peak depolarization produced by ionophoresis was longer for naphazoline (367 ms at about 35°C) than for noradrenaline (309 ms) and at room temperature the difference was enhanced and the total time to peak was 1268 ms and 989 ms respectively. In both cases the difference could be largely attributed to the larger latency of the naphazoline-induced response. Again, the increased latency of the naphazoline- (and oxymetazoline-) induced depolarization might be due to difficulty in ionophoresing the drug. However, assuming a linear relationship between latency and temperature the Q_{10} of the naphazoline latency between 25°C and 35°C is 3.9 and the Q_{10} for the noradrenaline latency is 3.4. Physical processes are not much affected by temperature and have a $Q_{10} < 1.5$ (Taylor, 1924; for example the half-decay time of the ionophoretically induced depolarizations) and so the above high Q_{10} values suggest that the latency is a characteristic of the drug-receptor interaction although the exact pharmacological significance of the latency remains unclear. It is possible that the difference in the latencies of the depolarization may represent a difference in the kinetics of the various drugs with the 'depolarizing receptor'. In any event it seems improbable that the difference in the rate of depolarization can account for the much slower rate of contraction produced by naphazoline in the organ bath. The ratio of the total time to peak contraction naphazoline: noradrenaline is between 3.4 and 4.2 (Gibson, 1981) whereas the ratio of the time to peak depolarization is 1.2. Also it is equally unlikely that free diffusion of noradrenaline and naphazoline are very different but buffered diffusion could account for the slowness of the contraction induced by naphazoline. If the concentration of receptors in the mouse anococcygeus is x_d then diffusion of a drug through the muscle will be slowed by a factor $(1 + \frac{x_d}{Kd})$, where Kd is equilibrium dissociation constant of the drug (see Colquhoun, Large & Rang, 1977). In the present context the diffusion of naphazoline would be slower than that of noradrenaline if naphazoline had a much greater affinity for the α-adrenoceptor and there is no information on the relative affinities of α-adrenoceptor agonists in the anococcygeus muscle. Of course if the contraction induced by the addition of naphazoline to the organ bath resulted as a consequence of binding to a receptor subtype whose activation causes contraction in the absence of a change in membrane potential then its rate of action would not be reflected in electrophysiological experiments.

This work was supported by the Medical Research Council.

References

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**, 544–552.

COLQUHOUN, D., LARGE, W.A. & RANG, H.P. (1977). An analysis of the action of a false transmitter at the neuromuscular junction. *J. Physiol.*, **266**, 361–395.

CREED, K.E. (1975). Membrane properties of the smooth muscle cells of the rat anococcygeus muscle. *J. Physiol.*, **245**, 49–62.

DREYER, F. & PEPER, K. (1974). Iontophoretic application of acetylcholine: Advantages of high resistance micropipettes in connection with an electric current pump. *Pflügers Arch.*, **348**, 263–272.

GIBSON, A. (1981). Contractile responses of the mouse anococcygeus muscle to some α-adrenoceptor agonists. *Br. J. Pharmac.*, **73**, 284–285P.

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

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

HIRST, G.D.S. & NEILD, T.O. (1980b). Matters arising: Noradrenergic transmission. *Nature*, **288**, 301–302.

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

HOLMAN, M. (1981). The intrinsic innervation and peristaltic reflex of the small intestine. In *Smooth Muscle: an Assessment of Current Knowledge.* ed. Bülbring, E., Brading, A.F., Jones, A.W. & Tomita, T. pp. 311–338. London: Edward Arnold.

KATZ, B. & THESLEFF, S. (1957). A study of the desensitization produced by acetylcholine at the motor endplate. *J. Physiol.*, **138**, 63–80.

LARGE, W.A. (1981). Membrane potential responses to ionophoretically applied α-adrenoceptor stimulants in the mouse anococcygeus muscle. *J. Physiol.*, **317**, 88P.

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

McGRATH, J.C. (1982). Evidence for more than one type of post-junctional α-adrenoceptor. *Biochem. Pharmac.*, **31**, 467–484.

TAYLOR, H.S. (1924). *Treatise on Physical Chemistry*, Vol. 2. London: Macmillan.

(Received October 18, 1982.
Revised November 23, 1982.)