J. Physiol. (1982), **326**, *pp.* 385–400
With 7 text-figures
Printed in Great Britain

MEMBRANE POTENTIAL RESPONSES OF THE MOUSE ANOCOCCYGEUS MUSCLE TO IONOPHORETICALLY APPLIED NORADRENALINE

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(*Received 2 September 1981*)

SUMMARY

1. Membrane potential responses to ionophoretically applied noradrenaline and to field stimulation were studied in the mouse anococcygeus muscle using intracellular recording techniques.

2. The ionophoretic application of noradrenaline produced charge-dependent depolarizations whose total duration was 1–2 s at room temperature and which were characterized by a delay between the start of the ionophoretic pulse and the onset of depolarization (termed the latency of the responses). On occasion ionophoresis of noradrenaline did not depolarize the muscle even though it seemed that successful ejection of noradrenaline had occurred as small localized contractions could be seen.

3. The characteristics of these depolarizations were unaffected by tetrodotoxin (10^{-7} M) and could not be reproduced when the ionophoretic pipette contained 2 M-NaCl rather than noradrenaline. Moreover noradrenaline still produced depolarizations in denervated muscle and thus it is concluded that the responses were caused by noradrenaline released from the ionophoretic micropipette and not from the intrinsic noradrenergic nerves.

4. Field stimulation of innervated muscle usually evoked excitatory junction potentials (e.j.p.s), but sometimes inhibitory junction potentials (i.j.p.s) or a mixture of e.j.p.s and i.j.p.s were observed. The time course of the e.j.p.s was slightly longer than that of the ionophoretic depolarizations which was accounted for by a smaller latency of the ionophoretically induced responses.

5. The pharmacology of the nerve-evoked e.j.p.s and the ionophoretically induced depolarizations was similar as both types of responses were antagonized by α_1-adrenoceptor blocking agents (phentolamine and prazosin) but were unaffected by the β-adrenoceptor antagonist, propranolol. It is probable that noradrenaline released from the intrinsic nerves and that from the ionophoretic micropipette were acting on the same adrenoceptors.

6. The latency and to a lesser extent the rise-time of the depolarizations produced by the ionophoretic application of noradrenaline was highly sensitive to changes in temperature of the bathing fluid (Q_{10}s > 2) whereas the half-decay time was relatively insensitive to temperature changes ($Q_{10} \sim 1{\cdot}5$). In addition the latency of the depolarizations was not altered by inhibiting the noradrenaline-uptake mechanism

with cocaine (2×10^{-6} M) or by α-adrenoceptor blocking agents. Thus it seems likely that the latency of the responses is a property of the noradrenaline–receptor interaction rather than being caused by other phenomena such as diffusion of noradrenaline.

INTRODUCTION

One advantage of ionophoresis, whereby drugs are expelled from micropipettes (Nastuk, 1953), over other methods of drug application is that the drugs can be applied for very brief periods of time giving the possibility of studying the kinetics of drug–receptor interactions. At the neuromuscular junction, however, it has proved difficult to mimic nerve-evoked synaptic potentials with ionophoretically induced responses (but see Kuffler & Yoshikami, 1975). Presumably one of the main reasons for this difficulty is that the time course of the acetylcholine (ACh) concentration in the region of the post-synaptic receptors following an ionophoretic pulse is slow compared with the ACh–receptor interaction. However, responses in some smooth muscles are of a much slower time course. For example, when acetylcholine or carbachol are applied by ionophoresis to guinea-pig taenia or ileum (Bolton, 1976) and cultured smooth cells (Purves, 1974), the time from the start of the ionophoretic pulse to peak depolarization is at least several hundred milliseconds, even when the duration of the application is relatively brief. This suggests that the duration of the response is not limited by diffusion of the drug, but is a characteristic of the drug–receptor interaction.

At the time of starting the present study, no similar investigation of noradrenaline-induced responses in smooth muscle had been published, but recently Hirst & Neild (1980, 1981) have shown ionophoretically applied noradrenaline does produce depolarizations in arterioles which last for about a second. In the present experiments the time course of the depolarizations produced by ionophoretically applied noradrenaline has been investigated and compared with nerve-evoked excitatory junction potentials (e.j.p.s) in the mouse anococcygeus muscle. A preliminary account of some of this work has already been published (Large, 1981).

METHODS

Experiments were carried out on the isolated mouse anococcygeus muscle dissected from male albino mice (LACA; 25–35 g). The mouse anococcygeus was selected because it is densely innervated by sympathetic nerves (Gibson & Wedmore, 1981) and has the advantage of being small (less than 1 mg wet weight), thus being suitable for Nomarski optics used in these experiments. Single muscles (about 2 mm in length) were stripped of connective tissue before being set up in the experimental chamber. The preparations were pinned on to a bed of Sylgard resin (Dow-Corning) and the base of the experimental chamber was a microscope slide mounted on the modified stage of a Zeiss Nomarski microscope. Muscles were superfused continuously with Krebs solution flowing at a rate of about 2 ml/min in a total bath volume of 1·25 ml. 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_2–95% O_2. Experiments were carried out either at room temperature (20–23 °C) or at 33–36 °C, the temperature being checked routinely with a thermistor probe placed close to the preparation.

Electrophysiological recording and ionophoresis

Micro-electrodes were pulled from capillary glass of 1·5 mm or 1·00 mm external diameter containing an internal filament (Clark Electromedical) on a Narishige horizontal puller. Recording electrodes were filled with 4 M-K-acetate and had resistances of 100–200 MΩ. Similar electrodes were used for ionophoresis, except that they were filled with 0·5 M-noradrenaline (the bitartrate salt was dissolved in distilled water). These electrodes had resistances of 175–500 MΩ but most that there were used had a resistance of about 300 MΩ and noradrenaline was ionophoresed as a cation using a constant current pump similar to that described by Dreyer & Peper (1974). With this current pump it is possible to measure the current at the electrode tip, but routinely the voltage applied to the ionophoretic electrode was monitored as this characteristic provides information on whether the micropipette is likely to block during passage of current. The time to peak for the current pulse was about 1 ms. To prevent leakage of noradrenaline, a steady 'braking' current was passed in the opposite direction to the ejection current. With acceptable electrodes a retaining current of about 0·7 nA prevented spontaneous release as judged by contraction of the muscle in direct proximity to the micro-electrode, so in practice 'braking' currents of 1·5–2·5 nA were used.

Nerve-evoked e.j.p.s were produced by field stimulation of the tissue with two Ag–AgCl electrodes 1–2 mm apart placed either side of the preparation. Parameters of stimulation used were: pulse width 0·5 ms, pulse amplitude of 40–70 V which produced maximal junctional potentials and various frequencies, to be discussed in the text. The membrane potential was recorded simultaneously on a chart recorder (Kipp and Zonen BD 41) and on an FM tape recorder (Racal Store 4D); subsequently data stored on the tape recorder was captured using a transient recorder (Datalab DL 901) and transferred to paper by an X–Y plotter (Bryans).

Some mice were pretreated with 6-hydroxydopamine in order to remove sympathetic innervation. The dose regimen was: 2 × 50 mg/kg I.P. on day 1; 2 × 100 mg/kg 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 in the mouse (Gibson & Wedmore, 1981).

In the tables, the mean ± S.E. of the mean are given.

Drugs used were noradrenaline bitartrate (Sigma); phentolamine mesylate (Ciba); cocaine hydrochloride (May and Baker); propranolol hydrochloride (ICI); prazosin hydrochloride (Pfizer); tetrodotoxin (Sigma); yohimbine hydrochloride (Sigma) and 6-hydroxydopamine hydrochloride (Sigma).

RESULTS

Ionophoretic application of noradrenaline

Ionophoresis of noradrenaline usually produced depolarization of the mouse anococcygeus muscle and Fig. 1 illustrates a series of depolarizations from one cell produced by the ionophoretic application of noradrenaline at room temperature. The responses usually were monophasic in shape and were characterized by a delay between the beginning of the ionophoretic pulse and the start of the depolarization; this latency was rarely less than 400 ms at room temperature and 100 ms at 31–34 °C (the temperature dependence of the time course will be discussed more fully later in the text). It was possible to obtain a dose-dependent relationship, as increasing the ionophoretic charge produced larger depolarizations, although doubling the pulse rarely doubled the amplitude of the response except sometimes with small (< 5 mV) depolarizations (e.g. see top two traces in Fig. 1). In ionophoretic studies it is common practice to express the sensitivity to the applied stimulant in terms of mV depolarization produced per nC charge passed through the ionophoretic electrode (ionophoretic charge = amplitude × duration of pulse). In the present experiments the sensitivity to noradrenaline was calculated from the smallest response (usually 3–5 mV) and values ranging from 12–150 mV/nC were obtained in innervated muscle.

There were occasions when depolarizations could not be obtained, although small localized contractions could be observed demonstrating that the ionophoretic pulse had successfully ejected noradrenaline from the micropipette. In one survey where twenty-nine cells were tested, depolarizations were seen in twenty-four cells although

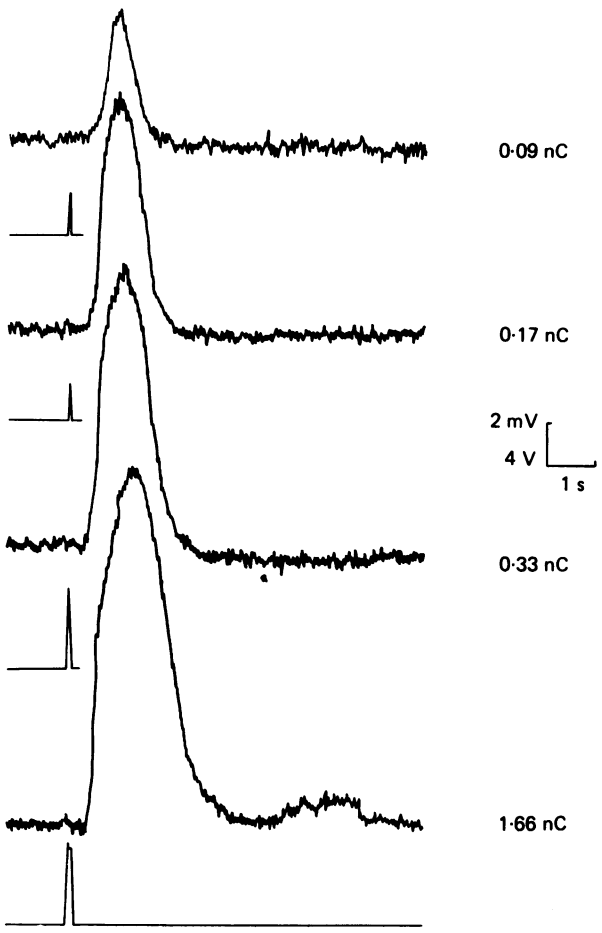

Fig. 1. Dose-related responses of the mouse anococcygeus muscle to ionophoretically applied noradrenaline. In each pair of traces the upper record is the membrane potential and the lower record monitors the voltage applied to the ionophoretic micro-electrode tip. Membrane potential (E_m), −62 mV, room temperature. The records of the voltage applied to the ionophoretic electrode appear triangular because the ionophoretic pulses are brief compared to the total sweep time and are limited by the frequency of the X–Y recorder.

all contracted. However, in some preparations depolarizations could be detected in all the cells.

The mouse anococcygeus muscle is densely innverated by sympathetic nerves (Gibson & Wedmore, 1981) so it is possible that the current passed through the ionophoretic electrode might have stimulated the noradrenergic fibres with the result that the depolarizations observed resulted from noradrenaline released from the

nerves rather than the micro-electrode. This seems unlikely as 10^{-7} M-tetrodotoxin (TTX) did not alter the sensitivity of the muscle to ionophoretically applied noradrenaline although this concentration of TTX totally abolished e.j.p.s evoked by field stimulation. Also when pipettes, filled with NaCl rather than noradrenaline, were used it was never possible to produce any depolarizations even with very large ionophoretic pulses. Finally when noradrenaline was applied to muscle in which the noradrenergic fibres had been destroyed with 6-hydroxydopamine it was still possible to produce depolarizations and the mean sensitivity to noradrenaline in two muscles was 200 ± 39 mV/nC ($n = 13$) which in fact was much larger than normally found in innervated tissue. In conclusion, the depolarizations produced by the ionophoretic application of noradrenaline are produced by noradrenaline released from the micro-electrode rather than from the noradrenergic nerves.

Responses to field stimulation

The amplitude of the e.j.p.s recorded at 33–36 °C was very variable as a single stimulus produced no response in some muscles, whereas in other preparations e.j.p.s of 25 mV in amplitude could be recorded in response to a single pulse (e.g. see Expt. 6 in Table 1). Much larger responses could be obtained by stimulating with short trains at high frequency, and Fig. 2 illustrates one experiment carried out at 34 °C. Record Fig. 2*A* was produced by a single pulse, Fig. 6*B*, *C* and *D* were evoked by field stimulation at 10, 20 and 30 Hz respectively for 150 ms. These larger responses had a similar time course to e.j.p.s evoked by a single stimulus, although at room temperature the time to peak was a little shorter for the larger responses. Table 1 describes the characteristics of e.j.p.s evoked by single stimuli and two stimuli at 10 Hz recorded from the same cells in various muscles; it was only possible to evoke e.j.p.s by a single stimulus in two out of eighteen muscles at room temperature so the data are a little sparse. Sometimes field stimulation at 33–36 °C produced more complicated responses as sometimes hyperpolarizations were observed and a mixture of depolarization and hyperpolarization could be recorded but these responses were not included in the analysis of the time course of the e.j.p.s. In experiments designed to study the time course or pharmacology of the e.j.p.s the tissue was stimulated at 10 Hz for 150 ms as these larger responses were easier to measure than the small depolarizations usually observed with a single stimulus.

Comparison of the time course of the ionophoretic responses and e.j.p.s

It was of interest to compare the e.j.p.s with the ionophoretic responses. Fig. 3 illustrates an e.j.p. and an ionophoretic response to noradrenaline recorded from the same cell at room temperature, and the two depolarizations have a similar time course. As with the ionophoretic responses, there is a latency between the stimulus artifacts and the onset of the e.j.p. and more details on the time course are given in Table 2. At 33–36 °C it was not always easy to detect the latency of the e.j.p.s as the stimulus artifacts obscured the base line and the latency is much shorter at these higher temperatures, and consequently the total time from the first artifact to the peak response was measured. Responses of similar amplitude were used for comparison in Table 2 because of the dependence of the response latency on amplitude. At room temperature (Table 2) the latency of the e.j.p.s was significantly longer than that of

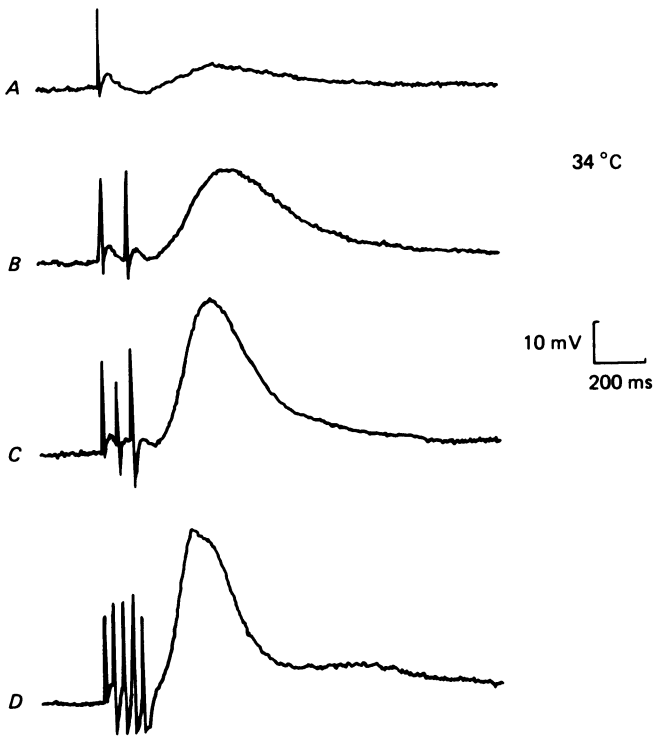

Fig. 2. Effect of various parameters of field stimulation in evoking e.j.p.s. The frequencies used in *B*, *C* and *D* were 10 Hz, 20 Hz and 30 Hz respectively for 150 ms. All records from the same cell. E_m, −70 mV; temperature, 34 °C.

TABLE 1. Characteristics of excitatory junction potentials produced by field stimulation

	Single stimulus		2 stimuli at 10 Hz	
Experiment	Total time to peak (ms)	Amplitude (mV)	Total time to peak (ms)	Amplitude (mV)
		Room temperature		
1	1294±16	1·44±0·12	1094±28	7·19±1·71
2	1255±44	2·28±0·34	1167±40	7·52±1·46
		34–36 °C		
3	455±5	3·44±0·66	508±12·5	12·50±2·07
4	410±25	1·50±0·29	460±10·0	5·30±1·25
5	545±24·2	1·67±0·18	515±6·12	6·19±0·88
6	431±33·4	17·47±2·51	395±31·5	24·65±1·3

Mean values were obtained from at least six observations.

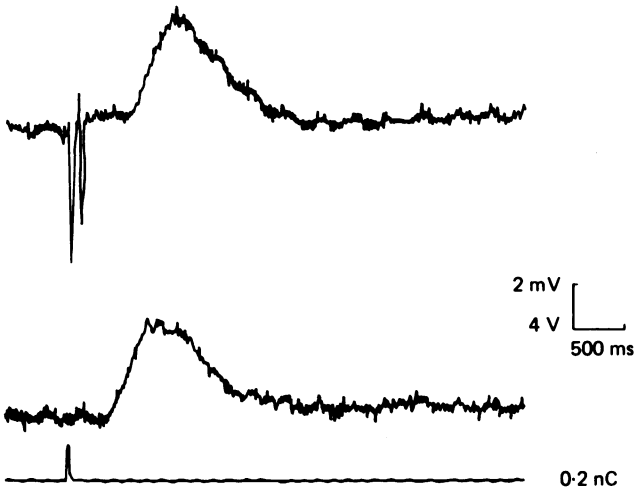

Fig. 3. Comparison of an e.j.p. and ionophoretic response to noradrenaline on the same cell. E_m, −65 mV; room temperature.

TABLE 2. Characteristics of responses produced by ionophoretic application of noradrenaline and of excitatory junction potentials produced by field stimulation (2 pulses at 10 Hz)

(*a*)

	Amplitude (mV) Room temperature	Latency (ms)	Rise time (ms)	Total time to peak (ms)
Ionophoretic responses	5·46 ± 0·51 (n = 12)	500 ± 41	544 ± 42	1044 ± 49
Excitatory junction potentials	6·17 ± 1·00 (n = 13)	646 ± 35*	626 ± 48	1272 ± 62*

(*b*)

	33–36 °C	
	Amplitude (mV)	Total time to peak (ms)
Ionophoretic responses	5·52 ± 0·85 (n = 15)	312 ± 19·3
Excitatory junction potentials	6·52 ± 1·23 (n = 14)	517 ± 13·9**

Statistically different from ionophoretic responses (* $P < 0.02$; ** $P < 0.001$).

Latency of e.j.p.s at room temperature was measured from first stimulation artifact to onset of stimulation.

Total time-to-peak of e.j.p.s was measured at 33–36 °C because the stimulation artifacts obscured the base line.

the ionophoretic response, but the rise times (onset of depolarization to peak response) were similar. This resulted in the total time to peak (latency and rise time) for the e.j.p.s being greater than the ionophoretically induced responses and this was also the case at 33–36 °C. Consequently the ionophoretic responses mimic the e.j.p.s quite successfully and since the duration of the ionophoretic pulse was short (1–50 ms) compared to the total time to peak (> 1 s) at room temperature, it is likely that the slow time course of the response is characteristic of the drug–receptor interaction which is confirmed in later experiments.

Pharmacology of the depolarizations

The pharmacology of the ionophoretically induced depolarizations are summarized in Table 3. In these experiments at room temperature the sensitivity of the muscle to ionophoretically-applied noradrenaline was estimated before and after addition of various drugs to the bathing solution. The α-blocking drug prazosin depressed markedly the responses to noradrenaline, whereas the β-blocker, propranolol and the α_2-receptor antagonist, yohimbine, were without effect. These results suggest that the responses are mediated via receptors that have been termed α_1. It was possible to construct dose–response curves in the presence and absence of the α_1-blocking agents prazosin and phentolamine and these are shown in Fig. 4. The dose–response curve is shifted to the right in a roughly parallel fashion which suggest that the antagonism is of a competitive nature. Using the curves in Fig. 4 it is possible to obtain an estimate of the equilibrium dissociation constants (K_D) for prazosin and phentolamine which are 10^{-9} and 10^{-8} M respectively. Interestingly, these values are an order lower than the values obtained when contractions are measured in the organ bath (A. Gibson, private communication).

Cocaine, a drug that inhibits neuronal uptake of noradrenaline, did not alter the sensitivity of the muscle to ionophoretically-applied noradrenaline. The sensitivity of the tissue in the absence and presence of 2 μM-cocaine was 33·7 and 36·4 mV/nC respectively at room temperature (Table 3).

In the organ bath, where contractions were studied, this concentration of cocaine at room temperature shifted the dose–response curve to the left (A. Gibson, private communication) in a parallel manner, the dose ratio being 1·9. If a similar shift to the left occurred in the present ionophoretic responses, then the sensitivity should have increased to 48·9 mV/nC (calculated from the control sensitivity and the control curve in Fig. 4). Clearly, the actual value obtained in the presence of cocaine is closer to the control value than the expected sensitivity.

At higher temperatures when the noradrenaline uptake system is more active (Green & Miller, 1966; Sachs, 1970), cocaine still does not alter the sensitivity to noradrenaline. In three experiments the mean control sensitivity to noradrenaline at 36 °C was $50{\cdot}8 \pm 14{\cdot}9$ mV/nC ($n = 9$) compared to $49{\cdot}3 \pm 10{\cdot}4$ mV/nC ($n = 16$) in the presence of 2×10^{-6} M-cocaine. In the organ bath experiments the shift to the left of noradrenaline dose–response curve is very large, the dose ratio being 10 (A. Gibson, personal communication). Thus it would seem that noradrenaline ejected from an ionophoretic electrode is not removed by the uptake mechanism, at least before it combines with the α-receptors.

The pharmacology of the e.j.p.s was similar to that of the ionophoretic responses, in that they were antagonized by α-blockers but were unaffected by the β-blocker

propranolol and, therefore, it is probable that the depolarizations induced by ionophoretically applied noradrenaline are mediated by the same receptors that are activated by noradrenaline released from the intrinsic nerves.

TABLE 3. Pharmacology of noradrenaline-induced depolarizations

	Muscle sensitivity to noradrenaline (mV/nC)	
	Control	Drug present
Prazosine (5×10^{-9} M)	$38{\cdot}9 \pm 9{\cdot}3$ ($n = 20$)	$4{\cdot}48 \pm 1{\cdot}06$ ($n = 23$)
Propranolol (2×10^{-6} M)	$80{\cdot}1 \pm 13{\cdot}2$ ($n = 10$)	$81{\cdot}9 \pm 16{\cdot}9$ ($n = 8$)
Yohimbine (2×10^{-6} M)	$54{\cdot}7 \pm 11{\cdot}3$ ($n = 10$)	$57{\cdot}5 \pm 8{\cdot}4$ ($n = 14$
Cocaine (2×10^{-6} M)	$33{\cdot}7 \pm 6{\cdot}3$ ($n = 12$)	$36{\cdot}4 \pm 5{\cdot}7$ ($n = 18$)

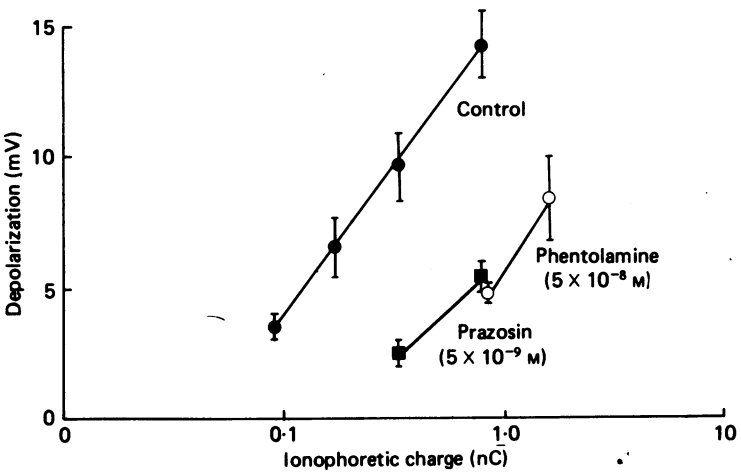

Fig. 4. Relationship between the ionophoretic charge applied to the noradrenaline micro-electrode and membrane depolarization in the absence and presence of the α-blockers prazosin and phentolamine. Each point is the mean of at least six measurements and the vertical bars represent the S.E. of the mean. Room temperature. Equilibrium dissociation constants were calculated from $K_D = X_b/(D_r - 1)$, where X_b is the antagonist concentration and D_r is the dose ratio calculated from the graph.

The latency of the depolarizations

One of the most interesting features of the e.j.p.s and ionophoretically-induced responses is the latency between the start of the pulse and the onset of the depolarizations. One of the main characteristics of this delay is that it can be reduced quite often by increasing the size of the ionophoretic charge as has been observed with ionophoretically applied ACh in taenia (Bolton, 1976). An example is shown in Fig. 5; when the ionophoretic charge was reduced in the lower record the latency is

increased as indicated by the arrow which indicates the beginning of the response. Table 4 summarizes results where ionophoretic responses and e.j.p.s have been grouped into 'bins' in terms of amplitude and the latencies of these different groups are compared. The latencies of the larger responses are statistically smaller than the latencies of the smaller depolarizations. A fuller analysis is illustrated in Fig. 6 and a similar relationship between response amplitude and latency exists for both the ionophoretic responses and the e.j.p.s.

TABLE 4. Relationship between amplitude and latency of depolarizations at room temperature

	Amplitude (mV)	Latency (ms)
Ionophoretic responses		
Depolarizations of 0–5 mV	3·86±0·26 (n = 19)	558±32**
Depolarizations of greater than 15 mV	19·0±0·69 (n = 21)	407±22**
Excitatory junction potentials		
Depolarizations of 0–3 mV	2·42±0·16 (n = 12)	715±36*
Depolarizations of greater than 9 mV	11·6±0·60 (n = 10)	579±27*

Statistically different * $P < 0·02$, ** $P < 0·01$.

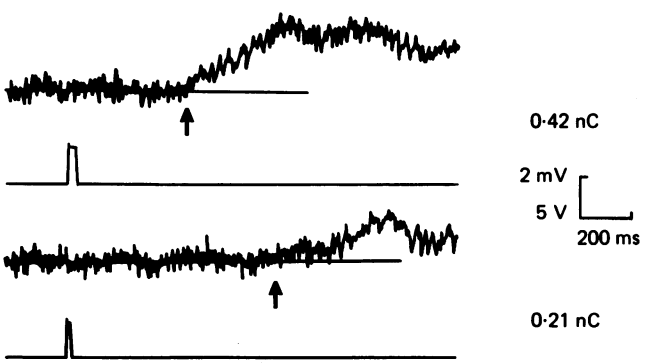

Fig. 5. Effect of pulse strength on the latency of the ionophoretic response. The horizontal straight line was drawn through the base line and the arrows point to the onset of depolarization. In the lower record the ionophoretic pulse was reduced by halving the duration.

One cause for the latency might be the presence of uptake or binding sites between the ionophoretic electrode and the receptors situated in the muscle membrane which would delay the diffusion of noradrenaline to the receptor sites. Table 5 summarizes the effect of cocaine and phentolamine on the latency of noradrenaline-induced responses. Neither drug reduced the latency and therefore neuronal uptake cannot account for the delay and if binding sites do exist, they cannot be blocked by phentolamine.

Effect of temperature on the time course of the ionophoretic responses

Despite these experiments there remains the possibility that the time course of the depolarization is governed by physical factors such as diffusion of the drug. One method of testing this is to study the effect of temperature on the time course of the depolarization as physical phenomena tend to be relatively insensitive to temperature

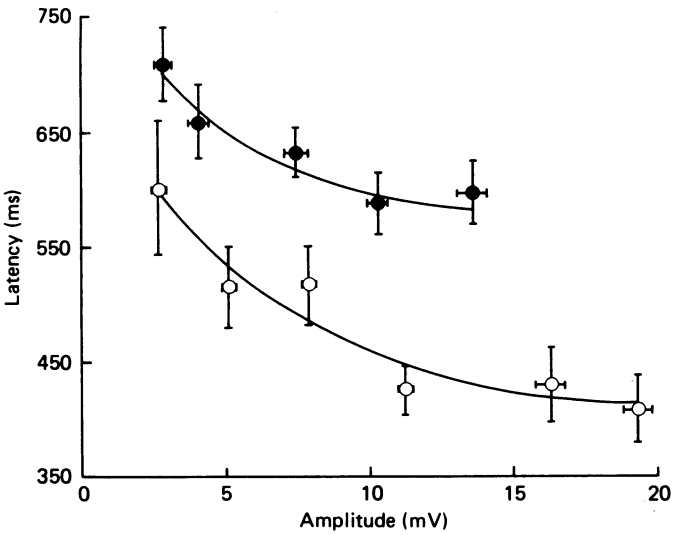

Fig. 6. Relationship between the amplitude of the depolarizations and latency. ●, e.j.p.s; ○, ionophoretic responses. Each point is the mean of at least twelve responses. Curves drawn by eye.

TABLE 5. Effect of drugs on the latency of the depolarization produced by the ionophoretic application of noradrenaline at room temperature

	Amplitude (mV)		Latency (ms)	
	Control	Drug	Control	Drug
Cocaine (2×10^{-6} M)	$3{\cdot}81 \pm 0{\cdot}47$ ($n = 8$)	$4{\cdot}45 \pm 0{\cdot}62$ ($n = 14$)	500 ± 27	482 ± 28
Phentolamine (5×10^{-8} M)	$10{\cdot}40 \pm 1{\cdot}30$ ($n = 8$)	$8{\cdot}90 \pm 1{\cdot}20$ ($n = 10$)	500 ± 20	542 ± 32

In the experiments with phentolamine larger ionophoretic charges were used in order to obtain responses of similar amplitude to those obtained in normal Krebs solution.

($Q_{10} \sim 1{\cdot}3$–$1{\cdot}5$) whereas the rate of chemical reactions is affected quite strongly by changes in temperature ($Q_{10} > 2$; Taylor, 1924). Ionophoretically induced depolarizations were recorded at room temperature and at 33–36 °C in the same muscles from at least six cells at each temperature. Examples of responses are shown in Fig. 7. Records *A* and *B* are the same but with different time scales as are *C* and *D*. The most marked effect was that increasing the temperature reduced markedly the

latency of the responses and to a lesser extent decreased the rise-time of the depolarization, whereas the decay phase was affected much less. The results from six experiments are summarized in Table 6 (*a*). Because of the dependence of the latency on amplitude, care was taken to compare responses of similar amplitude at low and high temperatures. Q_{10}s shown in Table 6 (*b*) were calculated from the figures in Table 6 (*a*) assuming a linear relationship between temperature and any given parameter. There was a high dependence of the latency of the depolarizations on temperature,

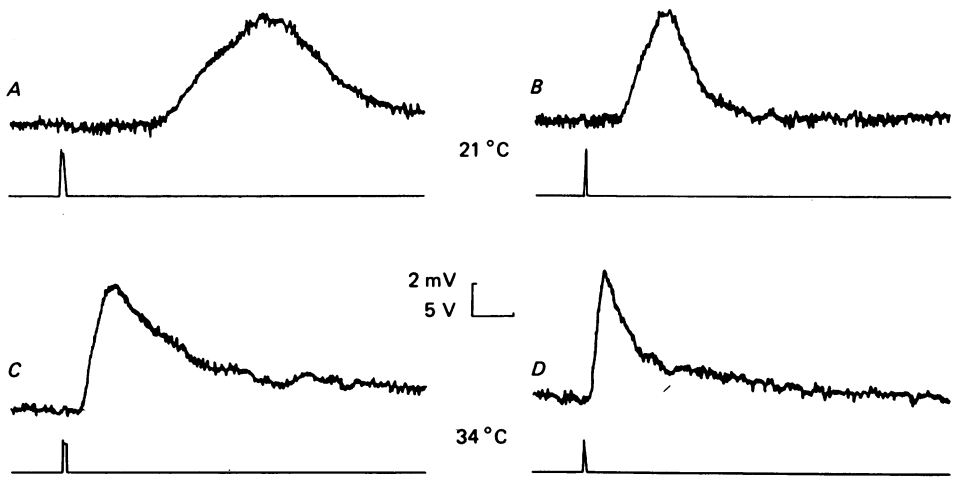

Fig. 7. Effect of temperature on the time course of the depolarizations produced by the ionophoretic application of noradrenaline. Traces *A* and *B* are the same, but on different time scales; the same applies to *C* and *D* which were recorded from another cell after the preparation had been warmed to 34 °C. Horizontal calibration, 200 ms for *A* and *C* and 500 ms for *B* and *D*. E_m, −64 mV for *A* and *B* and −68 mV for *C* and *D*.

the mean Q_{10} was 3·27 while the mean Q_{10} of the rise time was 2·08 suggesting that these characteristics of the responses are not governed by diffusion of noradrenaline in the vicinity of the receptors. In contrast, the mean Q_{10} of the half-decay time was lower and it seems unlikely that this process is governed by a chemical reaction, but may be determined by other processes such as the membrane time constant.

DISCUSSION

The results described in this paper show that the ionophoretic application of noradrenaline to the mouse anococcygeus muscle produces dose-related depolarizations. The fact that ionophoretic application of noradrenaline did not always depolarize the muscle even though contractions could be seen deserves some comment. Experiments, comparable to the present ones were carried out by Hirst & Neild (1980, 1981) who made similar observations in guinea-pig arterioles. They found that ionphoresis of noradrenaline sometimes depolarized the smooth muscle

(which produced contraction only if the threshold potential for spike generation was reached) but on many more occasions contractions were initiated with no change in membrane potential. They suggested that there might be two types of adrenoceptors; the combination of noradrenaline with one type results in depolarization, whereas

TABLE 6. (*a*) Effect of temperature on the characteristics of the responses induced by ionophoretic application or noradrenaline and (*b*) Q_{10}s

(*a*)

Experiment	Amplitude (mV)	Latency (ms)	Rise-time (ms)	Half-decay time (ms)	Sensitivity (mV/nC)
			20–22 °C		
1	4·60 ± 0·91	616 ± 69	393 ± 20	420 ± 54	8·97 ± 1·49
2	5·90 ± 0·58	653 ± 48	640 ± 69	550 ± 50	8·48 ± 0·73
3	5·20 ± 0·54	466 ± 25	734 ± 50	600 ± 55	6·96 ± 2·12
4	4·61 ± 0·84	408 ± 25	648 ± 52	677 ± 84	6·57 ± 1·55
5	13·40 ± 1·86	316 ± 21	487 ± 36	532 ± 81	50·85 ± 10·30
6	9·60 ± 1·44	416 ± 28	484 ± 35	472 ± 86	21·70 ± 3·88
Mean ± S.E. of mean	7·22 ± 1·45	479 ± 53	564 ± 53	542 ± 37	17·26 ± 7·10
			33–36 °C		
1	6·05 ± 0·68	120 ± 9·0	185 ± 15	267 ± 35	16·14 ± 2·62
2	5·33 ± 0·60	150 ± 7·0	232 ± 13	360 ± 0·90	7·63 ± 0·64
3	6·60 ± 0·56	106 ± 14	243 ± 23	303 ± 89	27·86 ± 3·65
4	5·85 ± 0·74	129 ± 19	196 ± 14	225 ± 38	23·90 ± 2·47
5	6·61 ± 0·97	93 ± 8·90	198 ± 16	247 ± 31	53·60 ± 10·15
6	9·50 ± 1·32	97 ± 11	222 ± 24	331 ± 20	24·02 ± 3·70
Mean ± S.E. of mean	6·65 ± 0·60	116 ± 8·82	213 ± 9·45	289 ± 21·1	25·53 ± 6·34

(*b*)

Experiment	Latency	Rise-time	Half-decay time	Sensitivity
1	3·95	1·63	1·21	1·55
2	3·22	2·04	1·13	0·89
3	3·26	2·24	1·47	2·28
4	2·43	2·54	(2·51)	2·17
5	2·83	2·05	1·79	1·05
6	3·90	1·98	1·30	1·10
Mean ± S.E. of mean	3·27 ± 0·24	2·08 ± 0·12	1·57 ± 0·21 (1·38 ± 0·12)	1·51 ± 0·24

The relation between the latency and the amplitude of the responses at 33–36 °C is similar to that at room temperature illustrated in Fig. 6. If the relation between a parameter and temperature was non-linear it is possible that the values of the Q_{10} might be over-estimated but in Expt. 6 (above) the temperatures were only 11 °C apart and on this occasion one of the highest Q_{10} values (3·90) was obtained.

the other type of receptor when activated can lead to contraction without depolarization. This explanation could account for the present results. However, the possibility that technical reasons account for the lack of depolarizations observed should not be excluded. For example, on those occasions the muscle might have been

damaged during impalement with the recording electrode which could markedly reduce the membrane resistance and hence transmembrane ion flow would not produce depolarization.

The receptors mediating depolarization are of the α_1 class as the responses could be blocked by prazosin and phentolamine, but not by propranolol or yohimbine. It is interesting that the depolarizations observed by Hirst & Neild (1980) in guinea-pig mesenteric arterioles could not be antagonized by phentolamine and therefore appear to be pharmacologically different from those in the mouse anococcygeus muscle.

It was surprising that the depolarization produced by the ionophoretic application of noradrenaline was briefer than the e.j.p., and this was largely accounted for by the shorter latency of the ionophoretic response, but part of the latency of the e.j.p. is due presumably to conduction of action potentials in the noradrenergic nerves. However, in a similar study, the inophoretically-induced depolarization was always slower than the e.j.p. (Hirst & Neild, 1981). The similarity of the time course of the e.j.p. and the ionophoretic response found in the present study can be explained by assuming that the ionophoretic electrode is as close to the muscle membrane as the nerve terminal. Some preliminary data from fluorescent micrographs does suggest that the varicosities are 500–1000 Å from the smooth muscle cell membrane which represents quite a wide synaptic gap (see Gabella, 1981). Also cocaine did not potentiate the responses to ionophoretically-applied noradrenaline either at room temperature or at 33–36 °C when the neuronal uptake mechanism is very active, as judged from the shift to the left of the dose–response curve for noradrenaline in organ bath experiments (Gibson & Wedmore, 1981). If noradrenaline released from an ionophoretic electrode had to diffuse past nerve terminals, presumably some would be taken up and less would reach the receptor and therefore inhibition of the uptake would increase the amount of noradrenaline able to bind to receptors. Consequently, it may be that in the present experiments the distance between the ionophoretic electrode and the smooth muscle membrane was not much greater than the synaptic gap.

Time course of depolarization induced by ionophoretically applied noradrenaline

The total duration of the noradrenaline response is 1–3 s at room temperature, even when relatively brief (as short as 1 ms) ionophoretic pulses are used. One of the most significant characteristics of this long time course is a delay between the start of the ionophoretic pulse and the first perceptible sign of depolarization (e.g. see Fig. 7). This type of latency has been observed in many other ionophoretic studies using different agonists, for example, muscarinic responses in cultured smooth muscle cells (Purves, 1974) guinea-pig taenia (Bolton, 1976) and mudpuppy parasympathetic ganglia (Hartzell, Kuffler, Stickgold & Yoshikami, 1977) and noradrenaline-induced responses in mammalian cardiac muscles (Reuter, 1974) cultured heart tissue (Hill-Smith & Purves, 1978) and guinea-pig arterioles (Hirst & Neild, 1980). Purves (1974) and Bolton (1976) have discussed in detail the time course of drug concentration after an iontophoretic pulse and even if the microelectrode tip was as far as 30 μm from the muscle membrane, noradrenaline would have reached its peak concentration 150 ms after the pulse (Hill-Smith & Purves, 1978) and since with small responses at room temperature the latency was as long as 900 ms, the free noradrenaline

concentration would have been negligible at the start of the depolarization. This argues against not only diffusion but also binding of noradrenaline to its receptors being the rate-limiting step in the time course of the response. The large Q_{10} of the latency, and to a lesser extent the rise time, also argues against diffusion being rate-limiting and moreover, this high temperature dependence rules out the possibility that the delay is governed by the membrane time constant as Lang (1979) found that the Q_{10} of the half-time of the electronic potential in guinea-pig ileum is about 1·4. The delay is not due to neuronal uptake or binding to α-receptors as cocaine and phentolamine did not reduce the latency. In conclusion, the most likely explanation is that the latency is a characteristic of events occurring after binding of noradrenaline to its receptors, and the α-receptor in the mouse anococcygeus muscle is another example of slow responding receptors.

This work was supported by the Medical Research Council.

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