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EFFECTS OF CATECHOLAMINES ON THE NEUROMUSCULAR JUNCTION IN THE RAT DIAPHRAGM

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SUMMARY

1. The effects of noradrenaline, adrenaline and isoprenaline on neuromuscular transmission in the rat diaphragm and the influence of adrenergic blocking agents on these actions were investigated.

2. The resting membrane potential of the muscle fibre was increased by adrenaline (5×10^{-6}–10^{-5} g/ml.) and isoprenaline (5×10^{-6} g/ml.) up to 3–4 mV, but noradrenaline (5×10^{-6}–10^{-5} g/ml.) had little effect.

3. The amplitude and the half-decay time of the end-plate potential (e.p.p.) were increased by noradrenaline (1×10^{-6} g/ml.), adrenaline (1×10^{-7}–10^{-5} g/ml.) and isoprenaline (1–5×10^{-6} g/ml.). The potentiation of the amplitude of the e.p.p. was greater with noradrenaline than with adrenaline and isoprenaline.

4. Noradrenaline (5×10^{-6} g/ml.) increased the frequency of miniature end-plate potentials (m.e.p.p.), but not their amplitude. However, isoprenaline (5×10^{-6} g/ml.) increased the amplitude of m.e.p.p.s without change in frequency. Adrenaline (5×10^{-6} g/ml.) increased both frequency and amplitude of m.e.p.p.s.

5. Adrenaline (5×10^{-6} g/ml.) and isoprenaline (5×10^{-6} g/ml.) increased the input resistance of the muscle membrane. The effect was blocked by the β-blocker, pronethalol (2×10^{-6} g/ml.), but not by the α-blocker, phentolamine (2×10^{-6} g/ml.). Noradrenaline did not change the input resistance of the muscle fibre.

6. Noradrenaline (5×10^{-6} g/ml.) and adrenaline (5×10^{-6} g/ml.) augmented the extracellularly recorded end-plate current (e.p.c.), but they had no effect on the half duration, nor on the action current (a.c.) of the nerve terminal, nor on the synaptic delay. Isoprenaline (5×10^{-6} g/ml.) had no effect on any of these parameters. The actions of noradrenaline and adrenaline on e.p.c. were abolished by phentolamine (2×10^{-6} g/ml.), but not by pronethalol (2×10^{-6} g/ml.).

7. Adrenaline (5×10^{-6} g/ml.) and isoprenaline (5×10^{-6} g/ml.) enhanced

the amplitude of the acetylcholine potential elicited by iontophoretic application of acetylcholine. No such effect was produced by noradrenaline (5×10^{-6} g/ml.).

8. It was concluded that noradrenaline acts on the nerve ending increasing the release of transmitter, and that isoprenaline acts on the post-synaptic membrane enhancing the input resistance, while adrenaline has both presynaptic and post-synaptic actions. The effect on the nerve ending is concerned with the α-action, whereas that on post-synaptic membrane with β-action of the catecholamines.

INTRODUCTION

It is known that catecholamines act on the neuromuscular junction of the skeletal muscle and facilitate its transmission (Orbeli, 1923; Toida, 1940; Burn, 1945). However, there are some differences between the reports concerning the sites of these actions.

Hutter & Loewenstein (1955) concluded that in frog skeletal muscle the facilitation of neuromuscular transmission by sympathetic stimulation was post-junctional, because noradrenaline sensitized the motor end-plates to acetylcholine. On the other hand, Jenkinson, Stamenovic & Whitaker (1968) observed in frog skeletal muscle that noradrenaline changed neither the amplitude of the miniature end-plate potentials nor the iontophoretically evoked acetylcholine potential, although the amplitude of the end-plate potential and the frequency of the miniature end-plate potentials were increased. From these results they concluded that noradrenaline increased the release of acetylcholine from the nerve endings. In rat skeletal muscle, it was also suggested by Krnjevic & Miledi (1958*b*) that adrenaline increased the amount of acetylcholine released from the nerve terminal.

Recently, Hidaka & Kuriyama (1969) observed, in fish red muscle, that adrenaline and noradrenaline produced different effects. In the presence of noradrenaline the frequency of the miniature junction potentials was increased, and the junction potentials and currents produced by nerve stimulation were potentiated without any change in the post-junctional properties. On the other hand, adrenaline enhanced the size of the junction potential evoked either by nerve stimulation or by iontophoretic application of acetylcholine, and it also increased both the input resistance and the resting membrane potential of the muscle fibre. They concluded that noradrenaline acts on the nerve terminal membrane, while adrenaline acts on the post-synaptic membrane.

The first object of the present experiments was to investigate the sites of the action of noradrenaline, adrenaline and isoprenaline on the neuro-

muscular junction in the rat diaphragm. The second aim was to determine electrophysiologically which adrenergic receptor, α or β, is involved in the mechanism of these actions. For this purpose, the effect of an α-blocker, phentolamine, and a β-blocker, pronethalol, on the various actions of the catecholamines was studied.

METHODS

The left hemidiaphragm-phrenic nerve preparation was dissected from Albino rats of either sex weighing 100–150 g under ether anaesthesia as described by Liley (1956*a*). The diaphragm strip, 1·5–2·0 cm wide, was cut out along the direction of the muscle fibres with the phrenic nerve attached and mounted in a lucite chamber with a capacity of about 5 ml. The bathing solution was a modified Krebs solution containing (mM) Na^+ 137, K^+ 5·9, Mg^{2+} 1·2, Ca^{2+} 2·5, Cl^- 134, $H_2PO_4^-$ 1·2, HCO_3^- 15·5, glucose 11·5. It was equilibrated with a mixture of 97 % O_2 and 3 % CO_2 (pH 7·2 at 33° C).

In order to record the end-plate potential, neuromuscular transmission was partially paralysed by reducing the Ca^{2+} concentration (0·5–2·5 mM) and increasing Mg^{2+} (1·2–6·0 mM) or by adding D-tubocurarine chloride (10^{-7}–10^{-6} g/ml.). The temperature was kept between 32 and 34° C except in the experiments in which the action current of the nerve terminal and the end-plate current were recorded extracellularly, which was done at room temperature (18–22° C).

Electrodes for intracellular recording were glass micropipettes filled with 3 M-KCl and a resistance ranging from 10 to 30 MΩ. After d.c. amplification with a preamplifier (Nihon Koden Ltd., MZ-3B), responses were displayed on a dual beam oscilloscope (Nihon Koden Ltd., VC-7).

The phrenic nerve was stimulated with two platinum wires (diameter 0·2 mm) in paraffin oil. Stimulating pulses of 0·02–0·04 msec duration and supramaximal strength were applied at intervals of 2–4 sec.

In order to investigate the membrane resistance of the muscle fibre, two microelectrodes were inserted (within 50 μ of each other) into a single fibre, one for passing current and the other for recording.

The action current of the nerve ending and the end-plate current were simultaneously recorded with a single extracellular glass electrode which had a tip diameter of about 10–30 μ and was filled either with 1·5–2·0 M-NaCl or with silver wire in the general manner described by Katz & Miledi (1965). For these recordings, an a.c. amplifier with a time constant of 0·01–0·03 sec was used.

Acetylcholine was applied iontophoretically to measure the sensitivity of the postjunctional membrane. Spontaneous diffusion was controlled by an adjustable inward current as described by Krnjevic & Miledi (1958*a*). (−)-Noradrenaline-HCl (Sankyo Ltd.), adrenaline-HCl (Sankyo Ltd.) and (−)-isoprenaline-HCl (Nikkenkagaku Ltd.) were added to the solution in the bath or to the Krebs solution in the reservoir. Sometimes, (−)-adrenaline cryst. (Merck) was used. The final concentration of the drugs was 10^{-7}–5×10^{-5} g/ml. (about $5{\cdot}0 \times 10^{-7}$–$2{\cdot}5 \times 10^{-4}$ mM). The α-blocker phentolamine methyl sulphate (Ciba Ltd.) and the β-blocker pronethalol (I.C.I.) were added to the Krebs solution in the reservoir at the concentration of 2×10^{-6} g/ml. (about $8{\cdot}0 \times 10^{-6}$ mM).

RESULTS

Resting membrane potentials

The effects of the catecholamines and of their antagonists on the resting membrane potential of the muscle fibres were determined by random penetrations using various drug concentrations as far as possible in the same preparation. The results are summarized in Table 1.

Both adrenaline and isoprenaline hyperpolarized the membrane of the muscle fibre by 3–4 mV at relatively high concentrations (5×10^{-6} g/ml. or more). Noradrenaline produced almost no change in the resting membrane potential up to a concentration of 5×10^{-6} g/ml. However, at 5×10^{-5} g/ml., there was possibly hyperpolarization, although the results were not statistically significant on account of the relatively small number of fibres sampled. In Table 1, the membrane potential changes marked by asterisks were statistically significant by Student's t test. Phentolamine and pronethalol had no effect on the resting potential.

End-plate potentials

End-plate potentials (e.p.p.) were produced by phrenic nerve stimulation at intervals of 2–4 sec, in Ca-deficient Mg-rich solution or in the presence of D-tubocurarine (10^{-7}–10^{-6} g/ml.). Fig. 1 shows typical effects of the catecholamines and of their antagonists on the e.p.p.s. Noradrenaline (1×10^{-6} g/ml.). and adrenaline (1×10^{-6} g/ml.) markedly increased the amplitude while isoprenaline in the same concentration caused a moderate increase in the size of the e.p.p. The order of potency at the concentration of 1×10^{-6} g/ml. was noradrenaline > adrenaline > isoprenaline (see Table 2). However, the effects were very variable. In higher concentrations (1×10^{-5} and 5×10^{-6} g/ml.) the potentiation caused by adrenaline and isoprenaline appeared to be smaller than that observed in the lower concentration (1×10^{-6} g/ml. and 1×10^{-7} g/ml.).

The half-decay time of e.p.p.s was slightly prolonged to almost the same extent by all three catecholamines, as shown in Table 2.

Table 2 also shows the effects of α- and β-blockers on the amplitude and half-decay time of the e.p.p.s. Phentolamine (2×10^{-6} g/ml.) increased the amplitude of e.p.p. and slightly lengthened its time course, while pronethalol (2×10^{-6} g/ml.) had no effect on e.p.p. (see also Fig. 1).

Miniature end-plate potentials

The effects of the catecholamines on the frequency and the amplitude of the miniature end-plate potentials (m.e.p.p.) are shown as histograms with sample records of m.e.p.p.s in Figs. 2, 3 and 4. Noradrenaline (5×10^{-6} g/ml.) increased the frequency of m.e.p.p.s from 1·32/sec to

TABLE 1. Effects of catecholamines, phentolamine and pronethalol on the resting membrane potential of the muscle fibre measured 15 to 20 min after application of each agent. (mV ± S.D.), (* = $P < 0.001$, ** = $P < 0.05$). Number of fibres in brackets

Concentration (g/ml.)	1×10^{-7}	1×10^{-6}	2×10^{-6}	5×10^{-6}	1×10^{-5}	5×10^{-5}
Control	83·4 ± 5·7 (40)	81·0 ± 6·1 (80)	—	80·9 ± 3·0 (70)	82·0 ± 6·2 (40)	—
Noradrenaline	83·0 ± 6·9 (40)	79·9 ± 5·8 (80)	—	81·3 ± 4·2 (72)	84·6 ± 7·4 (40)	—
Control	80·6 ± 6·8 (40)	82·6 ± 6·3 (40)	—	76·3 ± 5·5 (80)	—	82·4 ± 7·0 (40)
Adrenaline	80·5 ± 5·6 (40)	84·0 ± 6·7 (40)	—	*80·6 ± 5·6 (80)	—	**85·6 ± 6·8 (40)
Control	77·9 ± 5·9 (40)	78·4 ± 6·1 (40)	—	78·5 ± 4·5 (192)	—	—
Isoprenaline	77·5 ± 5·5 (40)	77·6 ± 7·9 (40)	—	*82·2 ± 5·3 (180)	—	—
Control	—	85·4 ± 6·2 (50)	78·6 ± 4·6 (50)	—	—	—
Phentolamine	—	83·9 ± 5·9 (50)	80·1 ± 5·4 (50)	—	—	—
Control	—	—	81·3 ± 4·5 (100)	—	—	—
Pronethalol	—	—	81·6 ± 5·0 (130)	—	—	—

TABLE 2. Effects of catecholamines, phentolamine and pronethalol on the end-plate potentials

Mean values of amplitude and half-decay time 3–5 min after application of catecholamines, phentolamine and pronethalol as percentage of control (± S.D.). n = number of observations. P = probability in Student's t test

Concentration (g/ml.)		Amplitude (% ± S.D.)	n	$P <$	Half-decay time (% ± S.D.)	n	$P <$
Noradrenaline	1×10^{-6}	167·3 ± 44·7	17	0·001	108·7 ± 13·3	17	0·05
Adrenaline	1×10^{-7}	141·5 ± 6·1	2	0·05	111·5 ± 21·0	2	0·5 >
	1×10^{-6}	154·3 ± 36·3	8	0·01	111·5 ± 8·1	8	0·02
	1×10^{-5}	125·8 ± 5·1	4	0·01	118·0 ± 7·1	4	0·05
Isoprenaline	1×10^{-6}	124·3 ± 19·9	8	0·02	108·4 ± 6·3	8	0·02
	5×10^{-6}	112·0 ± 0	2	—	117·5 ± 3·5	2	0·2
Phentolamine	2×10^{-6}	130·7 ± 8·4	3	0·05	120·0 ± 13·4	3	0·2
Pronethalol	2×10^{-6}	102·7 ± 4·5	3	0·5	102·3 ± 9·7	3	0·5 >

2·92/sec, while the mean amplitude remained almost unchanged (Fig. 2). Similar results were obtained from four different fibres.

These observations indicate that the action of noradrenaline is prejunctional, because the frequency of the m.e.p.p.s was increased but not the amplitude, in spite of the increase in the amplitude of the e.p.p. (cf. Fatt & Katz, 1952; Katz & Thesleff, 1957).

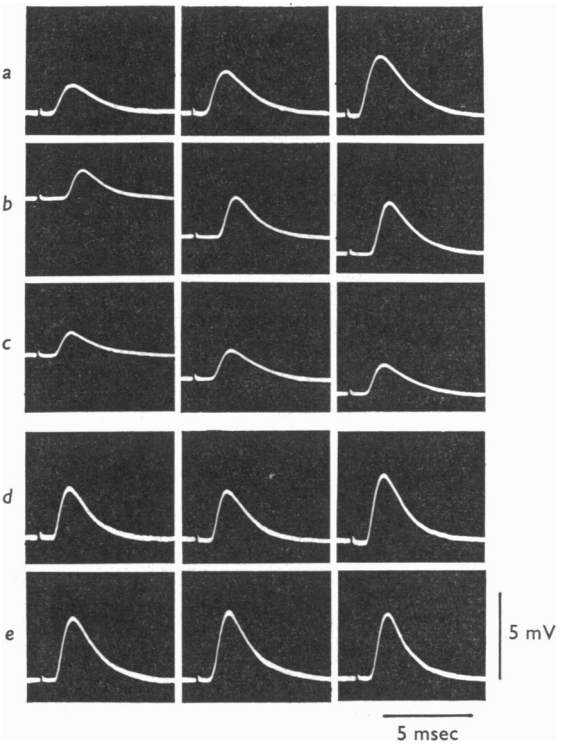

Fig. 1. Effects of catecholamines and of their antagonists on end-plate potentials in a solution containing D-tubocurarine 1×10^{-6} g/ml. *a*, noradrenaline 1×10^{-6} g/ml. *b*, adrenaline 1×10^{-6} g/ml. *c*, isoprenaline 1×10^{-6} g/ml. *d*, phentolamine 2×10^{-6} g/ml. *e*, pronethalol 2×10^{-6} g/ml. in five different preparations. First record, control; second, 5 min and third, 10 min after application of drugs taken from the same end-plate.

There was also a moderate increase in the frequency of the m.e.p.p. accompanied by an enhancement of the mean amplitude after application of adrenaline (5×10^{-6} g/ml.) in five fibres. A typical adrenaline effect is shown in Fig. 3. The frequency of m.e.p.p.s was increased from 5·03/sec to 7·03/sec. At the same time, the mean amplitude of m.e.p.p.s was increased from 1·06 to 1·32 mV. Neither the increased frequency nor the occurrence

of exceptionally large m.e.p.p.s can be the reason for the increased amplitude, because the difference between the mean amplitude excluding m.e.p.p.s larger than 2·1 mV with and without the presence of adrenaline

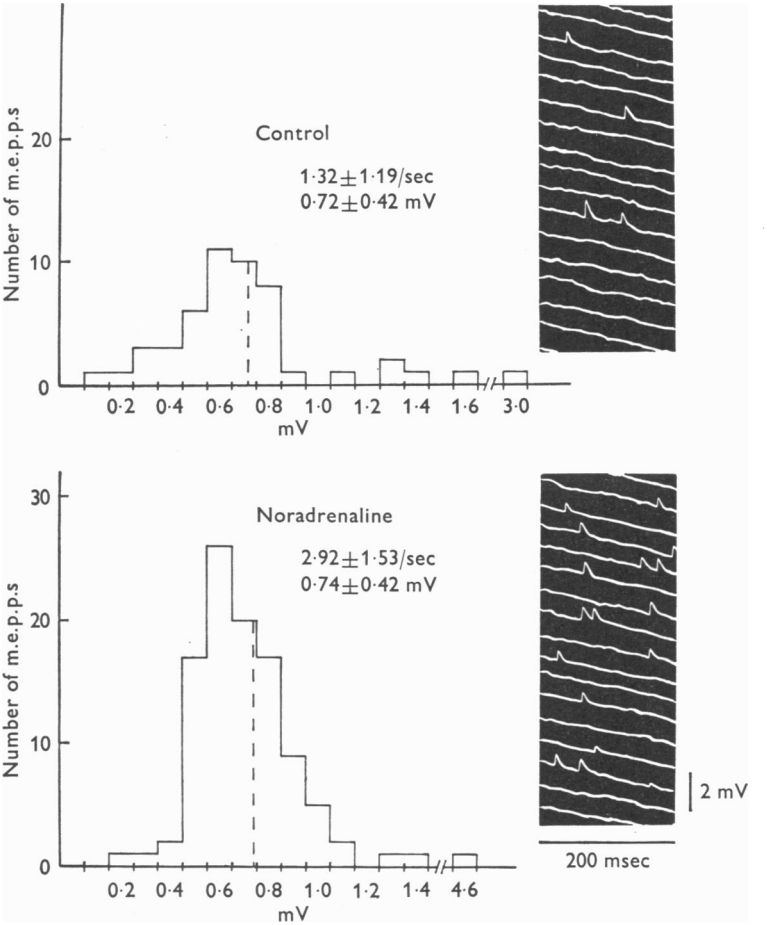

Fig. 2. Effects of noradrenaline 5×10^{-6} g/ml. on the amplitude and numbers of miniature end-plate potentials (m.e.p.p.s) recorded in normal Krebs solution during a 40 sec period. Right-hand side: sample records of m.e.p.p.s. Left-hand side: histograms of m.e.p.p.s. Results were obtained 5 min after noradrenaline application. The dashed lines indicate the mean amplitude of m.e.p.p.s (± S.D.) on the abscissa which was unchanged. The increase in frequency from 1·32/sec to 2·92/sec is significant ($P < 0·001$).

was statistically significant ($P < 0·05$). In the particular experiment shown in Fig. 3, in addition to the increase of the amplitude of m.e.p.p.s, adrenaline increased the frequency of exceptionally large m.e.p.p.s.

However, this was not always the case. Further experiments are necessary to determine if this action is significant or not.

A small augmentation of the amplitude of the m.e.p.p.s was also caused by isoprenaline (5×10^{-6} g/ml.) as shown in Fig. 4. However, the frequency of m.e.p.p.s was not significantly increased (three fibres). Since the increase in the size of the e.p.p. by adrenaline and isoprenaline was accompanied

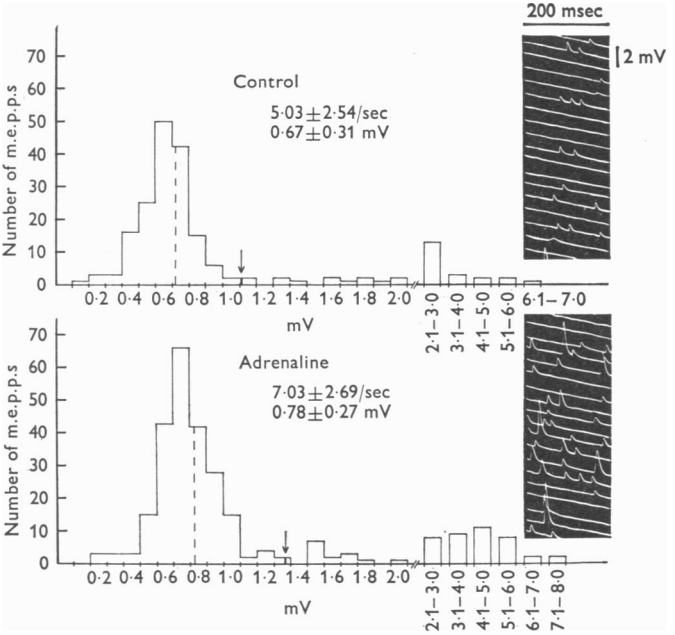

Fig. 3. Effects of adrenaline 5×10^{-6} g/ml. (5 min) on the amplitude and number of m.e.p.p.s recorded in normal Krebs solution during a 40 sec period. Right-hand side: sample records of m.e.p.p.s. Left-hand side: histograms of m.e.p.p.s. The continuous lines with arrows are the mean amplitudes of all m.e.p.p.s, $1{\cdot}06 \pm 1{\cdot}24$ (S.D.) mV and $1{\cdot}32 \pm 1{\cdot}44$ mV without and with adrenaline, respectively. The dashed lines and the values in the Figure are the mean amplitudes of the m.e.p.p.s excluding those larger than 2·1 mV. The increase in frequency is significant ($P < 0{\cdot}001$). The increase in amplitude is significant for all m.e.p.p.s ($P < 0{\cdot}05$) and also for the mean values in parenthesis ($P < 0{\cdot}001$).

by a parallel change in the amplitude of m.e.p.p.s, this may indicate a post-junctional action.

Phentolamine (2×10^{-6} g/ml.) increased the amplitude of the m.e.p.p. without changing the frequency as shown in Fig. 5. An increase in the amplitude of the m.e.p.p. is in accord with the augmentation of e.p.p. by treatment with phentolamine (Fig. 1). Since phentolamine had no effect

on the extracellularly recorded e.p.c. nor on the a.c. of the nerve ending (see Fig. 10), it may have some post-synaptic action.

Pronethalol (2×10^{-6} g/ml.) had no effect on the frequency and the amplitude of the m.e.p.p. as shown in Fig. 6. This agrees with the effect of

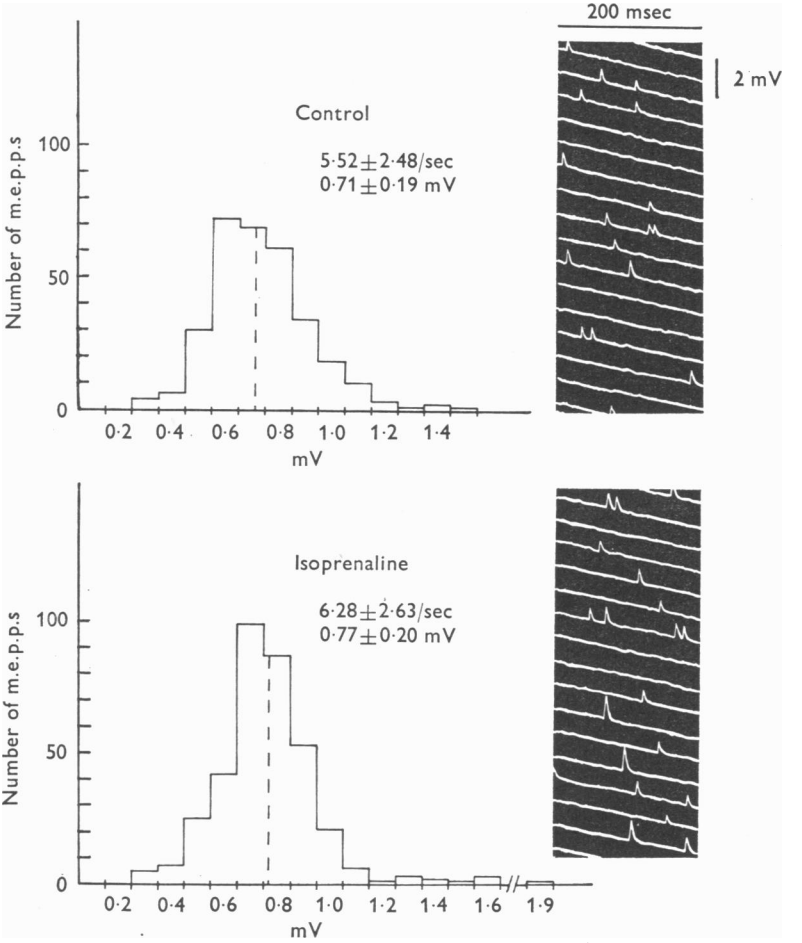

Fig. 4. Effects of isoprenaline 5×10^{-6} g/ml. on the amplitude and number of m.e.p.p.s recorded in normal Krebs solution during a 40 sec period. Right-hand side: sample records of m.e.p.p.s. The increase in the mean amplitude (± S.D.) is significant, ($P < 0{\cdot}001$). There is no increase in frequency.

pronethalol observed on the e.p.p. (Fig. 1), the e.p.c. and a.c. of the nerve ending (see Fig. 10). Therefore, it might be concluded that pronethalol has no effect on the neuromuscular transmission of the rat diaphragm.

The input resistance of the muscle fibre

The input resistance of the muscle varied greatly from fibre to fibre in the range between 3·75 and 9·42 $\times 10^5$ Ω. For this reason, the input resistance, after application of catecholamines, was always compared with the control in the same fibre. The current–voltage relationship was obtained from the amplitudes of the electrotonic potentials measured at the end of

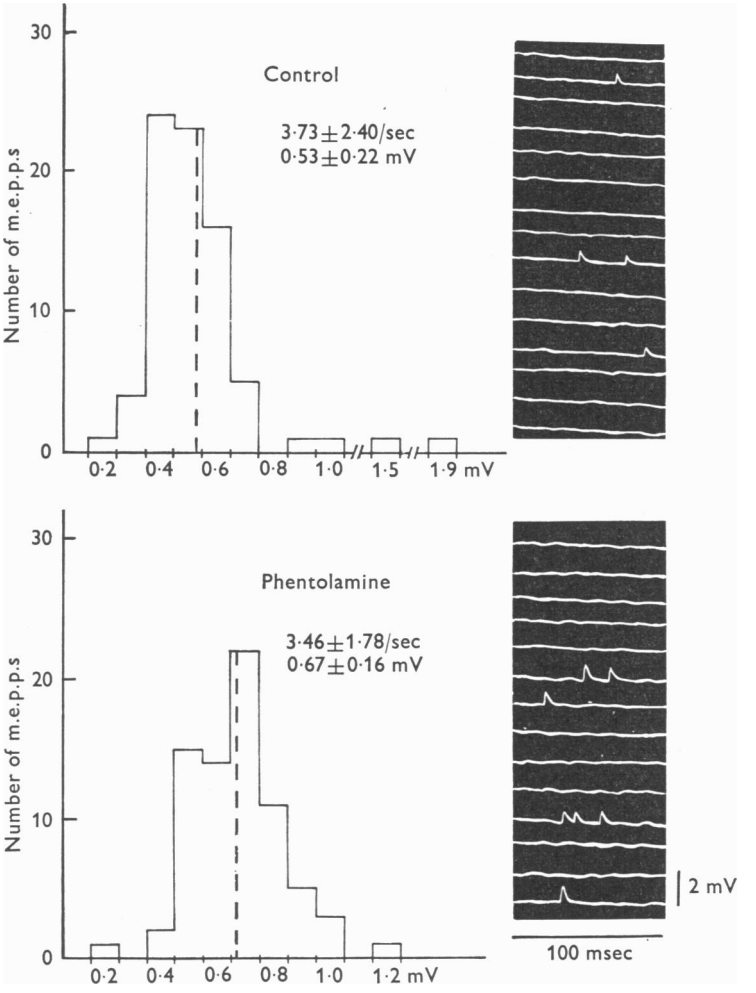

Fig. 5. Effects of phentolamine (2×10^{-6} g/ml.) on the amplitude and number of m.e.p.p.s recorded in normal Krebs solution during a 20 sec period. Illustrations are the same as in Fig. 4. Results were obtained 5 min after application of phentolamine. The increase in m.e.p.p. amplitude is significant ($P < 0{\cdot}001$).

the current pulses. Adrenaline (5×10^{-6} g/ml.) increased the input resistance of the muscle membrane as shown in Fig. 7. Isoprenaline (5×10^{-6} g/ml.) also increased the input resistance, but less than adrenaline, while noradrenaline (5×10^{-6} g/ml.) had no clear effect on the input resistance (Table 3).

The effects of phentolamine and pronethalol on the action of adrenaline are also shown in Table 3. The increase in the input resistance caused by

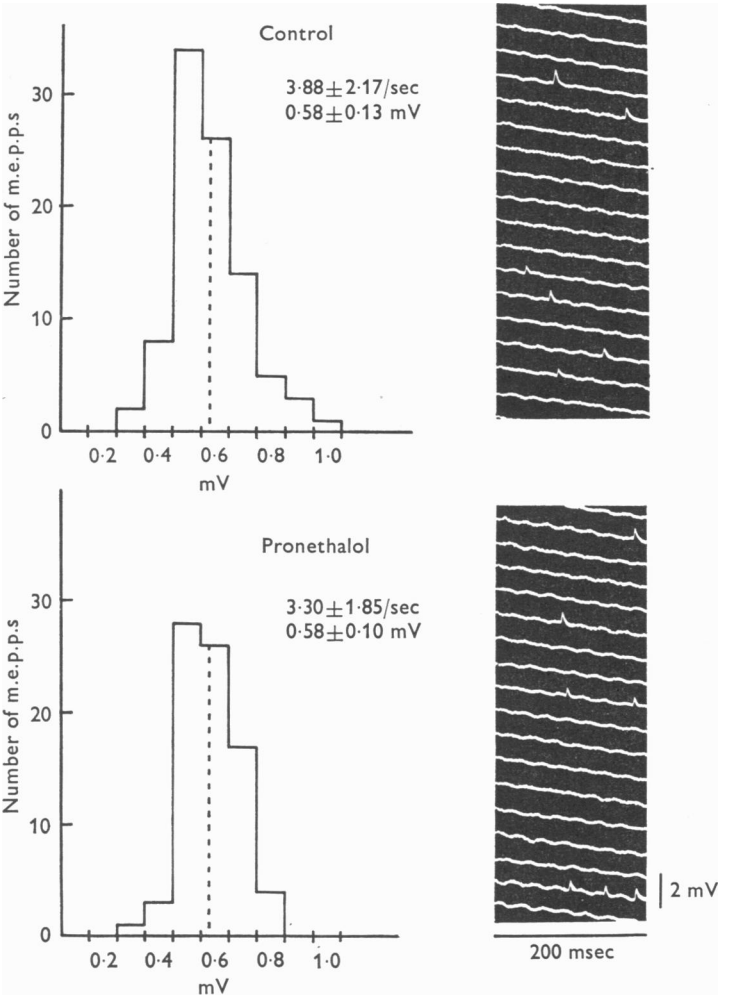

Fig. 6. Effects of pronethalol (2×10^{-6} g/ml.) on the amplitude and number of m.e.p.p.s recorded in normal Krebs solution during a 24 sec period. Illustrations in the Figure are the same as in Fig. 4. Results were obtained 5 min after application of pronethalol.

TABLE 3. Effect of catecholamines on the input resistance of the muscle membrane, and effect of phentolamine and pronethalol on the action of adrenaline

In the lower two series of experiments, the action of adrenaline 5×10^{-6} g/ml. was observed in the presence of phentolamine, 2×10^{-6} g/ml., or pronethalol, 2×10^{-6} g/ml. The values given are $\times 10^5\ \Omega$, and also calculated as percentage of control

No. of expts.	1	2	3	4	5	6	7	8	9	Mean ($\times 10^5\ \Omega$)
Control	9·41	9·13	6·90	6·76	5·24	5·30	4·42	4·00	3·78	6·10
Noradrenaline 5×10^{-6} g/ml.	10·23	9·45	6·54	6·93	4·71	5·30	4·46	4·07	4·13	6·20
%	108·7	103·5	94·8	102·5	89·0	100	100·9	101·8	109·3	101·3 ± 4·8 (S.D.)
Control	7·35	6·15	5·43	5·43	5·24	—	—	—	—	5·92
Adrenaline 5×10^{-6}	8·16	7·73	6·39	6·21	6·00	—	—	—	—	6·90
%	111·0	125·7	117·7	114·4	114·5	—	—	—	—	116·7 ± 5·0
Control	6·00	4·89	3·89	3·75	3·76	—	—	—	—	4·46
Isoprenaline 5×10^{-6}	6·33	5·23	4·54	4·20	4·20	—	—	—	—	4·90
%	105·5	117·0	116·7	112·0	111·7	—	—	—	—	112·6 ± 5·8
Phentolamine 2×10^{-6}	4·36	3·29	3·21	—	—	—	—	—	—	3·62
+ Adrenaline 5×10^{-6}	5·68	3·65	4·10	—	—	—	—	—	—	4·48
%	130·3	110·9	127·7	—	—	—	—	—	—	123·0 ± 8·6
Pronethalol 2×10^{-6}	6·99	6·06	5·06	—	—	—	—	—	—	6·04
+ Adrenaline 5×10^{-6}	7·27	5·98	5·06	—	—	—	—	—	—	6·10
%	104·0	101·3	100	—	—	—	—	—	—	101·8 ± 1·30

adrenaline was not affected in the presence of phentolamine 2×10^{-6} g/ml. (Fig. 8*b*), but it was blocked by pronethalol 2×10^{-6} g/ml. (Fig. 8*a*).

End-plate current and action current of the nerve ending

End-plate currents and the action currents of the nerve endings were simultaneously recorded with an extracellular glass electrode at room temperature (18–24° C). Records are shown on the right-hand side of Fig. 9.

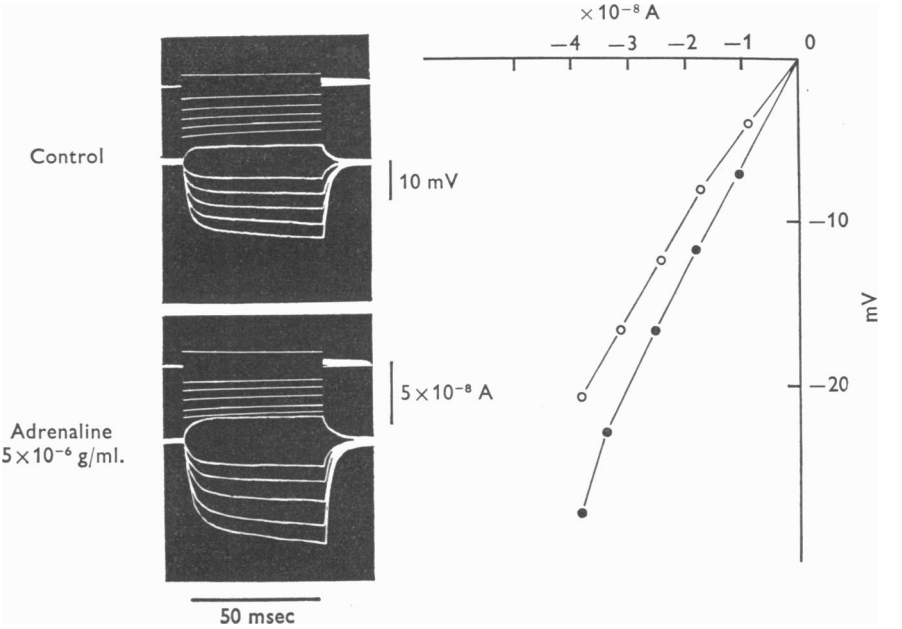

Fig. 7. Input resistance of muscle membrane. The current–voltage relationships before and 10 min after treatment with adrenaline 5×10^{-6} g/ml. are shown in the right-hand side. The input resistance in normal solution (○) was $5{\cdot}43 \times 10^5$ Ω, the input resistance 10 min after application of adrenaline (●), $6{\cdot}39 \times 10^5$ Ω. Left-hand side: electrotonic potentials evoked by five hyperpolarizing current pulses and one depolarizing pulse.

The first notch is the stimulus artifact. The second small downward deflexion is the action current of the nerve ending (a.c.) and the third large wave is the e.p.c. Both noradrenaline (5×10^{-6} g/ml.) and adrenaline (5×10^{-6} g/ml.) enhanced the amplitude of e.p.c.s 1·2–1·8 times, whereas isoprenaline (5×10^{-6}) had no effect. The potentiation of the e.p.c. was larger with noradrenaline than with adrenaline, which is consistent with the order of potency increasing the e.p.p. Since noradrenaline did not change the membrane resistance of the muscle fibre, these observations definitely indicate a presynaptic action, provided that the acetylcholine

sensitivity of the end-plate remains unchanged (Takeuchi & Takeuchi, 1959; Oomura & Tomita, 1961). The half duration of e.p.c.s measured at a half maximum amplitude and the amplitude of a.c.s was not affected by any of the catecholamines as illustrated on the left-hand side of Fig. 9.

The synaptic delays measured from the peak of the a.c. to the onset of the e.p.c. varied from 0·3 to 1·0 msec in different fibres and depended on the temperature. No significant difference could be detected between the synaptic delays before and after treatment with catecholamines (Fig. 9).

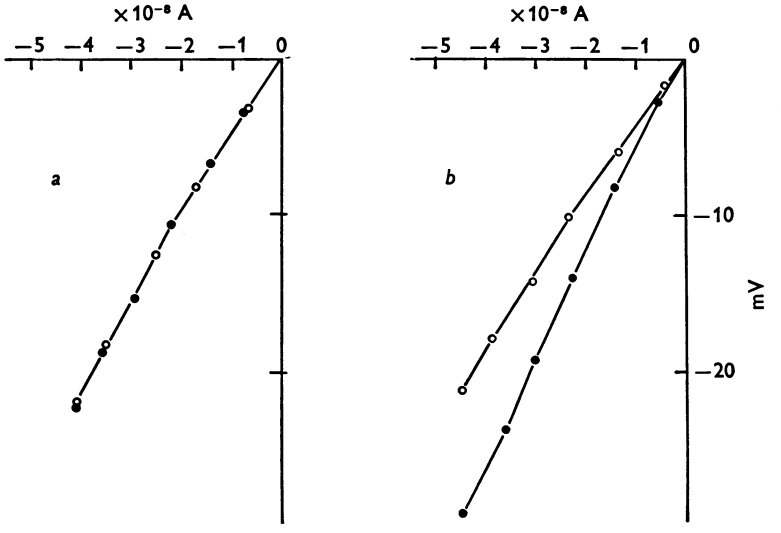

Fig. 8. The current–voltage relationships of the muscle fibre before and 10 min after treatment with adrenaline 5×10^{-6} g/ml. in the presence of pronethalol (*a*) or phentolamine (*b*) obtained in two different preparations. Either pronethalol or phentolamine had been added to the bathing solution at least 20–30 min before application of adrenaline. *a*, pronethalol 2×10^{-6} g/ml. (○), pronethalol 2×10^{-6} g/ml. + adrenaline 5×10^{-6} g/ml. (●); *b*, phentolamine 2×10^{-6} g/ml. (○), phentolamine 2×10^{-6} g/ml. + adrenaline 5×10^{-6} g/ml. (●).

Fig. 10 shows the effects of phentolamine and pronethalol on the action currents of the nerve endings and on the end-plate currents and their influence on the action of catecholamines. Neither phentolamine (2×10^{-6} g/ml.) nor pronethalol (2×10^{-6} g/ml.) themselves had any effect on e.p.c. and a.c. When noradrenaline (5×10^{-6} g/ml.) or adrenaline (5×10^{-6} g/ml.) was applied in the presence of phentolamine (2×10^{-6} g/ml.) the augmentation of the e.p.c. amplitude was almost abolished. However, in the presence of pronethalol (2×10^{-6} g/ml.), the e.p.c. amplitude was still

increased by noradrenaline (5×10^{-6} g/ml.) and by adrenaline (5×10^{-6} g/ml.), as in the normal solution.

It is of considerable interest that the increase in e.p.c. amplitude by noradrenaline and adrenaline is blocked by the α-blocker, while the increase of the input resistance of the muscle membrane by adrenaline and isoprenaline is blocked by the β-blocker.

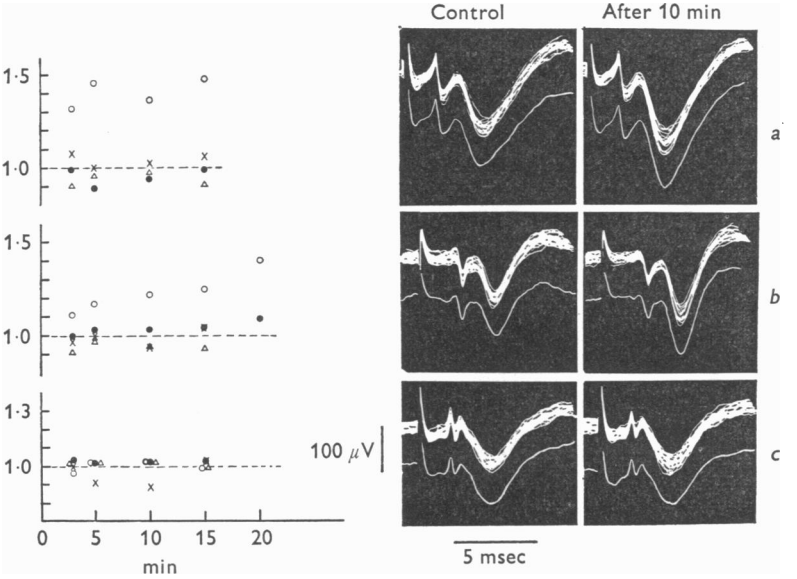

Fig. 9. Action currents (a.c.s) and end-plate currents (e.p.c.s) before and after treatment with catecholamines. Right-hand side: sample records. The upper traces are superimposed recordings of twenty sweeps, lower trace one sweep. *a*, noradrenaline 5×10^{-6} g/ml. *b*, adrenaline 5×10^{-6} g/ml. *c*, isoprenaline 5×10^{-6} g/ml. On the left-hand side the mean values of e.p.c. amplitude (○), e.p.c. half duration (●), a.c. amplitude (×), and synaptic delay (△) are plotted as multiples of the control values against the time of exposure from the same experiment as the records. Catecholamines were applied at the zero time. The solution contained Ca 1 mM and Mg 5 mM. Mean values of seven different experiments for *a*, six for *b* and four for *c*.

Acetylcholine potential

Fig. 11 shows the effects of catecholamines on the acetylcholine potentials evoked by iontophoretically applied acetylcholine. Both adrenaline (5×10^{-6} g/ml., five fibres) and isoprenaline (5×10^{-6} g/ml., three fibres) potentiated the amplitude of the acetylcholine potential to 1·2–1·5 times the control amplitude, while noradrenaline (5×10^{-6} g/ml., three fibres) had no effect. The increased response to acetylcholine caused by adrenaline

and isoprenaline is probably mainly due to the increased input resistance of the muscle membrane, because the externally recorded e.p.c.s were unchanged by isoprenaline, though adrenaline caused some potentiation.

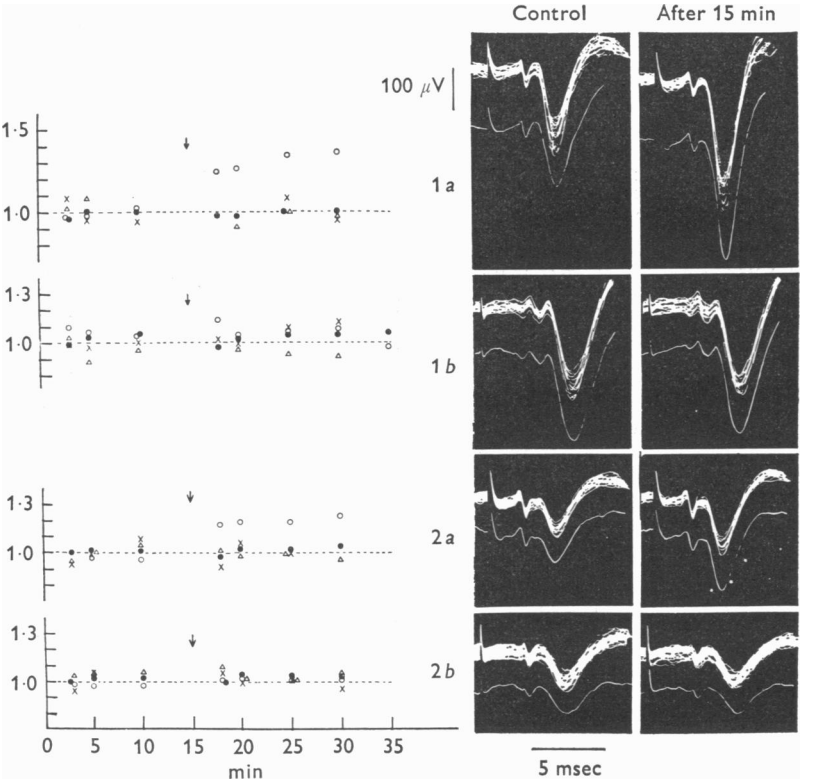

Fig. 10. The effects of phentolamine and pronethalol on a.c.s and e.p.c.s and the influences of these blockers on the action of noradrenaline and adrenaline. Illustrations as in Fig. 9. Records from four different preparations in solutions containing 1 mM-Ca and 5 mM-Mg. 1*a*, at the zero time, pronethalol (2×10^{-6} g/ml.) was applied; at the arrow, noradrenaline (5×10^{-6} g/ml.) was added to the solution containing phentolamine ($n = 3$). 1*b*, phentolamine (2×10^{-6} g/ml.) and noradrenaline (5×10^{-6} g/ml.) applied in the same way as in 1*a* ($n = 3$). 2*a*, pronethalol (2×10^{-6} g/ml.) and adrenaline (5×10^{-6} g/ml.) ($n = 6$). 2*b*, phentolamine (2×10^{-6} g/ml.) and adrenaline (5×10^{-6} g/ml.) ($n = 5$).

DISCUSSION

It can be concluded from the results obtained on the neuromuscular junction of the rat diaphragm, that noradrenaline acts mainly on the nerve terminal increasing the release of transmitter, that isoprenaline acts

solely on the muscle membrane increasing the input resistance with hyperpolarization of the membrane, and that adrenaline has both presynaptic and post-synaptic actions. The same site of the action of noradrenaline, facilitating transmitter release, has been suggested for frog skeletal muscle (Jenkinson *et al.* 1968), for fish red muscle (Hidaka & Kuriyama, 1969) and for the longitudinal somatic muscle (obliquely striated muscle) of the earthworm (Y. Ito & N. Tashiro, personal communication). In these

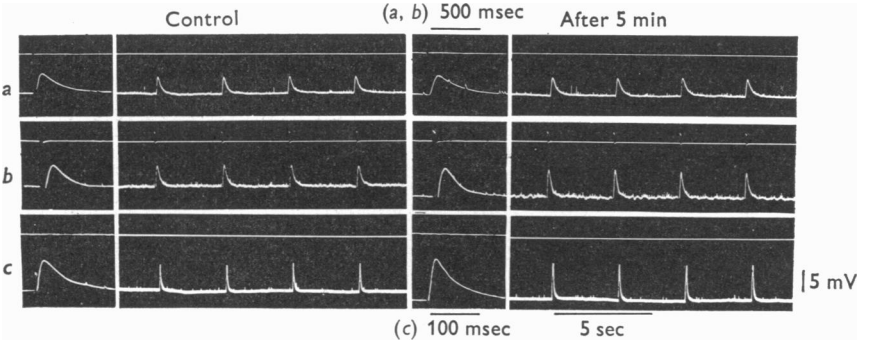

Fig. 11. Effects of catecholamines on acetylcholine potentials evoked by iontophoretically applied acetylcholine. *a*, noradrenaline 5×10^{-6} g/ml. *b*, adrenaline 5×10^{-6} g/ml. *c*, isoprenaline 5×10^{-6} g/ml. Intensity of current pulses was 1×10^{-7} A, and the duration was (*a*) 5 msec, (*b*) 8 msec, and (*c*) 1 msec. Three different preparations in normal solution.

muscles, noradrenaline increased the frequency of the m.e.p.p.s and increased the amplitude of the e.p.p. with little or no effect on the post-junctional membrane.

The presynaptic action of adrenaline in the rat diaphragm is in accordance with the results in the same preparation reported by Krnjevic & Miledi (1958*b*). However, in contrast to their finding that adrenaline has no effect on the post-junctional membrane, it was found in the present experiments that adrenaline increased both the membrane resistance and the resting potential of the muscle fibres. The cause of this difference is not known. In fish red muscle, Hidaka & Kuriyama (1969) demonstrated that the change in membrane resistance and resting potential contributed to the increase in the amplitude of the m.e.j.p. and e.j.p. Similarly, in the longitudinal muscle of the earthworm, adrenaline (10^{-5} g/ml.) enhanced the input resistance from 30–40 MΩ to 50–60 MΩ and hyperpolarized the membrane from -35 to -55 mV (Y. Ito & N. Tashiro, personal communication). This effect of adrenaline was not observed in Na-free solution and was blocked by propranolol (10^{-6} g/ml.), a β-blocking agent. Furthermore, in the longitudinal muscle of the earthworm the effect of isoprenaline

(1×10^{-6} g/ml.) on the post-junctional membrane was like that of adrenaline, as in the rat diaphragm.

It is known that noradrenaline has mainly an α-action, and that isoprenaline has a β-action, while adrenaline has both (Ahlquist, 1948). The potentiating action of noradrenaline on the amplitude of the end-plate current is blocked by phentolamine (α-blocker), while pronethalol (β-blocker) inhibits the effect of adrenaline and isoprenaline on the input resistance of the muscle fibre. Jenkinson *et al.* (1968) have also observed that, in frog skeletal muscle, the increase in the size of the e.p.p. caused by noradrenaline (10^{-5} M) is abolished by the α-blocker phentolamine (3×10^{-6} M) but not by the β-blocker pronethalol ($7{\cdot}5 \times 10^{-7}$ M). Furthermore, on the basis of recent experiments on mammalian skeletal muscle, Bowman & Raper (1967) postulated that adrenergic α-receptors are present in the motor nerve ending increasing transmitter release, while the β-receptors in the muscle fibres are associated with hyperpolarization of the muscle membrane.

Thus, in all the species so far studied (frog skeletal muscle, fish red muscle, earthworm longitudinal muscle and mammalian skeletal muscle including the rat diaphragm) the action of catecholamines can be explained by a presynaptic α-effect and a post-synaptic β-effect.

The mechanism by which the input resistance of the muscle membrane is increased has not been fully investigated in the present experiments. One possibility is that adrenaline and isoprenaline decrease the membrane permeability to Na ions.

Another possibility is suggested by the work of Somlyo & Somlyo (1969) who have reported that isoprenaline hyperpolarized the membrane of avian slow muscle by a few millivolts in normal solution. The hyperpolarization was not observed in solutions containing ouabain, or when Na was replaced by Li. It was therefore suggested that isoprenaline might stimulate an electrogenic Na pump. If this occurred also in the rat diaphragm, the increase in the resistance of the muscle fibre membrane might be a consequence of the hyperpolarization, rather than a reflexion of a permeability change.

The mechanism by which noradrenaline increases the release of transmitters from the nerve ending in the resting state and also by nerve impulses is still under investigation. It can, however, be said that the presynaptic actions of noradrenaline and adrenaline are unlikely to be due to a change of the resting potential of the nerve ending. If the increase in amplitude of the e.p.p. by noradrenaline were due to hyperpolarization of the nerve terminal (Hubbard & Willis, 1962; Takeuchi & Takeuchi, 1962) it would be difficult to explain the increase in the frequency of m.e.p.p.s since this is associated with depolarization of the nerve terminal (Liley,

1956*b*). Another reason for supposing that the membrane potential of the nerve ending is unchanged is the observation that catecholamines have no effect on the action current of the nerve ending. This presynaptic action of noradrenaline and adrenaline, i.e. potentiation of transmitter release without change in membrane potential of the nerve ending is similar to that of phenol on fish red muscle (Kuba, 1969).

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