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THE EFFECT OF ESERINE ON THE RESPONSE OF THE VAS DEFERENS TO HYPOGASTRIC NERVE STIMULATION

BY

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In experiments in which the vas deferens together with the hypogastric nerve of the guinea-pig was studied as an isolated preparation, the relation between frequency of electrical stimulation of the nerve and the height of contraction was determined. The optimal frequency was 20 shocks/sec. This value was the same when hyoscine was present to exclude the direct effect of any acetylcholine which might be released. When eserine was added to the bath in the presence of hyoscine, the response to stimulation at a frequency of 5 shocks/sec gradually increased until it became many times greater; the response to a frequency of 10 shocks/sec also increased, though to a less extent, but eserine decreased the amplitude of the contraction at a stimulus frequency of 20 shocks/sec. Neostigmine had the same effect as eserine. These findings indicate that, in the presence of hyoscine, eserine increased the noradrenaline released by low frequency stimulation, but decreased it for high frequency. If this is so, the results are compatible with the belief that there is a cholinergic link in the release of noradrenaline by hypogastric nerve stimulation.

The preparation of the vas deferens with the hypogastric nerve of the guinea-pig, for use in an isolated organ bath, was first made in 1959 by Huković, though his account was not published until 1961. Boyd, Chang & Rand (1960) showed that the contractions produced by stimulating the nerve were increased by eserine but were reduced in the presence of atropine. These observations suggested the presence of cholinergic fibres in the nerve. Boyd *et al.* (1960) also demonstrated that, even in the presence of atropine, eserine still increased the contractions. Since the contractions were gradually reduced and finally abolished by high concentrations of the anti-adrenaline agents, tolazoline (200 to 500 μg/ml.) and phenoxybenzamine (130 μg/ml.), it seemed likely that the chemical mediator was noradrenaline. These observations by Boyd *et al.* (1960) were incidental to the main part of their work, but in view of their importance we have repeated them, and in particular have studied the effect of eserine at different frequencies of stimulation and have investigated the effect of neostigmine.

METHODS

The isolated, innervated vas deferens preparation. Guinea-pigs, weighing 450 to 500 g, were killed by a blow on the head. The abdomen was opened in the midline and the distal colon was retracted to one side. The hypogastric nerves were identified and dissected free. The vas deferens on each side was cut from its attachments to the epididymis at one end and

the urethra at the other, and removed with its accompanying nerve. The preparation was mounted in a 150 ml. organ bath containing McEwen's (1956) solution at 29° C, through which a stream of 95% oxygen and 5% carbon dioxide was passed. The nerve was held in a stimulating electrode of the type described by Burn & Rand (1960); this consisted of a tube of 1 mm internal diameter containing two adjacent platinum rings. The electrode was submerged in the organ bath. Stimulation was carried out at 2 min intervals at known frequencies, the stimuli being rectangular, of 1 or 0.5 msec duration and of constant voltage. The stimuli were maximal. Contractions were recorded by an isotonic frontal writing lever, with a magnification of about five times.

For some experiments in which the direct effects of noradrenaline on the vas deferens were observed, a 10 ml. organ bath was used.

The drugs used include the following: eserine sulphate, hyoscine hydrobromide, neostigmine methylsulphate and atropine sulphate. Concentrations of these refer to the salts.

RESULTS

Effects of hyoscine at varying stimulus frequencies

The first experiments were directed to discovering the effect of hyoscine on the responses to low-frequency (10 shocks/sec) and high-frequency (40 to 50 shocks/sec) stimulation. In Fig. 1, the same number of pulses, 200, was used throughout, and these were given at the high rate of 50 shocks/sec for 4 sec, and at the low rate of 10 shocks/sec for 20 sec. The contraction at 50 shocks/sec was much

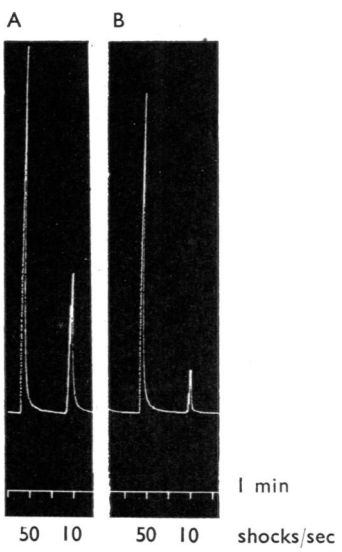

Fig. 1. Contractions of the isolated vas deferens of the guinea-pig, elicited by stimulation of the hypogastric nerve. Each stimulation was 200 shocks given either at 50 shocks/sec or at 10 shocks/sec. Between A and B hyoscine hydrobromide (0.05 μg/ml.) was added to the bath. It acted for 23 min during which stimulation continued at 2 min intervals. In B the contraction in response to 50 shocks/sec was 87% of that in A, while the contraction in response to 10 shocks/sec was only 30% of that in A.

greater than that at 10 shocks/sec (Fig. 1, A). These stimulations were repeated several times, with alternation of the two rates at intervals of 2 min. When a series of uniform responses at each rate had been obtained, hyoscine hydrobromide was added to the bath in a concentration of 0.05 μg/ml. This concentration reduced the response to stimulation at the lower rate, but had little effect on stimulation at the higher rate. Thus in Fig. 1, B, which shows the contractions 25 min after the addition of hyoscine, the response to stimulation at 10 shocks/sec was reduced to 27% of its former size whereas the response to stimulation at 50 shocks/sec was reduced only to 87% of its former size.

These results are summarized in Table 1. Concentrations of hyoscine from 0.05 to 1.0 μg/ml. had almost no effect on contractions in response to stimulus frequencies from 25 to 50 shocks/sec. The mean size of the contractions in the presence of hyoscine was 96% of the contractions before hyoscine was added. However, the

TABLE 1

EFFECTS OF HYOSCINE ON CONTRACTIONS OF THE VAS DEFERENS AT HIGH AND LOW STIMULUS FREQUENCIES

Expt.	Stimulus frequency per sec for 200 shocks		Concentration of hyoscine hydrobromide (μg/ml.)	Contraction after hyoscine (% of prior control)	
	High	Low		High frequency stimulation	Low frequency stimulation
1	50	10	0·05	87	41
2	40	10	0·1	104	24
3	30	12	1·0	95	56
4	50	10	0·05	90	57
5	50	10	0·1	95	45
6	25	5	0·05	108	34
7	32	8	0·2	90	45
Mean				96	43

same concentrations of hyoscine considerably reduced the responses to frequencies from 5 to 12 shocks/sec, the mean reduction being to 43%. Since the contractions in response to stimulations at 25 to 50 shocks/sec were unaffected by hyoscine, it was concluded that these contractions were almost entirely due to noradrenaline, whereas those in response to stimulations at 5 to 12 shocks/sec were considerably reduced in the presence of hyoscine, and were considered to be due in part to acetylcholine and in part to noradrenaline.

In several experiments we observed that, although the contractions in response to frequencies of 20 shocks/sec or more remained of the same height, those in response to lower frequencies, 10 or 5 shocks/sec, steadily declined in height although hyoscine had not been added to the bath. For example, after 22 min during which stimulations were carried out at intervals of 2 min, with groups of 200 shocks, alternately at 40 shocks/sec and 10 shocks/sec, there was no decline in the response to 40 shocks/sec, but that to 10 shocks/sec declined to about one-third of the initial size (Fig. 4, A and B). Hyoscine (0.1 μg/ml.) was then added to the bath and caused very little further reduction.

The relation between frequency and contraction amplitude

We determined the relation of stimulus frequency to height of contraction, and the results in three experiments are given in Table 2. In experiments 8 and 9 the greatest contraction was at a frequency of 20 shocks/sec, but in experiment 10 it

TABLE 2

RELATION OF FREQUENCY OF STIMULATION TO HEIGHT OF CONTRACTION

Stimulus frequency per sec for 200 shocks	Height of contraction (mm)		
	Expt. 8	Expt. 9	Expt. 10
5	22	0	8
10	64	31	34
20	122	84	69
25	117	81	71
40	115	77	77
50	110	70	74

was at a frequency of 40 shocks/sec. We have not seen a preparation in which maximal contractions were obtained at a frequency lower than 20 shocks/sec, but above this rate the optimal frequency varied. There was no diminution of the height of contraction with frequencies greater than the " optimal."

In one preparation the sequence of different frequencies was made random, and the mean size of contraction for each frequency was calculated. This was done before and after (Fig. 2) hyoscine was put in the bath. Only at a frequency of 10

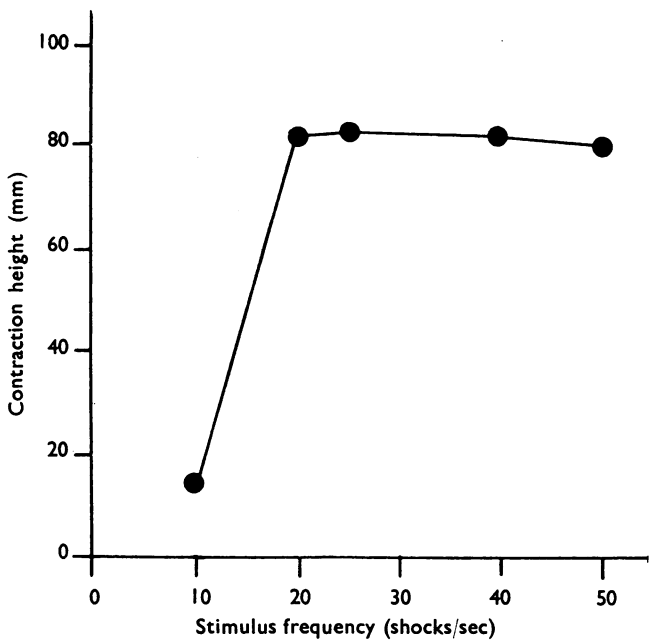

Fig. 2. The graph shows that when a fixed number of shocks were applied in the presence of hyoscine, the contractions (ordinate) increased in size as the stimulus frequency (abscissa) rose to 20 shocks/sec, but thereafter the contractions remained the same as the frequency rose to 50 shocks/sec. Each point was the mean of several contractions. The different stimulus frequencies were applied in random order.

shocks/sec was there any significant difference in the contractions with and without hyoscine; at this frequency in the absence of hyoscine the contractions were about twice the height of those with the drug. The presence of hyoscine did not alter the stimulus frequency required for the greatest contraction.

Effect of eserine

The next experiments were intended to repeat the finding of Boyd *et al.* (1960) that, in the presence of atropine, eserine increased the height of contraction caused by stimulation. These authors used a frequency of 10 shocks/sec, and added atropine in a concentration of 0.1 μg/ml. We used an atropine concentration of 1 μg/ml., and tested the response to acetylcholine (4 μg/ml.). Neither before eserine was added nor in its presence did acetylcholine cause contraction. When the frequency of stimulation was low, eserine increased the contraction, but failed to do so at higher frequencies (Table 3).

TABLE 3

INCREASE OF HEIGHT OF CONTRACTION CAUSED BY ESERINE IN THE PRESENCE OF ATROPINE

Expt.	Stimulus frequency per sec	Concentration of eserine (g/ml. $\times 10^{-5}$)	% increase in contraction
11	10	1·5	80
12	10	1·5	50
13	10	1·5	42
14	20	1·0	12
15	20	1·5	0
16	25	1·0	0
17	40	1·5	0

The increase in contraction might have been due to an effect of eserine in increasing the action of noradrenaline, and experiments were done to test this possibility. In the presence of atropine (1 μg/ml.), the contraction caused by noradrenaline (10 μg/ml.) was not increased by eserine, even in the concentration of 15 μg/ml. (Fig. 3). Therefore the effect of eserine could not be accounted for by potentiation

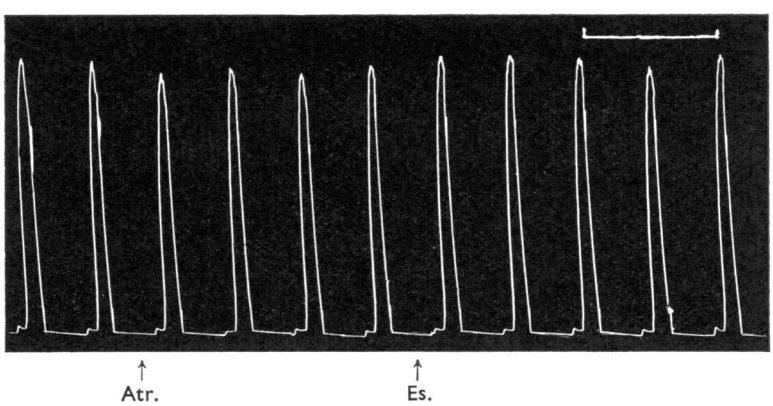

Fig. 3. Contractions in response to additions of noradrenaline to the bath to make concentrations of 10 μg/ml. The height of the contractions was not appreciably affected by adding atropine (1 μg/ml.) or, later, by adding eserine (15 μg/ml.) to the bath.

of the action of noradrenaline, but must have been due to stimulation releasing more noradrenaline in the presence of eserine.

The increased contractions in the presence of eserine were not seen at the higher stimulus frequencies (Table 3), and experiments were carried out to compare the effects of eserine at different stimulus frequencies.

One result is shown in Fig. 4. Each stimulation consisted of 200 shocks, given at 10 or at 40 shocks/sec. Fig. 4, A, shows the initial responses, and Fig. 4, B, those

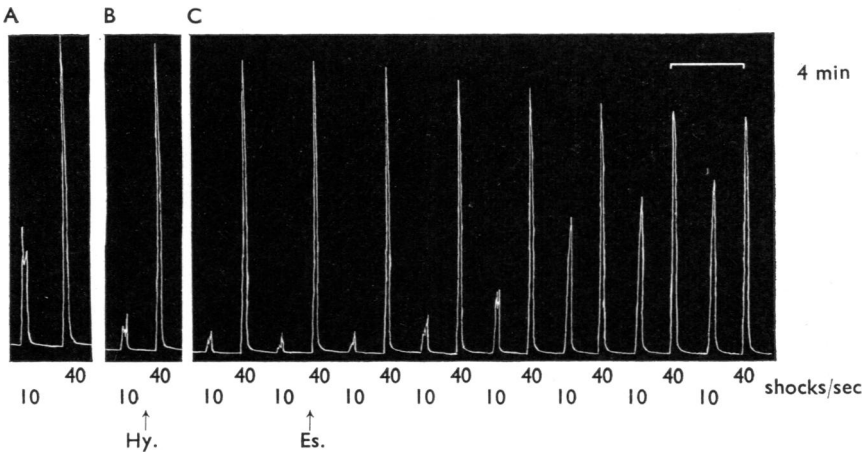

Fig. 4. A: control contractions caused by 200 shocks applied at the frequencies per sec shown. B: after 22 min the contraction caused by the lower frequency had declined, but that caused by the higher frequency had not altered. Hyoscine (0.1 μg/ml.) was added to the bath at the first arrow. C: 24 min later the contractions were similar to those in B. Eserine (5 μg/ml.) was added to the bath at the second arrow, and the effect of the lower frequency stimulation steadily increased. The effect of the higher frequency stimulation declined.

22 min later, stimulation having been given at 2 min intervals throughout this period. During the second record (Fig. 4, B) hyoscine (0.1 μg/ml.) was added. The third panel (Fig. 4, C) shows the responses 24 min later. Eserine (5 μg/ml.) was then added, the stimulation being continued as before. The height of the response to stimulation at 10 shocks/sec increased from 7 mm to 58 mm, while that to stimulation at 40 shocks/sec declined from 97 mm to 78 mm. Thus, before eserine, the contraction in response to stimulation at 10 shocks/sec was 7% of that in response to stimulation at 40 shocks/sec. When eserine had been present for 28 min the contraction at 10 shocks/sec was 73% of that at 40 shocks/sec.

In another experiment, stimulation with groups of 200 shocks was tested at three frequencies in the presence of hyoscine (0.1 μg/ml.). Fig. 5, A, shows the contractions before the addition of eserine, and Fig. 5, B, those 15 min after the addition of eserine. The contraction in response to stimulation at 5 shocks/sec was greatly increased; that in response to stimulation at 10 shocks/sec was slightly increased, and that in response to stimulation at 20 shocks/sec was diminished to about 60% of its previous height.

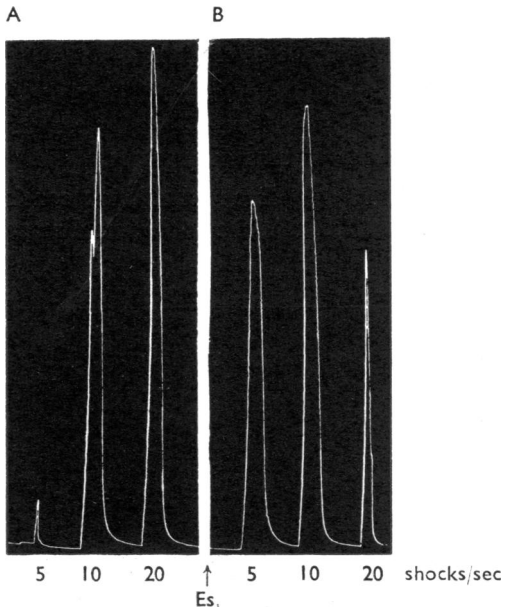

Fig. 5. A: contractions produced in the presence of hyoscine (0.1 μg/ml.) in response to 200 shocks at frequencies of 5, 10 and 20 shocks/sec. Eserine (5 μg/ml.) was then added to the bath and B shows contractions 15 min later. The contraction in response to 5 shocks/sec was greatly increased, that in response to 10 shocks/sec was slightly increased, while that in response to 20 shocks/sec was reduced.

The action of neostigmine

In another experiment in the presence of neostigmine (3 μg/ml.) the contractions in response to stimulations at 5 shocks/sec and 10 shocks/sec were increased, while those with 20 shocks/sec were decreased (Fig. 6).

DISCUSSION

Our results confirmed the findings of Boyd *et al.* (1960) that the contraction of the vas deferens caused by stimulation of the hypogastric nerve at 10 shocks/sec was increased by eserine even in the presence of atropine. The contraction was entirely due to noradrenaline. The effect of eserine was not due to some potentiation of the effect of noradrenaline itself, for in the presence of atropine the contractions caused by noradrenaline were not affected by eserine. Therefore the action of eserine in increasing the response to stimulation must have been due to an increase in the amount of noradrenaline released.

The addition of eserine or neostigmine to the bath caused the greatest increase in the response to stimulation when the frequency was 5 shocks/sec. The response at this frequency approximated more and more closely to the response at 20 shocks/sec in the absence of eserine. Thus it could be said that raising the frequency from 5 to 20 shocks/sec in the absence of eserine was equivalent to the effect of adding eserine with a stimulus frequency of 5 shocks/sec. Now the effect of adding eserine was to inhibit cholinesterase. It is clearly possible that the effect of raising the frequency from 5 to 20 shocks/sec was also an "anticholinesterase" effect in the

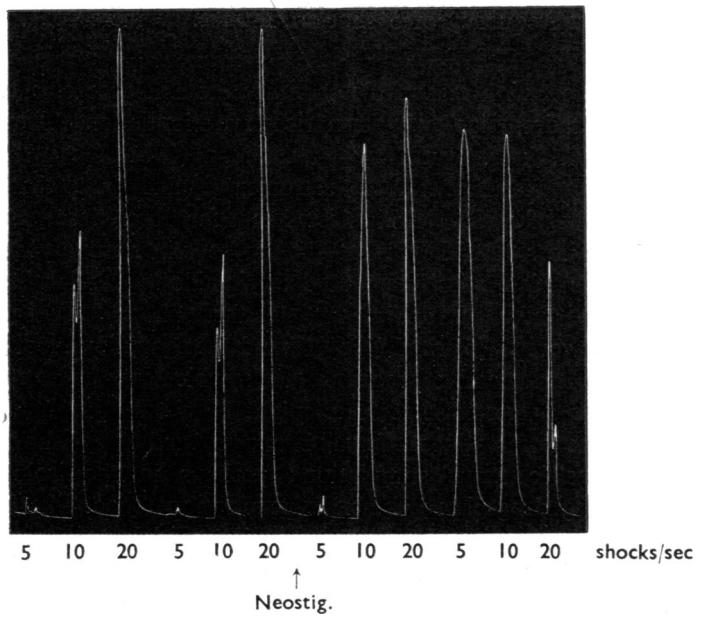

Fig. 6. Contractions produced by groups of 200 shocks in the presence of hyoscine (0.1 μg/ml.) at frequencies of 5, 10 and 20 shocks/sec. When each frequency had been used twice, neostigmine (3 μg/ml.) was added to the bath, and a change in contraction heights was seen almost at once. The contraction in response to 10 shocks/sec was increased and that in response to 20 shocks/sec was decreased. The next trial of 5 shocks/sec resulted in a very greatly increased contraction; the following contraction in response to 10 shocks/sec was increased a little more, while that in response to 20 shocks/sec was decreased still further.

sense that the time between successive pulses in which cholinesterase was able to act was diminished. If each pulse liberates so many quanta of acetylcholine, they will be destroyed by cholinesterase if a long period elapses before the next pulse comes; but with a higher frequency of stimulation, there will be less time for cholinesterase to act, and the concentration of acetylcholine will rise. On the assumption that a certain concentration of acetylcholine is required to release noradrenaline from a sympathetic postganglionic nerve ending, it can be understood why the optimal stimulus frequency of some sympathetic fibres is so high. Thus Gillespie & Mackenna (1961) used a frequency of 50 shocks/sec when stimulating the sympathetic fibres to the rabbit colon, but a frequency of 10 shocks/sec for the pelvic (parasympathetic) nerve. If a postganglionic fibre releases noradrenaline directly, it is hard to understand why a higher frequency should be required for the release of a more stable substance. The optimal stimulus frequency of different postganglionic fibres varies. This variation may depend on the amount of cholinesterase present, the optimal frequency being higher when the amount of cholinesterase is greater.

Eserine and neostigmine depressed the response to stimulation at 20 shocks/sec. There was a parallelism between the effect of these substances on the response to stimulation of the hypogastric nerve at different frequencies, and their action on the response of the rat diaphragm to stimulation of the phrenic nerve. Bülbring (1946)

showed that at a frequency of 5 shocks/min eserine increased the contractions of the diaphragm, but that at a frequency of 50 shocks/min eserine decreased them.

The results show with reasonable certainty the existence of a cholinergic link in the release of noradrenaline by hypogastric nerve stimulation. They do not, however, show where the link is. Burnstock & Holman (1961) measured the conduction velocity of the hypogastric nerve of the guinea-pig and found that it was 0.9 m/sec at a temperature of 35° C. This indicated that the fibres were sympathetic C fibres, and led the authors to believe that they were stimulating postganglionic fibres. (With intracellular electrodes they recorded " junction potentials " due to the release of noradrenaline, and it was of interest that these showed a considerable variation in latency.) However, Sjöstrand (1962) found that ganglion-blocking drugs such as hexamethonium and nicotine produced block, which was complete at low stimulus frequencies. He suggested that there might be a true ganglionic synapse at the hypogastric nerve terminations. Boyd, Chang & Rand (1961) showed that bretylium blocked hypogastric nerve stimulation; perhaps in the isolated vas deferens ganglion-blocking drugs have sufficient penetration to act like bretylium. There is no sharp distinction between hexamethonium and bretylium, for bretylium has a ganglion-blocking action (Boura & Green, 1959). It may be recalled that Sherif (1935) stimulated the hypogastric nerve to the uterus *in situ* of the dog and observed that transmission was not affected by nicotine given in successive doses until they had no further effect on blood pressure.

REFERENCES

BOURA, A. L. A. & GREEN, A. F. (1959). The actions of bretylium; adrenergic neurone blocking and other effects. *Brit. J. Pharmacol.*, **14**, 536–548.

BOYD, H., CHANG, V. & RAND, M. J. (1960). The anticholinesterase activity of some antiadrenaline agents. *Brit. J. Pharmacol.*, **15**, 525–531.

BOYD, H., CHANG, V. & RAND, M. J. (1961). The local anaesthetic activity of bretylium in relation to its action in blocking sympathetic responses. *Arch. int. Pharmacodyn.*, **131**, 10–23.

BÜLBRING, E. (1946). Observations on the isolated phrenic nerve diaphragm preparation of the rat. *Brit. J. Pharmacol.*, **1**, 38–61.

BURN, J. H. & RAND, M. J. (1959). Sympathetic adrenergic mechanism. *Nature* (*Lond.*), **184**, 163–166.

BURN, J. H. & RAND, M. J. (1960). The relation of circulating noradrenaline to the effect of sympathetic stimulation. *J. Physiol.* (*Lond.*), **150**, 295–305.

BURNSTOCK, G. & HOLMAN, M. E. (1961). The transmission of excitation from autonomic nerve to smooth muscle. *J. Physiol.* (*Lond.*), **155**, 115–133.

GILLESPIE, J. S. & MACKENNA, B. R. (1961). The inhibitory action of the sympathetic nerves on the smooth muscle of the rabbit gut, its reversal by reserpine and restoration by catechol amines and by DOPA. *J. Physiol.* (*Lond.*), **156**, 17–34.

HUKOVIĆ, S. (1961). Responses of the isolated sympathetic nerve-ductus deferens preparation of the guinea-pig. *Brit. J. Pharmacol.*, **16**, 188–194.

MCEWEN, L. M. (1956). The effect on the isolated rabbit heart of vagal stimulation and its modification by cocaine, hexamethonium and ouabain. *J. Physiol.* (*Lond.*), **131**, 678–689.

SHERIF, M. A. F. (1935). The chemical transmitter of the sympathetic nerve to the uterus. *J. Physiol.* (*Lond.*), **85**, 298–308.

SJÖSTRAND, N. O. (1962). Inhibition by ganglion-blocking agents of the motor response of the isolated guinea-pig vas deferens to hypogastric nerve stimulation. *Acta physiol. scand.*, **54**, 306–315.