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THE EFFECT OF PHYSOSTIGMINE ON THE CONTRACTION OF THE RETRACTOR PENIS MUSCLE OF THE DOG IN RESPONSE TO SYMPATHETIC STIMULATION

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The anatomy of the retractor penis muscle of the dog and of its nerve supply was described in detail by Langley & Anderson (1895). The preganglionic fibres leave the cord between the 12th thoracic and 3rd lumbar (inclusive) and run down to the sacral ganglia. The post-ganglionic fibres arise mainly from the 1st sacral ganglion, with additional fibres from the 2nd and 3rd. The post-ganglionic fibres reach the retractor in the pudic nerves. The retractor penis itself is a long thin band of smooth muscle which contracts within a sheath running along the ventral surface of the penis. The transmission of impulses from the sympathetic fibres to the muscle is by means of an adrenaline-like substance. Thus Elliott (1905) found that the retractor penis was more easily excited by adrenaline after denervation. In making this observation he compared the distal part of the retractor, denervated 14 days previously, with the normally innervated part, making the comparison *in vitro*. Dale (1906) recorded the contraction of the retractor in response to stimulation of the pudic nerves and in response to intravenous injection of adrenaline. He then injected " chrysotoxin " (a preparation of ergot equivalent to ergotamine) and observed that the contractions in response to nerve stimulation and to injection of adrenaline were abolished. The sympathetic nerves can, therefore, be assumed to be adrenergic.

We have not attempted to investigate whether the nerves to the retractor also liberate acetylcholine because we consider that it can be assumed that they do. It has been shown that post-ganglionic fibres which are functionally adrenergic also release acetylcholine in (a) the splenic nerves of the cat (Brandon & Rand, 1961), (b) the accelerator nerves to the heart of the cat and dog (Folkow, Frost, Haeger & Uvnäs, 1948), (c) the post-ganglionic fibres to the vessels of the hind leg of the dog (Bülbring & Burn, 1935) and of the cat (Folkow, Haeger & Uvnäs, 1948), (d) the post-ganglionic fibres to the pilomotor muscles of the tail of the cat (Wolner, 1965), (e) the post-ganglionic fibres to the vessels of the rabbit's ear (Burn & Rand, 1960). In all these cases acetylcholine has been demonstrated in the venous effluent when the post-ganglionic fibres were stimulated during perfusion with a modified Ringer solution containing an anticholinesterase. In other tissues evidence has been obtained by observing the response to stimulation when the animal has been treated with reserpine beforehand, the response being similar to that produced by acetylcholine and being increased by an anticholinesterase and being blocked

by atropine. In some tissues, evidence of acetylcholine release has been obtained when stimulation has been applied at low frequency, both acetylcholine and noradrenaline being released together (Burn, Dromey & Large, 1963). Hitherto no tissue has been found in which evidence of acetylcholine release was absent.

In the experiments to be described we have made some observations on the response of the retractor muscle to injections of acetylcholine, noradrenaline and 5-hydroxytryptamine, but our main purpose has been to discover whether the response to post-ganglionic stimulation, observed in the presence of hyoscine, was modified by the injection of physostigmine, and whether the change in the response which physostigmine caused was related to the frequency of stimulation.

METHODS

Dogs, 11–18 kg in weight, were anaesthetized with ether followed by chloralose (80 mg/kg). The retractor penis muscle was found by incising the skin along the midline of the ventral surface of the penis for about 2–3 cm. A thread was tied round the end of the muscle which is nearest to the end of the penis and, after the muscle was detached from its insertion, the thread was connected to a frontal writing lever, recording isotonically. The dog was eviscerated by dividing in succession the rectum, the inferior mesenteric artery, the mesentery up to the superior mesenteric artery, the superior mesenteric artery and the coeliac axis. The oesophagus and portal canal were also divided and the viscera were then removed. The lumbar vessels on the right side were then divided and the right and left sympathetic chains were cut just above the aortic bifurcation. The right chain was followed to the pelvic cavity after dividing the right external iliac vessels. When the 1st sacral ganglion was exposed, small shielded electrodes were placed around the post-ganglionic fibres running laterally from it. In some dogs these fibres were fastened so closely to the sacrum that this was not possible and the electrodes were then placed around the ganglion itself. A stream of Krebs solution irrigated the ganglion and the electrodes, the fluid being removed by constant suction from a pump. Blood pressure was recorded from a carotid artery. The electrodes were attached to a stimulator which gave supramaximal shocks of 1 msec duration at frequencies varying from 0.2/sec to 5/sec. In each experiment the number of shocks at each stimulation was kept constant, though it varied in different experiments.

The following drugs were used: acetylcholine perchlorate, hexamethonium bromide, 5-hydroxytryptamine creatinine sulphate, hyoscine hydrobromide, l-noradrenaline bitartrate, physostigmine sulphate. They were all made up in 0.9% saline and all doses in this paper refer to the base.

RESULTS

Acetylcholine and noradrenaline

When the sympathetic chains were intact, so that impulses of central origin reached the retractor muscle, it showed large spontaneous contractions. These conditions were not suitable for studying the effects of acetylcholine and noradrenaline and it was found that the spontaneous activity was reduced or abolished by a small intravenous dose of pentobarbitone sodium. We observed in two experiments that the injection of 50 μg acetylcholine caused a contraction, as shown in Fig. 1a. The injection of 50 μg noradrenaline also caused a contraction. When the sympathetic chains were cut, then the injection of acetylcholine did not cause a contraction, but the injection of noradrenaline caused a contraction as before (Fig. 1b). However, the injection of acetylcholine diminished the response to the injection of noradrenaline when the two substances were mixed together and injected simultaneously, as shown in Fig. 1c. Further injections of noradrenaline alone caused responses which increased until they were as great as, or

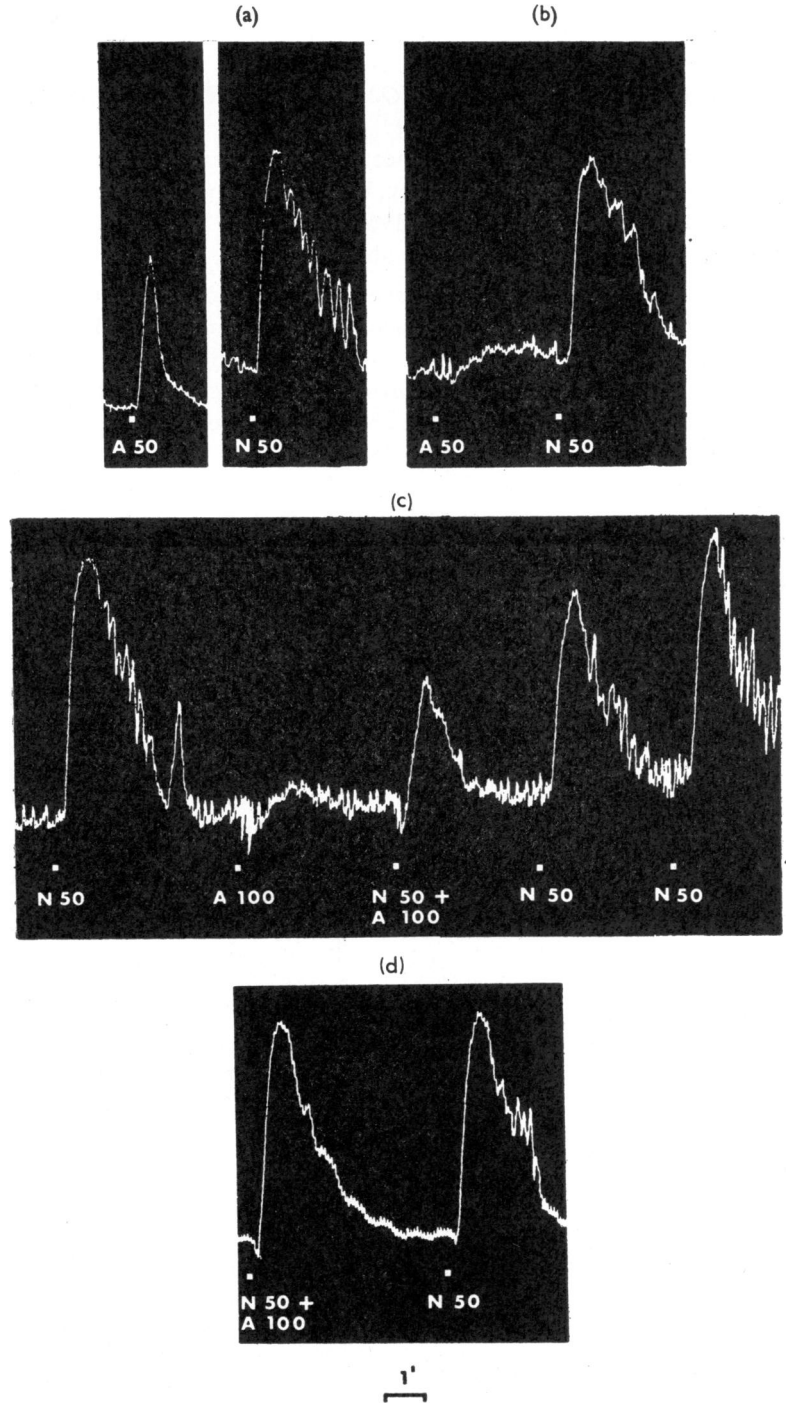

Fig. 1. Dog, 18 kg, chloralose anaesthesia, eviscerated. Record of isotonic contractions of the retractor penis muscle. A50=50 μg acetylcholine, N50=50 μg noradrenaline, A100=100 μg acetylcholine, injected intravenously. Between (a) and (b) both sympathetic chains were cut. This procedure abolished the effect of acetylcholine but noradrenaline still contracted the muscle. When acetylcholine and noradrenaline were injected together, however (c), the effect of noradrenaline was reduced. Between (c) and (d), 0.2 mg/kg hyoscine was given intravenously. The contraction caused by noradrenaline was unchanged, and the injection of acetylcholine together with noradrenaline did not reduce the effect of the noradrenaline.

even greater than, before acetylcholine was injected. This effect of acetylcholine in reducing the contraction caused by a simultaneous injection of noradrenaline was observed in three experiments. The effect was absent when hyoscine was given previously, as shown in Fig. 1d.

Effect of physostigmine on the response to noradrenaline

Burn, Philpot & Trendelenburg (1954) showed that physostigmine increased the contraction of the nictitating membrane of the cat caused by noradrenaline. This increase was abolished by the subsequent injection of atropine. Later it was shown that after the injection of 0.1 mg/kg hyoscine, the response of the nictitating membrane to noradrenaline was unaffected by the injection of 0.5 mg/kg physostigmine (Burn, Rand & Wien, 1963).

Experiments were therefore made, to see if the response of the retractor to noradrenaline was increased by physostigmine when hyoscine was injected previously. The results of six experiments are shown in Table 1, and in only one of these, when 0.1 mg/kg

TABLE 1

EFFECT OF PHYSOSTIGMINE ON THE RESPONSE TO NORADRENALINE BEFORE AND AFTER THE INJECTION OF HYOSCINE

Amount of hyoscine injected (mg/kg)	Effect of physostigmine (0·5 mg/kg) on response to 50 μg noradrenaline
0·1	No change
0·1	Response increased
0·15	No change
0·15	No change
0·15	No change
0·2	No change

hyoscine had been injected, was the response to noradrenaline increased by physostigmine. When larger amounts of hyoscine were injected, physostigmine did not affect the noradrenaline contractions, as shown in Fig. 2.

Response to stimulation at different frequencies

The response to stimulation of the post-ganglionic sympathetic fibres was determined in experiments performed after the injection of hyoscine. In each experiment a fixed number of supramaximal shocks was given, the frequency being varied in a range from 0.2/sec (1 shock in 5 sec) to 5/sec. This range was chosen because it is the range in which stimuli are delivered in physiological conditions (Folkow, 1955).

The contractions in response to different frequencies are shown in Table 2, which gives details of five experiments. The effect of the change in frequency on the height of the contraction in experiment 2 was to increase the height from 37 mm in response to a frequency of 0.2/sec, to 64 mm in response to a frequency of 2.0/sec. The increase in response was graded according to the frequency. A similar grading was seen in all five experiments.

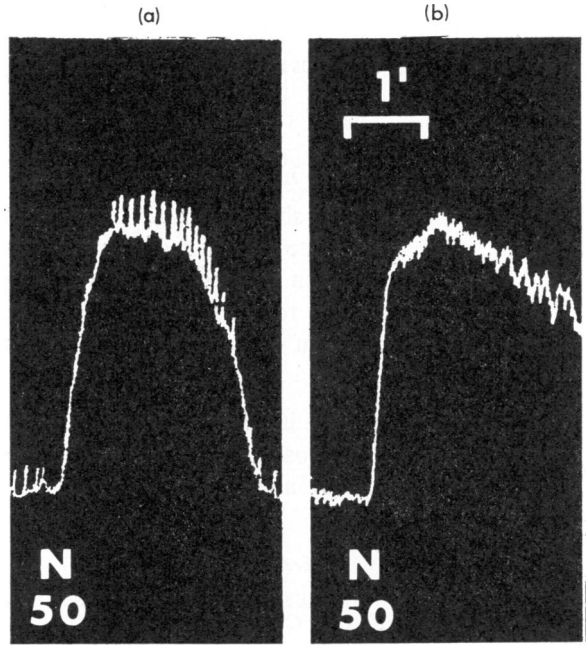

Fig. 2. Dog, 15 kg, chloralose anaesthesia, eviscerated, hyoscine (0.15 mg/kg) intravenously. Record of isotonic contractions of the retractor penis muscle. Between (a) and (b), 0.5 mg/kg physostigmine intravenously did not affect the contraction caused by 50 μg noradrenaline $\left(\frac{N}{50}\right)$.

TABLE 2

TO SHOW THE EFFECT OF INJECTING PHYSOSTIGMINE (0·5 MG/KG) ON THE RESPONSE OF THE RETRACTOR PENIS TO POST-GANGLIONIC STIMULATION

Expt.	Hyoscine (mg/kg)	Shocks (No.)	Frequency/ sec	Response (mm)		% increase after physostigmine
				Control	After physostigmine	
1	0·1	10	1	24·5	32	31
			5	35	36	3
2	0·15	5	0·2	37	74	100
			0·5	44	69	57
			1·0	51	71	39
			2·0	64	70	9
3	0·05	40	0·5	22	43	95
			1·0	26·5	45	70
			2·0	29	47	62
4	0·1	10	0·5	23	31	35
			1·0	28	36	28
			2·0	32	38	19
5	0·2	50	1·0	34	41	21
			2·0	41	53	29
			5·0	48	61	27

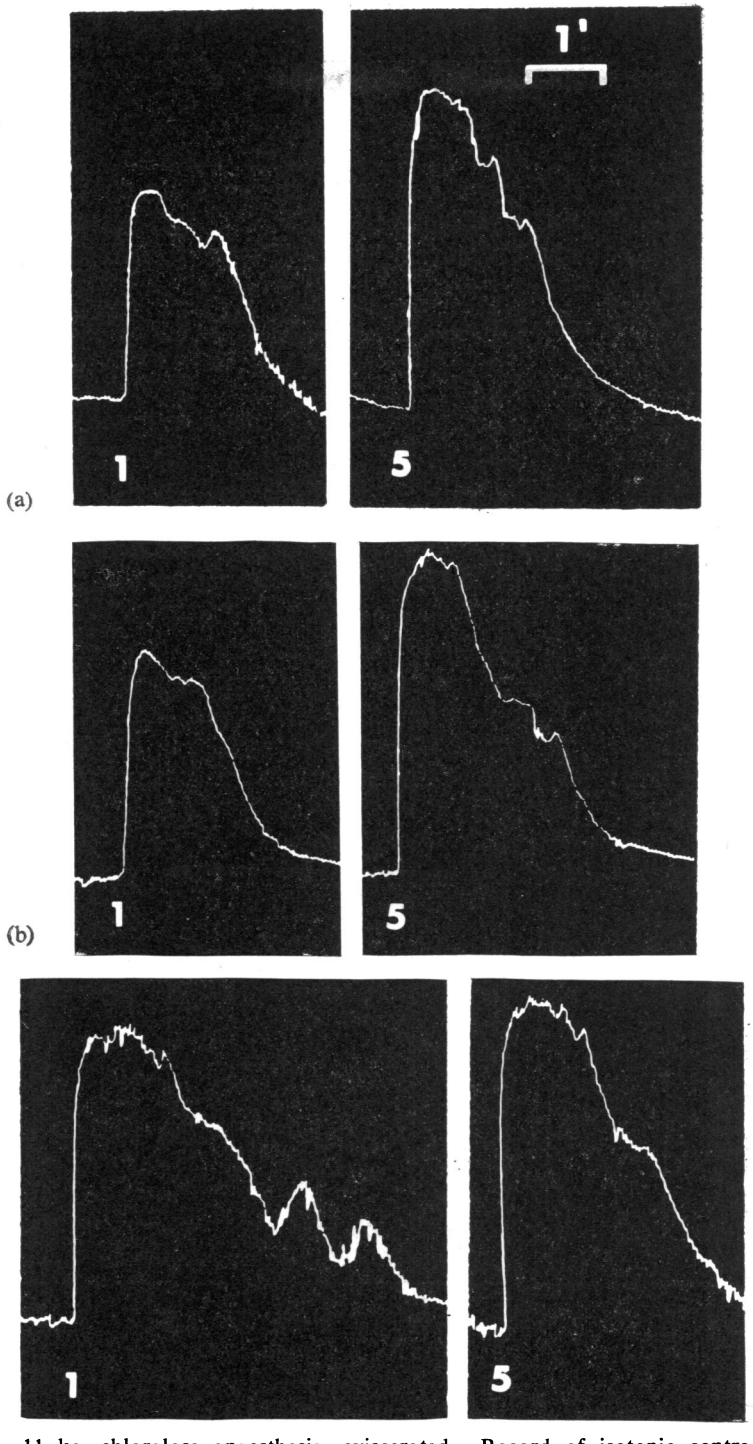

Fig. 3. Dog, 11 kg, chloralose anaesthesia, eviscerated. Record of isotonic contractions of the retractor penis muscle in response to stimulation of 1st sacral ganglion (1 or 5 shocks/sec, 1 msec, 20 V). The white figures in each panel show the stimulus frequency. At each stimulation, 10 shocks were given. (a) control responses, that to 5/sec being greater than that to 1/sec. (b) responses after 0.1 mg/kg hyoscine intravenously. (c) responses after 0.5 mg/kg physostigmine intravenously when the response to stimulation at 1/sec was increased.

Effect of physostigmine

After the intravenous injection of 0.5 mg/kg physostigmine the stimulation was repeated, and the contractions were found to be increased. In the five experiments set out in Table 2, the effect of physostigmine was to cause the greatest increase in the response to stimulation at the lowest frequency, and a progressively smaller increase as the frequency rose.

Figure 3 (b) and (c) shows the responses of experiment 1 in Table 2. The response to stimulation at 1/sec was increased from 24.5 mm to 32 mm, an increase of 31%, while the response to stimulation at 5/sec was increased only very slightly from 35 mm to 36 mm, an increase of 3%. Throughout the experiment the base line between stimulations remained almost constant, and the responses to stimulation at 1/sec and 5/sec were recorded several times. The response to a frequency of 1/sec was consistently less than that to a frequency of 5/sec before physostigmine was injected, but after the injection the responses to both frequencies were nearly the same. Thus physostigmine produced a greater increase at the lower frequency. The intravenous injection of hyoscine between (a) and (b) had very little effect on the response to stimulation.

Figure 4 shows the responses in experiment 2 of Table 2. The response to the lowest frequency was doubled by physostigmine, while the response to the highest increased by only 9%. All the responses after physostigmine were similar in height, so that physostigmine had the effect of abolishing the differences in response due to varying frequencies. Experiment 2 was the best experiment in the series because (1) there were

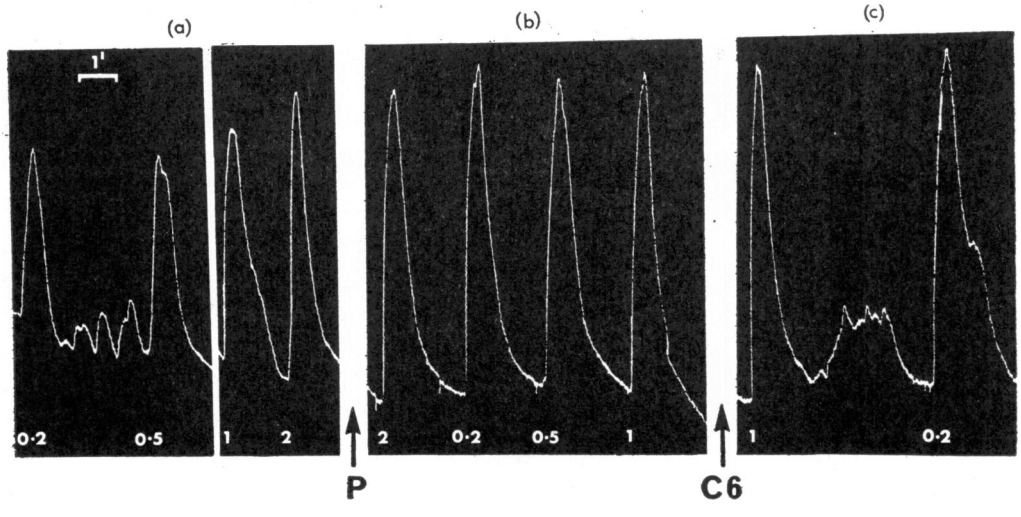

Fig. 4. Dog, 14.5 kg, chloralose anaesthesia, eviscerated, 0.15 mg/kg hyoscine intravenously. Record of isotonic contractions of the retractor penis muscle in response to stimulation of the post-ganglionic fibres from the 1st sacral ganglion (0.2, 0.5, 1 or 2 shocks/sec, 1 msec, 10 V). The white figures in each panel show the stimulus frequency. At each stimulation five shocks were given. (a) Control observations. Between (a) and (b) 0.5 mg/kg physostigmine was injected intravenously (P) and between (b) and (c) 0.2 mg/kg hexamethonium (C6). In the presence of physostigmine (b) are shown the increased responses all similar in height. Their size was not affected by hexamethonium (c).

fairly large differences between the responses to different frequencies before physostigmine was injected, (2) it was possible to apply the electrodes to the post-ganglionic fibres, rather than to the ganglion, (3) the amount of hyoscine injected was sufficient to ensure that physostigmine did not increase the response to noradrenaline, and (4) because, as Fig. 4 shows, the responses after the injection of physostigmine were unchanged when hexamethonium was injected.

Figure 5 shows the responses of experiment 3 in Table 2. In this experiment physostigmine produced a large increase in all the responses, but the amount of hyoscine was insufficient to ensure that the physostigmine did not increase the effect of the noradrenaline released. The increase was, however, greatest for the lowest frequency and least for the highest frequency, as in experiments 1 and 2. Thus the effect of physostigmine must have been due in part to an increase in the amount of noradrenaline released.

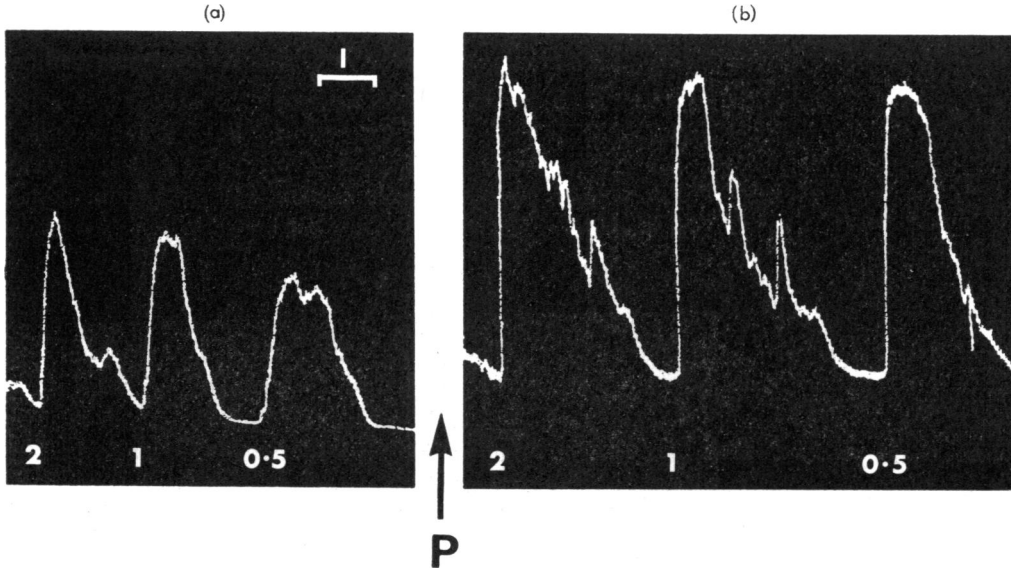

Fig. 5. Dog, 14 kg, chloralose anaesthesia, eviscerated, 0.05 mg/kg hyoscine intravenously. Record of isotonic contractions of the retractor penis muscle in response to stimulation of 1st sacral ganglion (0.5, 1 or 2 shocks/sec, 1 msec, 30 V). The white figures in each panel show the stimulus frequency. At each stimulation 40 shocks were given. Between (a) and (b) 0.5 mg/kg physostigmine was injected intravenously (P), in the presence of which the muscle contractions were greatly potentiated.

5-hydroxytryptamine

The contractions obtained in response to post-ganglionic stimulation were not due to acetylcholine because they were obtained in the presence of hyoscine. There was a possibility that they might have been due to the release of 5-hydroxytryptamine. However, the injection of 100 μg of this substance did not cause any contraction of the retractor, whereas 50 μg noradrenaline caused a large contraction.

DISCUSSION

There has long been general agreement that the cells of the adrenal medulla are derived from the embryonic sympathetic nervous system. At an early stage the intra-adrenal cells are very similar to those in the sympathetic ganglia. By the 30 mm crown-rump length stage in the human embryo some of the cells differentiate towards the structure of chromaffin cells, but most of them keep the general appearance of sympathetic neuroblasts (Boyd, 1960). The discovery of Feldberg & Minz (1933) that acetylcholine was the transmitter of splanchnic impulses to the adrenal medulla might therefore have given rise to the inquiry whether acetylcholine was also involved in the release of noradrenaline from the post-ganglionic sympathetic fibre.

It has been known since the work of Hoffmann, Hoffmann, Middleton & Talesnik (1945) that in the presence of atropine, acetylcholine causes an increase in the rate and force of the heart, and liberates an adrenaline-like substance from it. This action has been demonstrated in many tissues, and is due to a release of noradrenaline from sympathetic nerve endings. Ferry (1963) observed that acetylcholine injected into the splenic artery set up antidromic impulses in the fibres of the splenic nerve. From this he concluded that the action of acetylcholine on the adrenergic neurone was indirect, depending on its effect in setting up impulses in the nerve. However, it has been shown by Hertting & Widhalm (1965) in the perfused spleen, by Wolner (1965) in the pilomotor muscles of the cat tail, and also by Fischer, Weise & Kopin (1966) in the spleen that bretylium will block the release of noradrenaline by the nerve impulse in concentrations which do not affect the release by acetylcholine. It follows that the action of acetylcholine is not indirect as Ferry supposed.

The action of acetylcholine when injected has to be considered together with the observations described in the introduction, which have shown that stimulation of apparently all sympathetic post-ganglionic fibres releases not only noradrenaline but also acetylcholine. The function of this acetylcholine might clearly be related to the release of noradrenaline as in the adrenal medulla. It was, therefore, necessary to consider how to investigate this. The most obvious way seemed to be to test the effect of an anticholinesterase on the response to stimulation of adrenergic fibres in the presence of atropine or hyoscine to exclude any direct action of acetylcholine on the organ. In these circumstances an increase in the response to stimulation in the presence of the anticholinesterase would be direct evidence that acetylcholine was playing a part in the release of noradrenaline. If such an effect was found, then it could be predicted that it would vary with frequency of stimulation. When the interval between shocks was long, for example when the frequency was 0.2/sec, there would ordinarily be time for cholinesterase to destroy much of the acetylcholine liberated by one shock before the next shock arrived, and therefore the concentration of acetylcholine would not rise even when a series of shocks was given. According to the hypothesis the amount of noradrenaline released would then be small. However, when the frequency was increased, the concentration of acetylcholine would rise because there would be progressively less time for cholinesterase to act between the shocks. Consequently, with rising frequency of stimulation there should be a bigger response. In the presence of an anticholinesterase, the concentration of acetylcholine should rise because destruction is lessened, and in

particular the amount of noradrenaline released should be nearly as great at a low frequency as at a frequency ten times higher.

Experiments of this kind were made in the nictitating membrane of the cat (Burn *et al.*, 1963) in the femoral artery of the dog (Bernard & De Schaepdryver, 1964) in the innervated taenia of the guinea-pig (Ng, 1966) in the isolated rabbit heart (Huković, 1966) and they have now been made in the retractor penis of the dog. In all five organs it was found that anticholinesterases increased the response to post-ganglionic stimulation, and in all five organs the increase was graded according to the frequency, the increase being greatest for the lowest frequency and being less as the frequency rose. We wish to call attention to the experiment shown in the present paper as expt. 2 of Table 2, and Fig. 4, which affords an excellent example in the fullest agreement with the hypothesis.

The view that the sympathetic fibre releases noradrenaline through the mediation of acetylcholine is further supported by the blocking action of hemicholinium, which interrupts acetylcholine synthesis (Birks & MacIntosh, 1961). Recently Ludueña & Grigas (1966) have published observations on the retractor penis of the dog, most of which have been made on the muscle isolated in a bath. They found that the contractions produced by transmural stimulation were unaffected by hemicholinium, but the longest period for which they tested hemicholinium was 174 min. Chang & Rand (1960), Brandon & Rand (1961) and Rand & Ridehalgh (1965) found that in the isolated atria of the cat, in the perfused spleen of the cat and in the isolated colon of the guinea-pig, the time required was 4.5 to 5.5 hr, and only in the perfused vessels of the rabbit ear was 3 hr sufficient to observe a block by hemicholinium. In all cases they restored the effect of stimulation with choline. Ludueña and Grigas quoted Birmingham & Wilson (1963) as having failed to observe a block of the contractions of the vas deferens by hemicholinium when the contractions were caused by transmural stimulation. In fact Birmingham and Wilson said, "A 15% reduction was produced by hemicholinium (5×10^{-4}) at the end of 1 hr contact." Recently Burn (1966) has considered the results of those who have failed to observe a block with hemicholinium, and in view of the evidence of Rand and his colleagues, the failures were all due to insufficient time being allowed for the hemicholinium to act.

Finally, it may be added that the evidence for the part played by acetylcholine in the release of noradrenaline is greatly strengthened by the work of Rand & Whaler (1965), who observed that botulinum toxin blocked the effect of sympathetic stimulation in the ileum of the rabbit, in the pilomotor muscles of the cat's tail and in the guinea-pig vas deferens. Since it was shown by Burgen, Dickens & Zatman (1949) that botulinum toxin blocks the release of acetylcholine, the findings of Rand and Whaler also indicate that acetylcholine plays a part in the release of noradrenaline.

The evidence from the sources described is now so uniformly in favour of the hypothesis that it is well on its way to becoming a theory.

SUMMARY

1. Experiments have been carried out on dogs anaesthetized with chloralose in which the contractions of the retractor penis have been recorded in response to stimulation of the sympathetic post-ganglionic fibres leaving the 1st sacral ganglion.

2. Hyoscine was injected to exclude the action on the retractor muscle of any acetylcholine released by stimulation.

3. During each period of stimulation, a fixed number of supramaximal shocks was applied at frequencies varying from 0.2/sec to 5/sec. The resulting contractions were proportional to the frequencies, being smallest for the lowest frequency.

4. Stimulation was then repeated at the same frequencies after the injection of physostigmine. The contractions were then increased, the increase being greatest for stimulation of the lowest frequency, and being progressively less as higher frequencies were used. The effect of physostigmine was to make the responses alike at all frequencies.

5. The evidence supports the view that in the adrenergic fibre, the release of noradrenaline is mediated by acetylcholine.

REFERENCES

BERNARD, P. J. & DE SCHAEPDRYVER, A. F. (1964). Adrenergic mechanisms in the dog hindleg. *Archs. int. Pharmacodyn. Ther.*, **148**, 301–305.

BIRKS, R. & MACINTOSH, F. C. (1961). Acetylcholine metabolism of a sympathetic ganglion. *Can. J. Biochem. Physiol.*, **39**, 787–827.

BIRMINGHAM, A. T. & WILSON, A. B. (1963). Preganglionic and postganglionic stimulation of the guinea-pig isolated vas deferens preparation. *Br. J. Pharmac. Chemother.*, **21**, 569–580.

BOYD, J. D. (1960). Origin, development and distribution of chromaffin cells. In *Adrenergic mechanisms*, pp. 63–82 ed. by VANE, J. R., WOLSTENHOLME, G. E. W. & O'CONNOR, M. Ciba Foundation Symposium. Churchill, London.

BRANDON, K. W. & RAND, M. J. (1961). Acetylcholine and the sympathetic innervation of the spleen. *J. Physiol., Lond.*, **157**, 18–32.

BÜLBRING, E. & BURN, J. H. (1935). The sympathetic dilator fibres in the muscles of the cat and dog. *J. Physiol., Lond.*, **83**, 483–501.

BURGEN, A. S. V., DICKENS, F. & ZATMAN, L. J. (1949). The action of botulinum toxin on the neuromuscular junction. *J. Physiol., Lond.*, **109**, 10–24.

BURN, J. H. (1966). Adrenergic transmission. *Pharmac. Rev.*, **18**, 459–470.

BURN, J. H., DROMEY, J. J. & LARGE, B. J. (1963). The release of acetylcholine by sympathetic nerve stimulation at different frequencies. *Br. J. Pharmac. Chemother.*, **21**, 97–103.

BURN, J. H., PHILPOT, F. J. & TRENDELENBURG, U. (1954). Effect of denervation on enzymes in iris aud blood vessels. *Br. J. Pharmac. Chemother.*, **9**, 423–428.

BURN, J. H. & RAND, M. J. (1959). Sympathetic postganglionic mechanism. *Nature, Lond.*, **184**, 163–165.

BURN, J. H. & RAND, M. J. (1960). Sympathetic postganglionic cholinergic fibres. *Br. J. Pharmac. Chemother.*, **15**, 56–66.

BURN, J. H., RAND, M. J. & WIEN, R. (1963). The adrenergic mechanism in the nictitating membrane. *Br. J. Pharmac. Chemother.*, **20**, 83–94.

CHANG, V. & RAND, M. J. (1960). Transmission failure in sympathetic nerves produced by hemicholinium *Br. J. Pharmac. Chemother.*, **15**, 588–600.

DALE, H. H. (1906). On some physiological actions of ergot. *J. Physiol., Lond.*, **34**, 163–206.

ELLIOTT, T. R. (1905). The action of adrenalin. *J. Physiol., Lond.*, **32**, 401–467.

FELDBERG, W. & MINZ, B. (1933). Das Auftreten eines acetylcholinartigen Stoffes im Nebennierenvenenblut bei Reizung der Nervi splanchnici. *Pflugers. Arch. ges. Physiol.*, **233**, 657–682.

FERRY, C. B. (1963). The sympathomimetic effect of acetylcholine on the spleen of the cat. *J. Physiol., Lond.*, **167**, 487–504.

FISCHER, J. E., WEISE, V. K. & KOPIN, I. J. (1966). Interactions of bretylium and acetylcholine at sympathetic nerve endings. *J. Pharmac. exp. Ther.*, **153**, 523–529.

FOLKOW, B. (1955). Nervous control of the blood vessels. *Physiol. Rev.*, **35**, 629–663.

FOLKOW, B., FROST, J., HAEGAR, K. & UVNÄS, B. (1948). Cholinergic fibres in the sympathetic outflow to the heart in the dog and the cat. *Acta physiol. scand.*, **15**, 421–426.

FOLKOW, B., HAEGAR, K. & UVNÄS, B. (1948). Cholinergic vasodilator nerves in the sympathetic outflow to the muscles of the hind limbs of the cat. *Acta physiol. scand.*, **15**, 401–411.

HERTTING, G. & WIDHALM, S. (1965). Uber den Mechanismus der Noradrenalin-Freisetzung aus sympathischen Nervenendigungen. *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.*, **250**, 257–258.

HOFFMANN, F., HOFFMANN, E. J., MIDDLETON, S. & TALESNIK, J. (1945). The stimulating effect of acetylcholine on the mammalian heart and the liberation of an epinephrine-like substance by the isolated heart. *Amer. J. Physiol.*, **144**, 189–198.

HUKOVIĆ, S. (1966). The effect of anticholinesterases on the increase in rate of the isolated heart in response to sympathetic stimulation. *Br. J. Pharmac. Chemother.*, **28**, 273–281.

LANGLEY, J. N. & ANDERSON, H. K. (1895). The innervation of the pelvic and adjoining viscera. Part 2. The bladder. *J. Physiol., Lond.*, **19**, 71–139.

LUDUEÑA, F. P. & GRIGAS, E. O. (1966). Pharmacological study of autonomic innervation of the dog retractor penis. *Amer. J. Physiol.*, **210**, 435–444.

NG, K. K. F. (1966). The effect of some anticholinesterases on the response of the taenia to sympathetic nerve stimulation. *J. Physiol., Lond.*, **182**, 233–243.

RAND, M. J. & RIDEHALGH, A. (1965). Actions of hemicholinium and triethylcholine on responses of guinea-pig colon to stimulation of autonomic nerves. *J. Pharm. Pharmac.*, **17**, 144–156.

RAND, M. J. & WHALER, B. C. (1965). Impairment of sympathetic transmission by botulinum toxin. *Nature, Lond.*, **206**, 588–591.

WOLNER, E. (1965). Versuche über die Innervation der Pilomotoren. *Naunyn-Schmiederbergs Arch. exp. Path. Pharmak.*, **250**, 437–450.