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THE RELEASE OF ACETYLCHOLINE BY SYMPATHETIC NERVE STIMULATION AT DIFFERENT FREQUENCIES

BY

J. H. BURN, J. J. DROMEY AND B. J. LARGE

From the Research Laboratories, May & Baker, Dagenham, Essex

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Supramaximal stimulation of the periarterial nerves in the mesentery to the rabbit isolated ileum causes inhibition which is increased when hyoscine is added to the bath. This increase is, however, usually seen only when the stimulus frequency is low, and the addition of hyoscine usually makes no difference when the frequency is 10 shocks/sec or more. The observations suggest that, at low frequency, stimulation releases both acetylcholine and noradrenaline, but that at higher frequencies only noradrenaline is released. Similar observations have been made for the nictitating membrane of the cat. When the postganglionic fibres from the superior cervical ganglion were stimulated supramaximally, the contractions of the membrane increased in size as the frequency rose. In the presence of hyoscine the contractions were smaller, the greatest difference being for the lowest stimulus frequency, the difference diminishing as the frequency rose. These observations are consistent with the view that sympathetic cholinergic fibres in many situations release acetylcholine to act directly only at low frequencies, and that at higher frequencies the acetylcholine is almost entirely used to release noradrenaline.

When sympathetic postganglionic nerve fibres are stimulated, noradrenaline is released, and in addition acetylcholine may be released. In a recent paper (Burn & Weetman, 1963) experiments were described in which it appeared that acetylcholine was released together with noradrenaline when stimulation was applied at lower frequencies but that, at higher frequencies, only noradrenaline was released. These observations were made with the isolated vas deferens of the guinea-pig, stimulation being applied to the hypogastric nerve.

While the hypogastric nerve seems to be largely composed of postganglionic fibres, preganglionic fibres are also present. Moreover Vogt (1963) has found chromaffin cells in the hypogastric nerve of the dog. Sjöstrand (1962) observed that hexamethonium blocked the response to stimulation of the hypogastric nerve in the guinea-pig. In addition, Ohlin & Strömblad (1963) reported that hexamethonium (3.7×10^{-4} M) blocked the response to stimulation of the hypogastric nerve when the electrodes were 3 to 5 cm from the organ, but not when the electrodes were 1 to 5 mm from the organ. Experiments have therefore been carried out with other organs where the fibres stimulated were all postganglionic. The rabbit isolated ileum was used, stimulation being applied to the periarterial nerves to inhibit the rhythmical contractions, and in addition the nictitating membrane of the cat anaesthetized with chloralose was used, stimulation being applied to the fibres leaving the superior

cervical ganglion. In some of these experiments the preganglionic fibres had been cut 7 days previously.

METHODS

Rabbit ileum. Pieces of ileum, about 2 in. long when relaxed, were removed from a freshly killed rabbit, together with the adjacent mesentery containing the arteries. Each piece was transferred to a Petri dish containing Locke's solution, and a silk thread was tied round the main artery. This was pulled through the hole at the end of a pair of electrodes of the pattern described by Burn & Rand (1960), so that the artery was held firmly in place. The piece of ileum and the electrodes were then set up in an organ-bath of 100 ml. capacity. The bathing fluid was Locke's solution at 32° C, bubbled with 95% oxygen and 5% carbon dioxide. An isotonic lever with a frontal writing point was used to record the pendular movements. Supramaximal stimuli were given of 0.5 msec duration at various frequencies using a fixed number of shocks. The Locke's solution contained (in 1 l.) NaCl 8.5 g, KCl 0.42 g, $CaCl_2$ (anhydrous) 0.24 g, $NaHCO_3$ 0.5 g and dextrose 2 g. Hyoscine hydrobromide was added so that the final concentration was 0.1 μg/ml., except in the experiment of Fig. 2, in which it was 0.01 μg/ml.

Nictitating membrane. In each of three cats an aseptic division of the right cervical sympathetic chain was performed 7 days before the experiment. For the main experiment the cat was anaesthetized with ether followed by chloralose (80 mg/kg), and the right nictitating membrane was attached by a thread to an isotonic lever with a frontal writing point which gave seven-fold magnification. The right superior cervical ganglion was exposed so that shielded electrodes could be applied to the fibres leaving the ganglion, and when the electrodes were fixed in position they were submerged in liquid paraffin (B.P.). Stimuli were applied at various frequencies using a fixed number of shocks of 0.5 msec duration. To ensure that the stimuli were supramaximal, the voltage was increased until there was no further increase in the height of contraction, and then a voltage about 50% greater than this was used in the experiment. Hyoscine hydrobromide was injected intravenously in the dose of 0.1 mg/kg of body weight.

RESULTS

The rabbit ileum. When a fixed number of shocks were given at different frequencies, inhibition of the movements was greatly increased after the addition of hyoscine to the bath if the stimulus frequency was low. This effect is illustrated in Fig. 1 when 500 shocks were given at each stimulation. In Fig. 1*a* stimulation at a frequency of 3 shocks/sec caused an initial increase followed by a general fall in tone during the period of stimulation. Hyoscine (0.1 μg/ml.) was added and, after 10 min, the stimulation was repeated. As seen in Fig. 1*b*, there was a large inhibition. Fig. 1*a* also records the response to stimulation at 5 shocks/sec before hyoscine, and Fig. 1*b* shows the response after hyoscine had been added. The addition of hyoscine had a greater effect at the frequency of 3 shocks/sec than at 5 shocks/sec.

When the stimulus frequency was raised to 10 shocks/sec, the addition of hyoscine usually had no effect on the response to stimulation, as is shown in Figs. 2 and 3. In both of these experiments the addition of hyoscine increased the inhibitory effect of stimulation at 5 shocks/sec, but not that at 10 shocks/sec which indeed appeared to be less in the presence of hyoscine. This diminution in the response was explained by the reduction in the mean tone of the ileum which hyoscine nearly always caused.

Thus when the stimulus frequency was 10 shocks/sec or higher, the addition of hyoscine to the bath did not usually increase the inhibitory response. However, this result was not invariable, and in one out of a total of sixteen experiments the addition of hyoscine increased the response to frequencies as high as 100 shocks/sec, though the increase was not great.

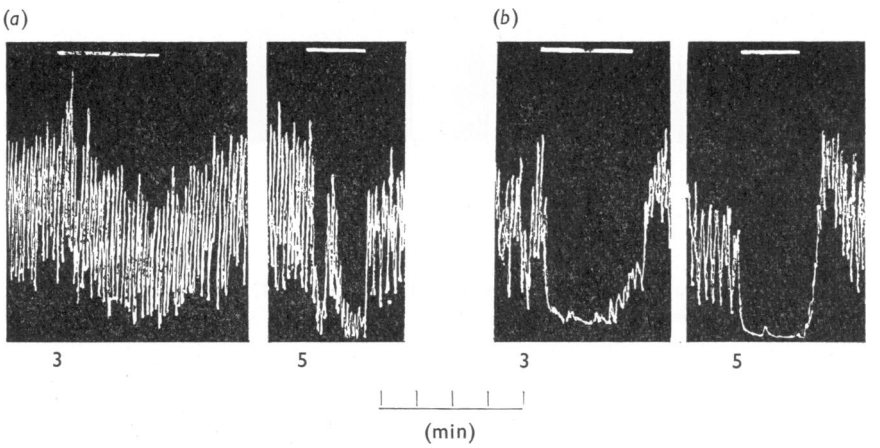

Fig. 1. Responses of a loop of rabbit ileum suspended in a 100 ml. organ-bath to stimulation of the periarterial nerves with 500 supramaximal shocks of 0.5 msec duration. Lines indicate periods of stimulation and numbers give stimulus frequencies. Observations in (*a*) were made at frequencies of 3 and 5 shocks/sec respectively, before the addition of hyoscine. Observations in (*b*) were made after the addition of hyoscine (0.1 μg/ml.). Hyoscine, by excluding the effect of acetylcholine, increased the inhibition caused by stimulation.

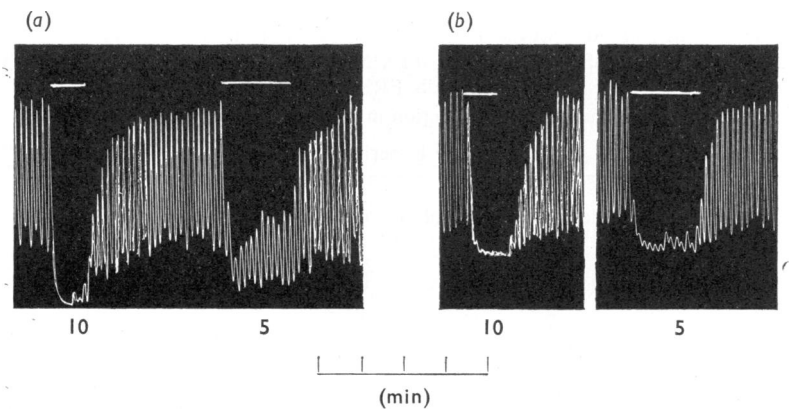

Fig. 2. Responses of a preparation similar to that of Fig. 1. In (*a*) are shown the responses to periarterial nerve stimulation with 500 supramaximal shocks at 10 and at 5 shocks/sec. In (*b*) are shown the responses after the addition of hyoscine (0.01 μg/ml.) to the bath. Hyoscine increased the inhibition due to stimulation at 5 shocks/sec, but did not increase that due to stimulation at 10 shocks/sec. The inhibition was indeed less, because the mean tone of the ileum was less.

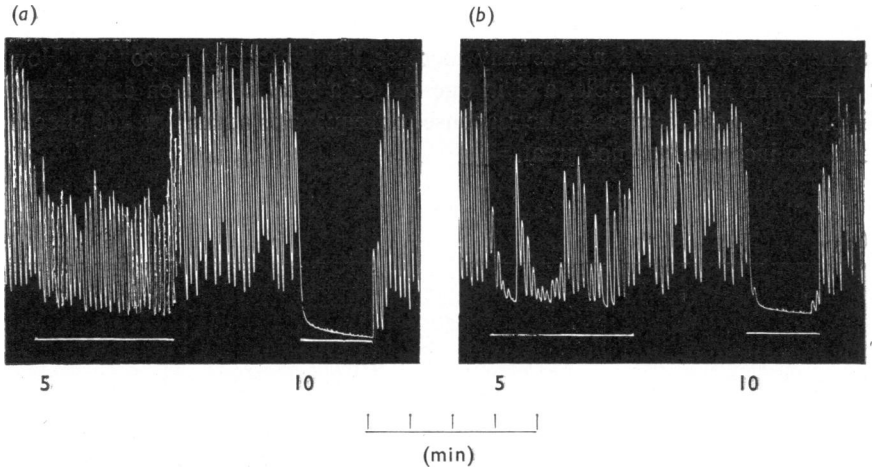

Fig. 3. Responses of a preparation similar to that of Fig. 1. In (*a*) are shown the responses to 1,000 shocks given at frequencies of 5 and of 10 shocks/sec. In (*b*) hyoscine (0.1 μg/ml.) was present. The inhibition produced by a frequency of 5 shocks/sec was then greater, but that produced by a frequency of 10 shocks/sec was not greater. In the presence of hyoscine the mean tone of the ileum was lower.

The nictitating membrane. In three experiments the cervical sympathetic chain had been cut seven days previously. This ensured that the fibres stimulated were all postganglionic, for any preganglionic fibres which did not terminate in the ganglion would have degenerated as a result of the section. The results of these experiments were similar to those of experiments in which this operation was not performed. The details of seven experiments are given in Table 1. The stimulus

TABLE 1

EFFECT OF HYOSCINE (0.1 MG/KG) ON THE CONTRACTION OF THE NICTITATING MEMBRANE IN RESPONSE TO SUPRAMAXIMAL POSTGANGLIONIC STIMULATION AT VARIOUS FREQUENCIES

Values are heights of contraction in mm for trains of 200 shocks

Frequency (shocks/sec)	Experiment 1			Experiment 2			Experiment 3		
	Control	Hyoscine	% decrease	Control	Hyoscine	% decrease	Control	Hyoscine	% decrease
0·5	26	20	23	—	—	—	7	1·5	78·5
1·5	36·5	30·5	16	33	28	15	16	11	31
5·0	41	37	10	43	38·5	10·5	27	27	0
10	42	40	5	50	48	4	33	33	0
20	44	44	0	52	50·5	3	—	—	—

Frequency (shocks/sec)	Experiment 4			Experiment 5		
	Control	Hyoscine	% decrease	Control	Hyoscine	% decrease
0·5	36	28·5	21	25	20·5	18
1·5	52	49	6	63·5	56·5	11
5·0	63	62	1·6	75·5	68·5	9·3
10	75	75	0	81	78	4
20	82·5	82·5	0	83·5	83·5	0

Frequency (shocks/sec)	Experiment 6			Experiment 7		
	Control	Hyoscine	% decrease	Control	Hyoscine	% decrease
0·5	—	—	—	—	—	—
1·5	34	31·5	7	22	18	18
5·0	43	42	2·3	37	33	10
10	44	43·5	1	49	47	4
20	42·5	42	1	69	67	2

frequency ranged from 0.5 to 20 shocks/sec, and the column headed "control" gives the height of the contractions (in mm on the kymograph) in response to a fixed number of shocks at the frequency indicated when the control responses had been obtained. Hyoscine was injected (0.1 mg/kg) and stimulation at the different frequencies was repeated. In each experiment in Table 1, hyoscine decreased the contraction most in response to stimulation at the lowest frequency and as the frequency rose the effect of hyoscine became less. In experiments 3 and 4 the effect of hyoscine was very small or absent at a frequency of 5 shocks/sec, though the frequency above which the contraction did not increase in experiment 3 was at least 10 shocks/sec, and in experiment 4 was 20 shocks/sec.

DISCUSSION

The results on the ileum and on the nictitating membrane show a close similarity. Stimulation produced a response which was modified by hyoscine when the stimulus frequency was low, but not when it was high.

In the ileum, the inhibition produced by stimulation at 3 to 5 shocks/sec was relatively small and ill-defined. In the presence of hyoscine, which excluded any action of acetylcholine, the inhibition was much greater. Thus the low-frequency stimulation appeared to have released both noradrenaline and acetylcholine, the latter counteracting the former before hyoscine was added. However, the response to stimulation at 10 shocks/sec or higher was nearly always unaffected by hyoscine, and therefore appeared to be due to noradrenaline only or at least to a heavy preponderance of noradrenaline.

In the nictitating membrane noradrenaline and acetylcholine do not act in opposition; each causes contraction. Therefore if stimulation released both substances, the contraction would be less when the presence of hyoscine had excluded the action of acetylcholine. It was observed that hyoscine reduced the contraction produced by stimulation at a low frequency, but this reduction diminished as the stimulus frequency rose.

The results are thus similar to those seen in the vas deferens when the hypogastric nerve was stimulated. The contraction of the vas deferens in response to stimulation at 10 shocks/sec was reduced to less than half in the presence of hyoscine, while the contraction in response to 25 to 40 shocks/sec was unaffected by the presence of hyoscine (Burn & Weetman, 1963).

What appears to be a fourth situation of the same kind is the submaxillary gland. Sympathetic nerve stimulation, when applied at 10 or 20 shocks/sec, produced a secretion of saliva which, however, was not maintained for very long. The secretion was not arrested by atropine. However, stimulation at a low frequency, less than

5 shocks/sec, produced a flow of saliva which continued for a long time (Emmelin & Engström, 1960). This secretion was stopped by atropine (Emmelin, personal communication). Again it would appear that low-frequency stimulation of the sympathetic fibres released acetylcholine, but that stimulation at a higher frequency released little acetylcholine or none at all.

The precise interpretation of the observations on the submaxillary gland is difficult because acetylcholine may be released from specific secretory fibres in the sympathetic nerves or it may perhaps be released from the nerves supplying the blood vessels. If, however, attention is confined to the observations on the nictitating membrane and on the rabbit ileum, it can be said that these observations do not readily agree with the idea that cholinergic and adrenergic fibres are separate. If they were, a rise in the frequency of stimulation would be expected to have the reverse effect, namely to increase the release of acetylcholine in relation to the release of noradrenaline. When a fixed number of stimuli is used the concentration of noradrenaline at the nerve ending should not greatly differ at a low frequency from that at a high frequency, since noradrenaline is fairly stable. But the concentration of acetylcholine should increase as the stimulus frequency rises since cholinesterase would have less time to act between the pulses. Hence stimulation of a mixed nerve supply should give an increasing effect due to acetylcholine as the stimulus frequency increases.

In experiments on the rabbit colon in which they stimulated the sympathetic nerves, Gillespie & Mackenna (1961) obtained a good motor response when stimulating at 50 shocks/sec when the rabbit from which the colon was taken had previously been treated with reserpine. Thus the release of acetylcholine by sympathetic fibres was evident at a high stimulus frequency when noradrenaline was absent.

Similarly Day & Rand (1961) carried out experiments on cat isolated atria in which they stimulated the sympathetic fibres at 50 shocks/sec. When guanethidine was added to the bath to block the release of noradrenaline, stimulation produced well-marked inhibitory effects. Thus sympathetic nerve stimulation at a high frequency released acetylcholine when noradrenaline could not be released because of the presence of a blocking agent.

These observations suggest that the apparent failure of sympathetic nerve stimulation to release acetylcholine when the stimulus frequency is high or moderately high is connected with the release of noradrenaline. The hypothesis has been put forward that, as the stimulus frequency rises, the concentration of acetylcholine which is released also rises and more acetylcholine is then used in the release of noradrenaline. Direct support for this interpretation has been given by the observations of Burn, Rand & Wien (1963) on the effect of physostigmine and neostigmine on the contraction of the nictitating membrane in response to postganglionic nerve stimulation. When hyoscine was injected to exclude any direct action of acetylcholine on the nictitating membrane, the injection of physostigmine (or of neostigmine) increased the contraction, the increase being greatest for stimulation at the lowest frequency. The increase gradually diminished as the stimulus frequency increased, probably because inhibition of cholinesterase was of less importance when, due to the increased frequency, there was less time for cholinesterase to act.

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