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Research Papers

Actions of hemicholinium and triethylcholine on responses of guinea-pig colon to stimulation of autonomic nerves

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Hemicholinium caused a failure of responses of the guinea-pig colon to stimulation of extrinsic parasympathetic and sympathetic nerves: failure of the parasympathetic responses occurred the more readily. In seven of 20 experiments, hemicholinium did not block the inhibitory response to sympathetic nerve stimulation but the latent period between the start of a train of stimuli and the first sign of relaxation was prolonged after repeated stimulation in the presence of hemicholinium in all 20 experiments. Triethylcholine caused failure of responses of the guinea-pig colon and a reduction of responses of rabbit ileum to sympathetic nerve stimulation. Choline sometimes reversed the blocking action of hemicholinium on responses to parasympathetic and sympathetic nerve stimulation.

THE action of hemicholinium on the responses to stimulation of sympathetic adrenergic nerves was examined by Chang & Rand (1960) to test for the presence of a cholinergic link in the release of noradrenaline. They found that hemicholinium indeed caused a failure of response. Others have observed a failure (Brandon & Rand, 1961; Wong & Long, 1961), or reduction (Bentley & Sabine, 1963; Birmingham & Wilson, 1963; Bevan & Su, 1964) of sympathetic responses, however, there are reports that hemicholinium is ineffective (Wilson & Long, 1959; MacIntosh, 1959; Gardiner & Thompson, 1961; Bentley, 1962; Long & Highgenboten, 1964).

The only tissue in which the effects of hemicholinium have been tested on responses to both cholinergic and adrenergic nerve stimulation is the dog bladder (Wong & Long, 1961). These authors found that the contractions of the bladder in response to stimulation of the hypogastric (adrenergic) nerve failed with lower frequencies of nerve stimulation and smaller doses of hemicholinium than did the contractions induced by stimulation of the pelvic (cholinergic) nerves.

Recently, Huković showed us how to prepare the guinea-pig isolated colon with both sympathetic and parasympathetic nerves. It was decided to test the action of hemicholinium on the responses of the colon to stimulation of each nerve.

Triethylcholine acts like hemicholinium in producing failure of response to stimulation of cholinergic nerves at a sufficiently high rate (Bowman & Rand, 1961), and it has been shown that it impairs the synthesis of acetylcholine (Bull & Hemsworth, 1963). Therefore experiments were also made with triethylcholine.

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Finally, we wish to report a few additional experiments using preparations of sympathetically innervated rabbit intestine.

Methods

Huković's preparation of the isolated guinea-pig colon with both the sympathetic and parasympathetic extrinsic nerves intact is essentially similar to the preparation of the dually innervated rabbit colon described by Garry & Gillespie (1955). Adult male guinea-pigs were killed by a blow on the head and bled out. The nerves were identified and dissected free from the surrounding fascia. As long lengths as possible were removed together with 3 to 4 cm of the terminal colon. The colon was suspended anal end down in McEwen's (1956) solution at 32° in a 50 ml bath, bubbled with 95% oxygen and 5% carbon dioxide. Bipolar stimulating electrodes of the type described by Burn & Rand (1960) were placed on the nerves as far away from the colon as possible, in order to reduce current spread and to allow free movement of the tissue. Stimuli were given from an electronic stimulator generating rectilinear pulses. In every instance the voltage was adjusted to be supramaximal, usually 10 V was used, 6 to 8 V giving a maximal response. Other details of the parameters of stimulation are given in Results. Movements of the colon were recorded using a frontal writing lever with a magnification of 8 times and exerting a tension of 1·5 g. A few observations were made with guinea-pig ileum, without extrinsic nerves, in Tyrode solution in a 10 ml bath. Segments of rabbit colon and ileum were used with sympathetic, but not parasympathetic nerves. They were set up in a 50 ml bath of McEwen's solution at 37°. The writing lever had a magnification of 4 times and exerted a tension of 3·5 g.

The drugs used were hemicholinium dibromide (Aldritch Chemical Co.), triethylcholine chloride (Ward Blenkinsop), choline chloride, acetylcholine chloride, noradrenaline bitartrate, nicotine acid tartrate, atropine sulphate, hyoscine hydrobromide, guanethidine sulphate and hexamethonium bromide. The amounts stated refer to these salts.

Results

RESPONSES OF THE COLON TO NERVE STIMULATION

Stimulation of the pelvic nerve produced a contraction which rapidly reached a peak and was not sustained (Figs 1 and 2), occasionally a small component of relaxation was seen (Fig. 2), which was presumably due to the inclusion of stray sympathetic fibres in the electrodes. The threshold pulse duration was 200 μsec and the threshold frequency was 5/sec. The maximal response was obtained by stimulation with pulses of 2 msec duration at a rate of 50/sec in a train lasting 10 or 20 sec. This could be repeated at 2 min intervals without any decline in response during more than 4 hr.

Stimulation of the sympathetic nerve caused the colon to relax, and this response often lasted for a minute or more after stimulation had ceased (Figs 1 and 2). The threshold pulse width and frequency, and the

parameters of stimulation giving maximal relaxation, were the same as those for the pelvic nerve. Constant responses to regular periods of stimulation of the sympathetic nerve (2 msec, 50/sec for 10 sec every 2 min) could be obtained for more than 7 hr.

There was a difference in the latency of the responses to stimulation of the pelvic and sympathetic nerves. The contraction caused by the pelvic nerve started immediately the stimulation was begun, but the sympathetic relaxation started only after an appreciable latent period (2 or 3 sec). The persistence of the response during stimulation differed between the two nerves. As noted above, the contraction to pelvic nerve stimulation was not sustained, and if the train of stimulation was continued the contraction began to decline after about 10 sec, and had often fallen back to the baseline by 20 sec. However, the sympathetic relaxation was remarkably well sustained. For example, during stimulation at 50/sec for 30 min the colon remained relaxed; the tone recovered when the stimulation was stopped, and then the colon responded again to stimulation as well as it had done before.

Acetylcholine in a concentration of 0·02 μg/ml regularly caused contraction to about the same height as the maximal contraction to pelvic nerve stimulation. Noradrenaline (0·1 μg/ml) regularly caused relaxation.

EFFECTS OF ATROPINE, HYOSCINE, HEXAMETHONIUM AND GUANETHIDINE

Atropine or hyoscine in a concentration of 1 μg/ml blocked completely the responses to pelvic nerve stimulation and to acetylcholine, but were without effect on the responses to sympathetic stimulation.

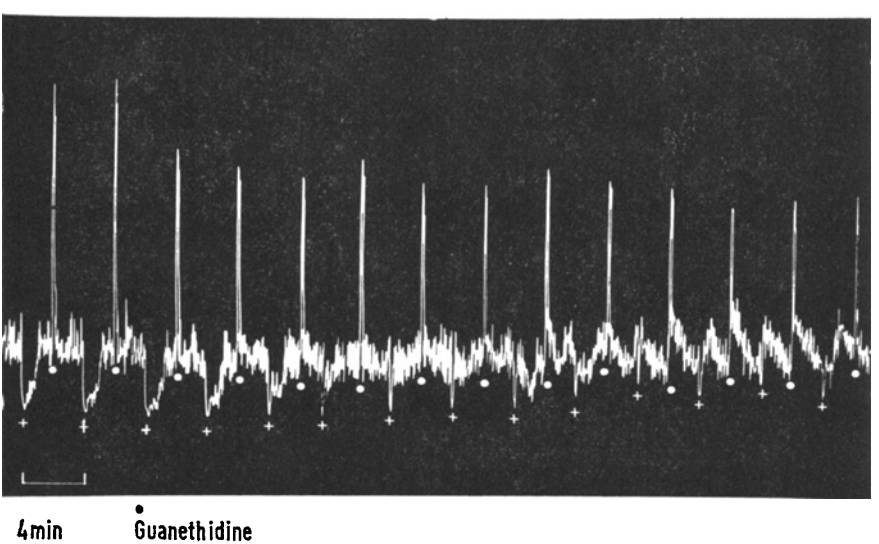

FIG. 1. Responses of guinea-pig colon to alternate stimulation of pelvic nerve (at white dots) and of sympathetic nerve (at +). Each nerve was stimulated with 2 msec pulses at 50/sec for 10 sec every 4 min. Guanethidine (2 μg/ml) almost abolished the inhibitory responses to sympathetic stimulation and reduced contractions to pelvic nerve stimulation.

Guanethidine (1 to 2 μg/ml) reduced the response to sympathetic nerve stimulation and the blockade was almost complete after 30 min (Fig. 1). This blockade persisted after washing out the guanethidine, but was reversed by dexamphetamine. The responses to pelvic nerve stimulation were reduced by guanethidine (Fig. 1), but were restored after washing it out.

Hexamethonium (1 to 5 μg/ml) blocked the contractions produced by pelvic nerve stimulation but was without effect on responses to sympathetic stimulation (Fig. 2).

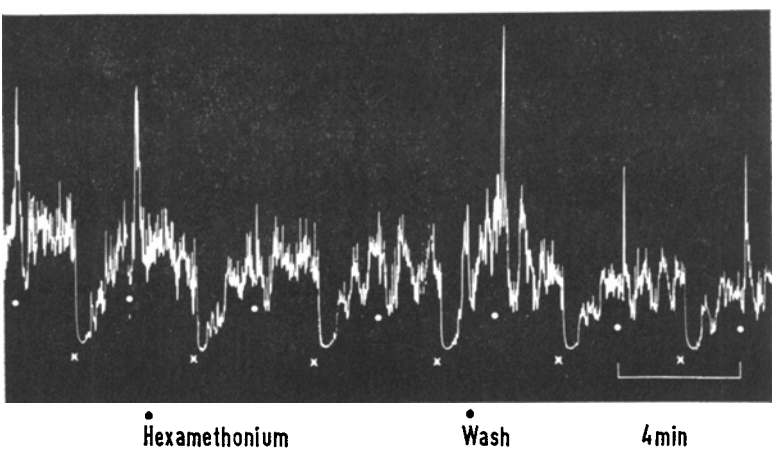

FIG. 2. Guinea-pig colon: nerve stimulation as in Fig. 1. Hexamethonium (5 μg/ml) blocked the contractions to pelvic nerve stimulation.

EFFECTS OF HEMICHOLINIUM AND TRIETHYLCHOLINE ON RESPONSES TO PELVIC NERVE STIMULATION

Hemicholinium in concentrations of 20 to 100 μg/ml caused a gradual failure of the contractions in response to pelvic nerve stimulation in 13 experiments. In one experiment 50 μg/ml did not cause failure, and in two experiments 10 μg/ml of hemicholinium were without action. There was little or no correlation between the concentration and the time taken for the contractions to fail. Table 1 gives the total number of pulses applied until failure developed with stimulation at 50/sec for 10 sec every 2 min. The responses did not recover after washing out the hemicholinium from the bath.

Triethylcholine (100 to 200 μg/ml) caused an immediate blockade of the responses to pelvic nerve stimulation, which were restored on washing out the bath. This effect can possibly be attributed to blockade of the parasympathetic ganglia, since a lower concentration of triethylcholine (10 to 50 μg/ml) blocked the contractions of ileum caused by nicotine (6 μg/ml). The contractions of ileum caused by acetylcholine were not affected by triethylcholine (200 μg/ml) present in the bath for 2 min. Triethylcholine caused a slow contraction of ileum and of colon in a concentration of 300 μg/ml.

EFFECTS OF HEMICHOLINIUM AND TRIETHYLCHOLINE ON RESPONSES TO SYMPATHETIC NERVE STIMULATION

Hemicholinium (20 to 100 μg/ml) caused a slowly developing failure of the extent of the relaxation produced by sympathetic nerve stimulation in 13 out of 20 experiments. The shortest time for complete failure of the inhibitory response, with stimulation at 50/sec for 20 sec periods every 4 r degree of stimulation necessary to cause

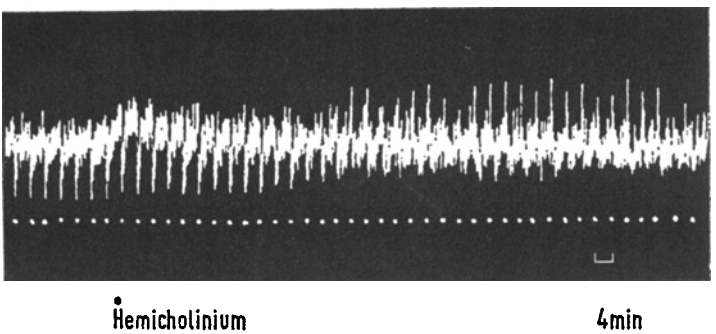

FIG. 3. Guinea-pig colon. Stimulation of sympathetic nerves with 2 msec pulses at 50/sec for 20 sec every 4 min at white dots. Hemicholinium (20 μg/ml) caused a reduction in inhibitory responses and the appearance of a motor response.

failure varied greatly. In some experiments prolonged periods of continuous stimulation lasting for up to 30 min were necessary to produce failure. However, even this was not always successful. If there were no definite signs of a reduction in response within 6 hr of adding hemicholinium the experiment was terminated. There was no consistent relationship between the concentration of hemicholinium and the number of pulses given until complete failure of response (Table 1).

TABLE 1. NUMBER OF PULSES APPLIED TO THE NERVES UNTIL THE RESPONSE FAILED COMPLETELY

Pelvic nerve		Sympathetic nerve	
Concentration of hemicholinium (μg/ml)	Total number of pulses $\times 10^3$	Concentration of hemicholinium (μg/ml)	Total number of pulses $\times 10^3$
20	80	20	14
40	10·5	20	56·5
50	10	40	16
50	15·5	50	43
50	18	50	48
50	22	50	50
50	24	50	51·5
50	30	50	86
50	37	50	95·5
50	48	50	100
50	50	75	30
75	9	100	32
75	15·5	100	36

In every experiment the latent period between the start of a train of stimuli and the first sign of relaxation was prolonged after repeated stimulation in the presence of hemicholinium. This occurred even in

preparations in which the extent of the inhibitory response was not diminished by hemicholinium. Sometimes the prolongation of the latent period was so marked that the relaxation only began at the end of a 20 sec train of stimulation. Nevertheless, experiments in which the extent of the delayed relaxation was the same as in the control period have been counted as ones in which no failure was produced. The prolongation of the latent period was the first effect observed, even in experiments in which relaxation ultimately failed completely.

In seven of the experiments in which the relaxations were blocked by hemicholinium a transient motor response occurred on stimulation of the sympathetic nerve (Fig. 3). Sometimes, at first, this was followed by relaxation, and then the relaxor component slowly failed (Fig. 4). It seemed unlikely that the motor response was due to stimulation of cholinergic fibres for the following reasons. It persisted after hemicholinium had caused a failure of contractions to pelvic nerve stimulation, as shown in Fig. 4, and it was unaffected by atropine or hyoscine in concentrations up to 10 μg/ml. The threshold frequency for eliciting the motor response differed from that for the usual responses of the two automatic nerves; it did not appear at less than 20/sec. The pulse width for eliciting it was almost the same as for the usual responses.

Washing out the hemicholinium did not result in restoration of inhibitory responses when these had been lost or replaced by motor responses.

Noradrenaline had the same action after failure was produced with hemicholinium as it had before.

Triethylcholine in concentrations of 100 to 250 μg/ml caused a slowly developing failure of responses to sympathetic stimulation in five out of seven experiments. The relaxations partially returned on washing out triethylcholine. A motor response, similar to that seen in some experiments with hemicholinium, appeared in one experiment.

EFFECTS OF HEMICHOLINIUM AND TRIETHYLCHOLINE ON PREPARATIONS WITH BOTH NERVES

In two experiments in which the pelvic and sympathetic nerves to the colon were stimulated alternately a parallel failure of the two responses was seen. Thus, in the experiment illustrated in Fig. 4, the contractions caused by pelvic nerve stimulation and the relaxations caused by sympathetic stimulation were diminished at about the same rate. In this experiment a motor response to sympathetic stimulation was observed. In three experiments in which the two nerves were stimulated alternately the responses to sympathetic stimulation failed more slowly and in two experiments they did not fail.

The latent period between the beginning of a train of sympathetic stimulation and the first appearance of the response was prolonged after hemicholinium, but, with parasympathetic stimulation, there was little prolongation of the latent period, the failure being manifested from the start as a diminution in the height of the contraction.

It was not possible to do experiments with triethylcholine using both sympathetic and parasympathetic nerves, because the ganglion blocking

activity of triethylcholine resulted in an immediate blockade of responses to pelvic nerve stimulation.

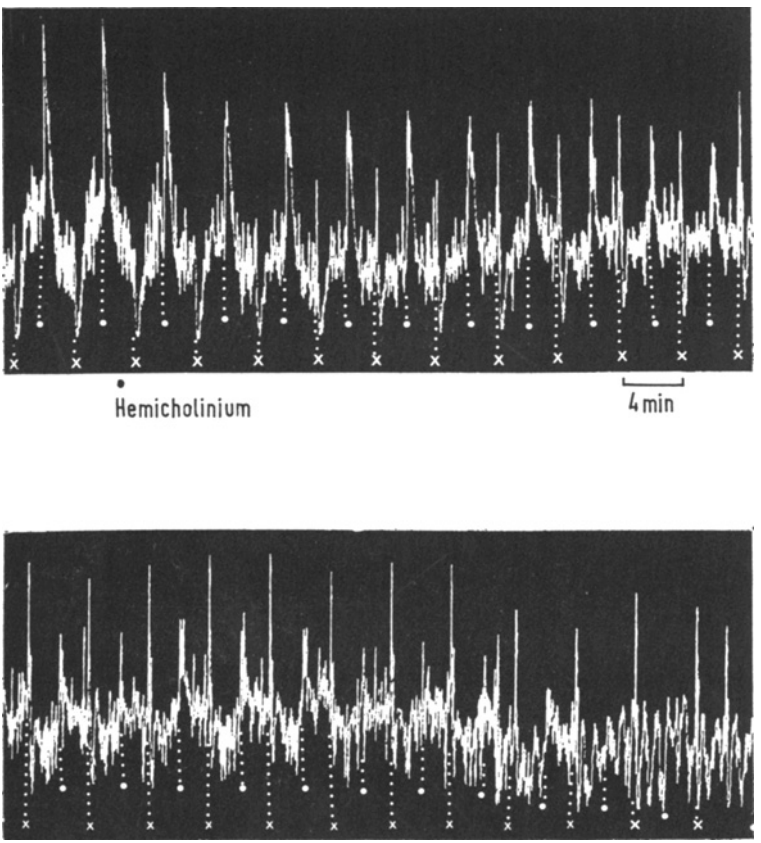

FIG. 4. Responses of guinea-pig colon to alternate stimulation of pelvic nerve (at white dots) and sympathetic nerve (at ×) using the same parameters of stimulation as in Fig. 1. The lower record is continuous with the upper. Hemicholinium (20 μg/ml) caused simultaneous failure of contractions to pelvic nerve stimulation and relaxations to sympathetic nerve stimulation: the relaxations were replaced by a motor response.

ACTIONS OF CHOLINE AFTER HEMICHOLINIUM AND TRIETHYLCHOLINE

Choline caused some degree of reversal in half of the experiments, but it was relatively ineffective where exposure to hemicholinium had been prolonged. It was more effective in restoring responses to pelvic nerve stimulation than those to sympathetic nerve stimulation, but this may be attributed to the longer exposure to hemicholinium which was required to produce failure of sympathetic responses. Fig. 5 illustrates an experiment showing a clear restoration of the inhibitory response to sympathetic stimulation by choline after failure had been produced in the presence of hemicholinium.

Choline enhanced the rate of recovery of the responses to sympathetic nerve stimulation which occurred on washing out triethylcholine.

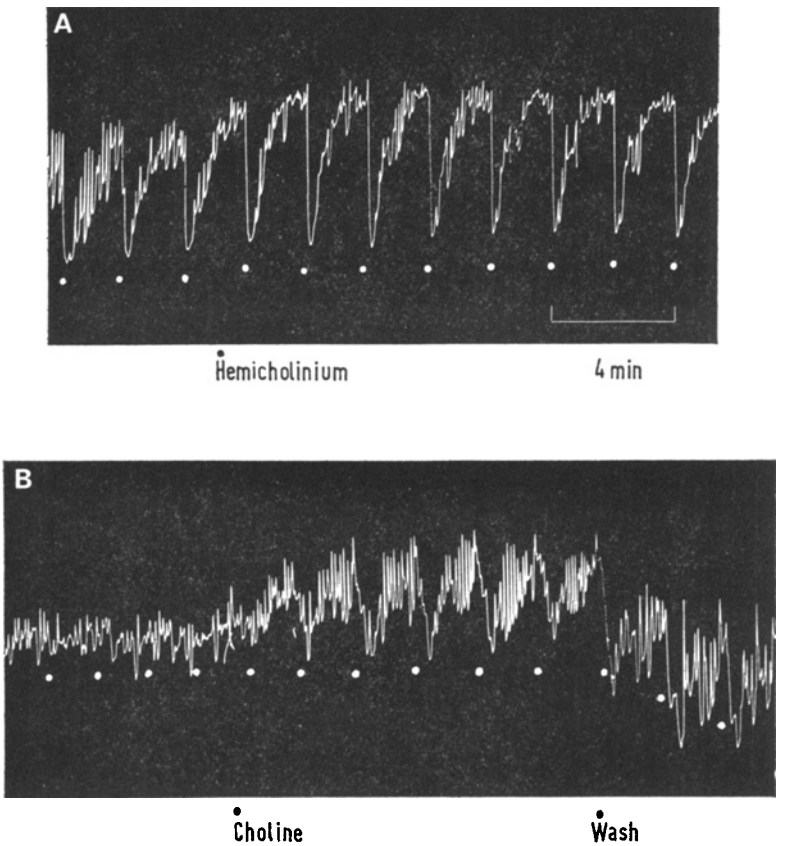

FIG. 5. Responses of guinea-pig colon to sympathetic nerve stimulation, with 2 msec pulses at 50/sec for 10 sec every 2 min at white dots. In A, hemicholinium (50 μg/ml) was added to the bath. The regime of stimulation was continuous between A and B. In B, 335 min later, the inhibitory responses were absent, then they were partly restored by choline (100 μg/ml).

ACTION OF HEMICHOLINIUM AND TRIETHYLCHOLINE ON SYMPATHETIC RESPONSES IN RABBIT COLON AND ILEUM

Chang & Rand (1960) found that hemicholinium produced a failure of the inhibitory responses to sympathetic nerve stimulation in the rabbit colon. However, Bentley (1962) reported that the inhibitory responses of rabbit ileum were not affected by 100 μg/ml of hemicholinium when the nerve was stimulated with 50 pulses/sec for 20 sec every 2·5 min for up to 45 min. It seemed possible that Bentley's finding may have been due to insufficiently vigorous stimulation. Our colleague, M. D. Day

(1963), therefore made experiments with rabbit ileum. His findings are as follows:

"Hemicholinium (50–200 μg/ml) appeared to cause a slight impairment of the responses to sympathetic nerve stimulation in 4 out of 6 preparations tested. However, in only 2 experiments did hemicholinium produce a greater impairment of nerve function than was observed in control segments taken from the same rabbits and subjected to the same amount of sympathetic nerve stimulation. The impairment of response to sympathetic nerve stimulation produced by hemicholinium was of slow onset and was increased when the nerves were stimulated either at a high frequency (50 or 100 pulses/sec) or continuously for prolonged periods (1–9 minutes). However, in no case did the impairment result in a complete abolition of the inhibitory response to sympathetic nerve stimulation even after 3 hours contact with hemicholinium."

Chang & Rand (1960) did not make any observations on the effect of choline after hemicholinium. This omission has now been remedied. Fig. 6 shows an increase in the relaxations which have become partly reduced in the presence of hemicholinium after adding choline to the bath. In the absence of hemicholinium, choline never resulting in greater relaxations, and high concentrations (200–400 μg/ml) caused reduction in responses to sympathetic nerve stimulation.

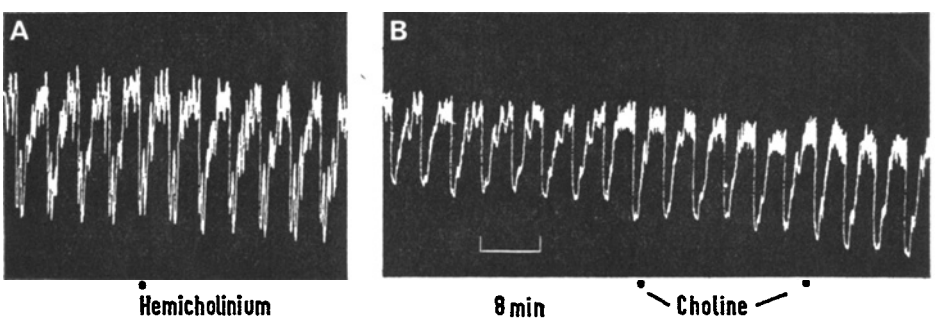

FIG. 6. Responses of rabbit colon to stimulation of sympathetic nerve with 2 msec pulses at 50/sec for 30 sec every 4 min. In A, hemicholinium (100 μg/ml) was added to the bath, and 132 min later, in B, the relaxations were less. Then choline (50 μg/ml), added at the two dots, increased the relaxations.

Triethylcholine caused a reduction in inhibitory responses to sympathetic nerve stimulation in rabbit colon and in ileum (Fig. 7).

Discussion

The responses of the guinea-pig colon to stimulation of the pelvic nerve and the sympathetic nerve closely correspond to those reported for the rabbit colon by Garry & Gillespie (1955) in all except one respect, namely the relationship between the response and the frequency of stimulation. In the rabbit colon, the threshold frequencies of stimulation were

1 every 2 sec for the pelvic and 5/sec for the sympathetic nerve; the maximal responses were obtained with 10/sec for the pelvic and 100/sec for the sympathetic nerve. However, the guinea-pig colon responded to stimulation of either nerve at a threshold frequency of 5/sec, and a frequency of 50/sec gave maximal responses. Our observations confirm Huković's findings (personal communication).

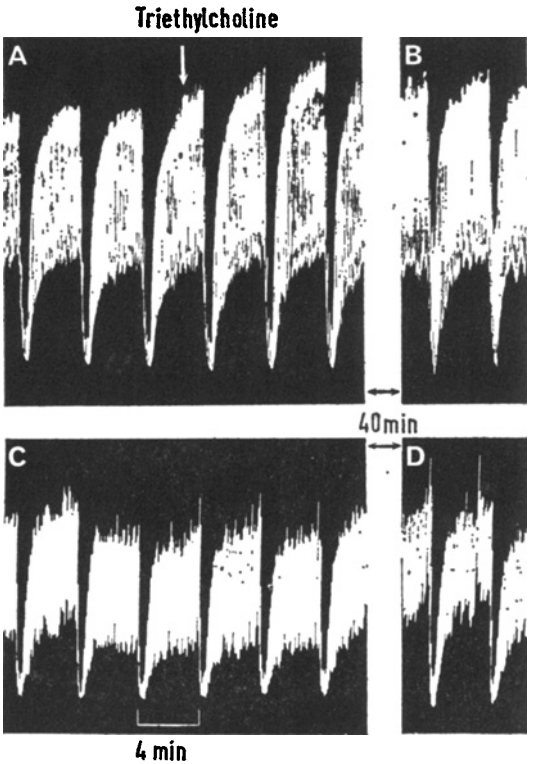

FIG. 7. Responses of rabbit ileum to sympathetic nerve stimulation with 2 msec pulses at 50/sec for 30 sec every 4 min. The records in the upper and lower rows are from two adjacent segments from the same rabbit set up at the same time in twin baths. The preparation in the upper row was treated with triethylcholine (400 μg/ml), the other preparation served as a control. Between A and B, and between C and D, 40 min elapsed during which two 12 min periods of continuous stimulation at 50/sec were given. This caused no impairment of the inhibitory response in the absence of triethylcholine, but there was a substantial reduction in response in its presence.

It was fortunate for our purposes that the guinea-pig colon responded maximally to exactly the same stimulation applied to either nerve, since it allowed a direct comparison of their relative susceptibilities to the blocking action of hemicholinium. In general, the responses to stimulation of the parasympathetic nerve are more easily and more regularly blocked than those of the sympathetic nerve. The difference in the effects of hemicholinium on response to the two nerves may be explained by the observations on the initial responses. Thus, pelvic nerve stimulation

produced a transient contraction which faded during the continued application of a train of stimuli, but sympathetic stimulation produced a response which recovered slowly after stimulation and which persisted throughout continuous stimulation of 30 min.

In some experiments a motor response was observed on stimulation of the sympathetic nerve when the relaxation had been partly or completely blocked by hemicholinium. It is unlikely that the motor response was obscuring the inhibitory response since it was so brief, and at first it was followed by inhibition. This motor response was not due to stimulation of cholinergic fibres, and differed from the motor response produced under certain circumstances by stimulation of the sympathetic nerves to the rabbit intestine (Gillespie & Mackenna, 1961; Day & Rand, 1961). Munro (1953) sometimes observed a contraction of guinea-pig intestine to periarterial nerve stimulation which was not blocked by atropine, anti-adrenaline or antihistamine drugs.

The responses of the guinea-pig colon to stimulation of the pelvic nerve involved cholinergic transmission at two junctions: the ganglionic synapse and the postganglionic endings. Transmission in the ganglionic synapses is easily blocked by hemicholinium (MacIntosh, Birks & Sastry, 1956), and it is possible that the greater sensitivity to blockade of the pelvic nerves to the colon is due to the presence of the ganglionic synapse: the facility of blockade of the responses of the guinea-pig vas deferens to hypogastric nerve stimulation has been explained in this way (Bentley & Sabine, 1963; Birmingham & Wilson, 1963). Armaly, Whinery & Long (1963) reported that there was no difference in the rate of development or the extent of the blockade of ocular response produced by stimulation of the pre- and postganglionic ciliary nerves in the cat. However, some postganglionic cholinergic nerves may be relatively resistant to hemicholinium since high doses of hemicholinium and vigorous stimulation are required to cause failure of the responses of the rat bladder to stimulation of the pelvic nerves (Huković, Rand & Vanov, 1964). Furthermore, the contractions of the guinea-pig ileum produced by Paton's method of transmural stimulation showed no signs of failure in the presence of either hemicholinium or triethylcholine (B. Hemsworth, unpublished observations), although it is believed that this stimulation excites postganglionic cholinergic nerves (Paton, 1955).

The blocking action exerted by hemicholinium on transmission is thought to be due to the depletion of acetylcholine from reserves in the nerve endings, as a result of the impaired resynthesis being inadequate to replace the amounts released by repeated stimulation (MacIntosh, Birks & Sastry, 1956; Lewartowski & Bielecki, 1963). The reversal of hemicholinium-induced blockade by choline provides good evidence that the failue is due to impaired synthesis of acetylcholine, since the inhibition of synthesis *in vitro* is prevented by choline (Gardiner, 1961), and it is believed that hemicholinium acts by competing with choline for a membrane transport system (see Schueler, 1960 and MacIntosh, 1961). Bevan & Su (1964) observed a reduction in contractions of the rabbit isolated pulmonary artery elicited by sympathetic nerve stimulation, but they

maintained that this was no evidence for a cholinergic step being involved because choline did not cause a restoration of contractions. However, Long (1961) in a brief note, gave the opinion that reversal of hemicholinium blockade of autonomic nerves with choline was less effective and required larger doses than does reversal of blockade of a somatic motor nerve. Our findings support this conclusion. We have been successful on occasions in overcoming the depression of sympathetic responses with choline, and we have occasionally been unsuccessful in overcoming depression of parasympathetic responses. There was no qualitative difference in the efficacy of choline in reversing the hemicholinium on either nerve.

An explanation that has been put forward to account for the failure of sympathetic responses caused by hemicholinium is that the sensitivity of the tissue to direct electrical stimulation is reduced at the same time (Bentley & Sabine, 1963; Bevan & Su, 1964). Our finding was that noradrenaline had the same actions after sympathetic failure as before, therefore we cannot support the explanation of reduced sensitivity in the instance of the guinea-pig ileum.

The comment we wish to make on the present findings is the same as that made by Chang & Rand (1960). It is possible that hemicholinium acts in one way at sympathetic nerve endings and in a different way at cholinergic nerve endings, but if a single mechanism is responsible the following argument holds. Either hemicholinium causes transmission failure by interfering with acetylcholine synthesis, in which case it provides evidence for a cholinergic link at sympathetic nerve endings, or its actions are unrelated to acetylcholine, in which case a new explanation must be found for the blockade of repeatedly stimulated motoneurones, preganglionic nerves and parasympathetic postganglionic nerves. In the light of other evidence (recently reviewed by Burn & Rand, 1964) the first alternative has much to recommend it.

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