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AN ANALYSIS OF THE SYMPATHOMIMETIC EFFECTS OF ACETYLCHOLINE ON THE RAT ILEUM

BY

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In 1959 Burn & Rand postulated that impulses passing along post-ganglionic sympathetic nerves released acetylcholine at their terminals, which in turn liberated noradrenaline as the final sympathetic transmitter.

In subsequent years considerable evidence has been marshalled in support of this hypothesis (Koelle, 1962; Burn, 1963; Burn & Rand, 1965). On the other hand, several alternative interpretations of the observations on which the hypothesis of Burn & Rand (1959) is based have been put forward (Gillespie & Mackenna, 1961; Ferry, 1963; Leaders, 1963). A growing amount of experimental evidence which tends to deny the general applicability of the hypothesis of Burn & Rand (1959) has also accumulated during the last few years (Gardiner, Hellman & Thompson, 1962; Nystrom, 1962; Leaders & Long, 1962; Trendelenburg, 1962; Bentley, 1962; Blakeley, Brown & Ferry, 1963; Leaders, 1963; Bentley & Sabine, 1963; Adams & Bay, 1963; Whelan & Skinner, 1963; Bevan & Su, 1964; Dorr & Brody, 1964; Leaders, 1965; Leaders & Dayrit, 1965).

One of the main lines of evidence for the concept that there exists a cholinergic link between the nerve impulse and the release of noradrenaline is that exogenous acetylcholine causes sympathomimetic effects in the presence of atropine in a number of organs. That this action of acetylcholine is dependent on noradrenaline stored in post-ganglionic sympathetic fibres and that it is blocked by adrenergic neurone blocking agents like xylocholine and bretylium, which also block the effects of sympathetic nerve stimulation, are advanced as major arguments in favour of the involvement of acetylcholine in post-ganglionic sympathetic mechanisms.

The sympathomimetic action of acetylcholine has been investigated in the heart (Hoffman, Hoffman, Middleton & Talesnik, 1945); blood vessels (Kottegoda, 1953; Burn & Rand, 1958); pilomotor muscles (Coon & Rothman, 1940; Burn & Rand, 1960) and spleen (Brandon & Rand, 1961). However, there is a paucity of information on the sympathomimetic effect of acetylcholine on the intestine, probably because it is often difficult to abolish the strong muscarinic stimulant action of acetylcholine and thus to unmask the sympathetic component of action (Burn & Gibbons, 1964).

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During routine exploratory experiments in our laboratory we observed that in the rat ileum acetylcholine elicited clear-cut, consistent and dose-related sympathomimetic effects in the presence of hyoscine. This afforded us an opportunity of analysing the sympathomimetic effects of acetylcholine on intestinal smooth muscle and seeing whether the results fitted the hypothesis of Burn & Rand (1959).

METHODS

Isolated rat ileum preparation

Healthy albino rats of either sex and weighing between 150 g and 200 g were used for obtaining the ileum preparation. All animals were starved for 18 hr but had free access to water before being used for experiment. The animals were stunned by a blow on the head and bled by cutting neck vessels. The abdominal wall was opened and the lower 15 to 20 cm of ileum was removed and washed in Tyrode solution (sodium chloride 8 g; potassium chloride 0.2 g; calcium chloride 0.2 g; magnesium chloride 0.1 g; sodium bicarbonate 1 g; sodium dihydrogen phosphate 0.05 g and dextrose 1 g and distilled water up to 1 l.). A piece of ileum 2 cm long was suspended in an organ bath of 35 ml. capacity. The tissue was suspended in an organ bath containing Tyrode solution (containing hyoscine 1 μg/ml.) at 37° C and was bubbled with air. The movements were recorded on a smoked drum with isotonic frontal writing lever that placed the tissue under 250 mg of tension in the horizontal equilibrium position. The lever system employed gave an eightfold magnification. The preparation was allowed to stabilize for 15 min before testing was begun.

Reserpine treatment. Rats were given reserpine 2 mg/kg subcutaneously for eight successive days and were killed 24 hr after the last injection.

Drugs. Acetylcholine chloride, noradrenaline bitartrate, bretylium tosylate, guanethidine sulphate, choline 2:6 xylyl ether (TM 10), dexamphetamine sulphate, methylamphetamine hydrochloride, hexamethonium chloride, mecamylamine hydrochloride, pempidine tartrate, hyoscine hydrobromide, cocaine hydrochloride, reserpine (Serpasil, Ciba), pronethalol hydrochloride and phentolamine methane sulphonate were used throughout and their doses are expressed in terms of the salt. Adrenaline base and nicotine base were used and their doses refer to the base.

All drugs were dissolved in Tyrode solution immediately before use. The total volume of fluid added to the bath in any given response cycle did not exceed 1 ml.

RESULTS

Sympathomimetic effect of acetylcholine on the rat ileum

In the presence of hyoscine (1 μg/ml.), acetylcholine (4 to 8 μg/ml.) caused a clear-cut and dose-related inhibition of the tone and movements of the rat ileum.

Added at 6-min intervals acetylcholine elicited consistent and reproducible responses throughout the duration (4 hr) of individual control experiments. For further analysis of the effect of acetylcholine a dose of 6 μg/ml. was chosen.

Effect of adrenaline receptor blocking drugs

In eight experiments either pronethalol (5 μg/ml. in four experiments) or phentolamine (10 μg/ml. in four experiments) was placed in the bath 5 min before the addition of acetylcholine. Pronethalol or phentolamine only partially blocked the relaxant effect of acetylcholine or of adrenaline (0.025 μg/ml.). Simultaneous exposure of the ileum to both phentolamine and pronethalol, however, resulted in total block of the responses to both acetylcholine and adrenaline (Fig. 1).

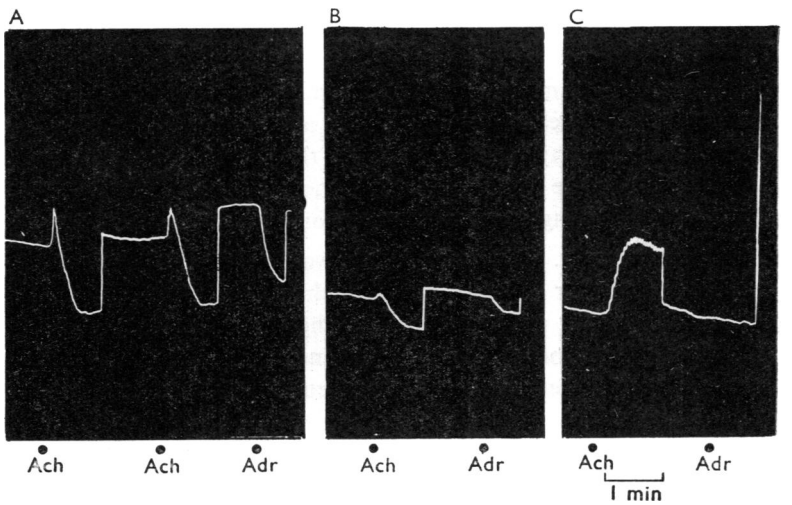

Fig. 1. Rat isolated ileum (suspended in Tyrode solution containing hyoscine, 1 μg/ml). Responses to acetylcholine (6 μg/ml. at Ach); and to adrenaline (0.025 μg/ml. at Adr). Panel A shows control responses; panel B shows responses after the addition of pronethalol (5 μg/ml.) for 5 min to the bath; and panel C shows responses after the addition of pronethalol (5 μg/ml.) and phentolamine (10 μg/ml.) for 5 min to the bath. Time mark, 1 min.

Effect of adrenergic neurone blocking agents

Xylocholine. The preparation was exposed to xylocholine (20 μg/ml.) for a period of 20 min, at the end of which acetylcholine was added. Of a total of 11 experiments, xylocholine completely blocked the effect of acetylcholine in four, considerably reduced it in six and had no effect in one. Responses to adrenaline (0.025 μg/ml.) were unaffected.

In the majority of experiments the blocking action of xylocholine could be reversed by washing out the drug; in a few instances it lasted for 10 to 15 min after removal of the drug from the bath.

Bretylium. After a 20-min exposure to bretylium (20 μg/ml.) responses of the rat ileum to acetylcholine were either totally blocked (11 experiments), considerably reduced (nine experiments) or unaltered (four experiments). Responses to adrenaline (0.025 μg/ml.) were either potentiated (16 experiments) or unaffected (eight experiments). In most instances where bretylium caused total blockade, a contractile response was observed after the addition of acetylcholine. This response was inhibited by the addition of hyoscine (total concentration 2 μg/ml.). In these experiments the blocking effect persisted for 10 to 15 min after washing. When bretylium only reduced the effect of acetylcholine, the blocking action was reversed on washing.

Guanethidine. Guanethidine (20 μg/ml. for 20 min) totally blocked the response to acetylcholine (10 experiments). The blocking action could not be readily reversed by washing and persisted for 60 to 90 min despite repeated washing. Responses to adrenaline (0.025 μg/ml.) were either potentiated (six experiments) or unaffected (four experiments).

Reversal of the blocking action of xylocholine, bretylium and guanethidine by dexamphetamine and methylamphetamine

Dexamphetamine, methylamphetamine and certain other indirectly acting sympathomimetic amines are capable of reversing the adrenergic nerve blocking action of bretylium, guanethidine and xylocholine. It was, therefore, of interest to see if a similar reversal of blockade of the effects of acetylcholine by bretylium, guanethidine or xylocholine occurred following dexamphetamine or methylamphetamine.

Xylocholine and bretylium. The preparation was exposed to xylocholine (20 μg/ml.) or bretylium (20 μg/ml.) for 20 min, after which it was washed. The blocking drug again added and, after 5 min, responses to acetylcholine were elicited and found blocked. This was followed by addition of dexamphetamine (10 μg/ml.) or methylamphetamine (10 μg/ml.); responses to acetylcholine were redetermined 10 min later.

Dexamphetamine totally reversed the blocking effect of xylocholine in six experiments but did not have any effect in two. With bretylium reversal of blocking action following dexamphetamine was observed in 10 out of 11 experiments (Fig. 2).

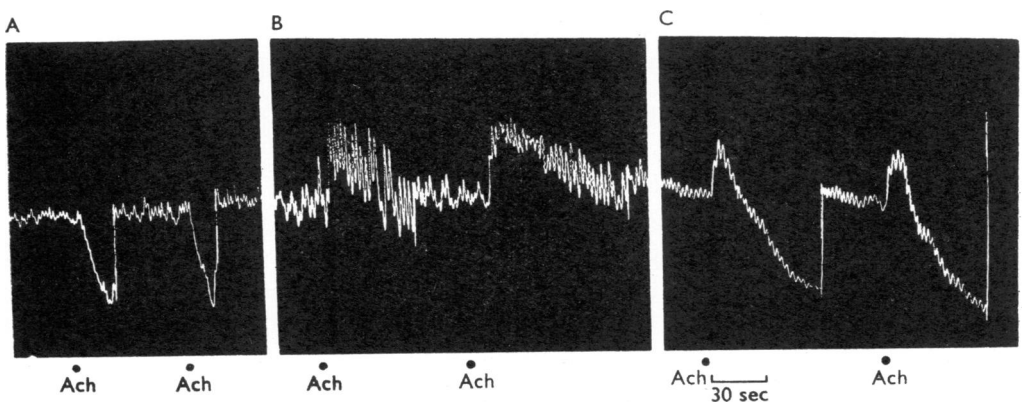

Fig. 2. Rat isolated ileum (suspended in Tyrode solution containing hyoscine, 1 μg/ml.). Responses at dots to acetylcholine (6 μg/ml.). Panel A shows control responses; and panel B shows responses after the preparation was exposed to bretylium (20 μg/ml.) for 20 min, washed, and again exposed to bretylium (20 μg/ml.) for 5 min. Without giving a wash dexamphetamine (10 μg/ml.) was now placed in the bath for 10 min and responses shown in panel C were taken. Time mark, 30 sec.

In two experiments methylamphetamine totally reversed the blocking effect of xylocholine, the blocking effect of bretylium was similarly reversed in five out of six experiments.

Guanethidine. The ileum was exposed to guanethidine (20 μg/ml.) for 20 min and then it was washed. Responses to acetylcholine were now totally blocked. Dexamphetamine or methylamphetamine was added to the bath and was allowed to act for 10 min; responses to acetylcholine were then redetermined.

Dexamphetamine reversed the blocking effect of guanethidine in only one out of three preparations, whereas with methylamphetamine reversal was observed in three out of five preparations (Fig. 3).

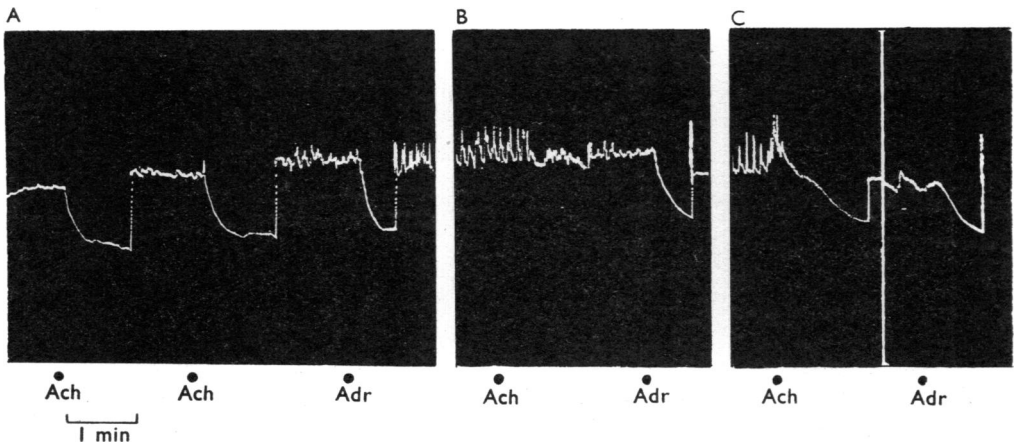

Fig. 3. Rat isolated ileum (suspended in Tyrode solution containing hyoscine, 1 μg/ml.). Responses to acetylcholine (6 μg/ml. at Ach); and to adrenaline (.025 μg/ml. at Adr). Panel A shows control responses; and panel B shows responses after the preparation was exposed to guanethidine (20 μg/ml.) for 20 min and was then washed. Without giving a wash methylamphetamine (10 μg/ml.) was now placed in the bath for 10 min and responses shown in panel C were taken. Time mark, 1 min.

Effect of pretreatment with reserpine

There was a tendency for some preparations obtained from reserpine-treated animals to lose tone as the experiment progressed.

The relaxant effect of acetylcholine was almost totally absent in preparations removed from rats with reserpine. Out of a total of 12 preparations studied, acetylcholine produced no effect in two, a contraction in five and a slight relaxation in five.

After an initial testing of the effect of acetylcholine, the preparations were exposed to noradrenaline (2 μg/ml.) for a period of 30 min, during which the ileum relaxed; the noradrenaline was then removed and the preparations repeatedly washed for 20 min. In 11 out of the 12 preparations the relaxant effect of acetylcholine was now fully restored.

In some of these experiments bretylium (20 μg/ml.), xylocholine (20 μg/ml.) or guanethidine (20 μg/ml.) was added after acetylcholine responses were fully restored to see if these could now be blocked. Bretylium (three experiments), xylocholine (three experiments) and guanethidine (two experiments) completely blocked the relaxant effect of acetylcholine restituted following exposure to noradrenaline. Figure 4 shows such a blocking action of xylocholine.

Effect of ganglion blocking drugs

The preparation was exposed to the ganglion blocking drugs hexamethonium (10 μg/ml.), pempidine (5 μg/ml.) and mecamylamine (5 μg/ml.) for 10 min and responses to acetylcholine were determined in the presence of the blocking drug; effects on responses to nicotine (2 μg/ml.) and adrenaline (0.025 μg/ml.) were also tested simultaneously. Hexamethonium (12 experiments), pempidine (four experiments) and

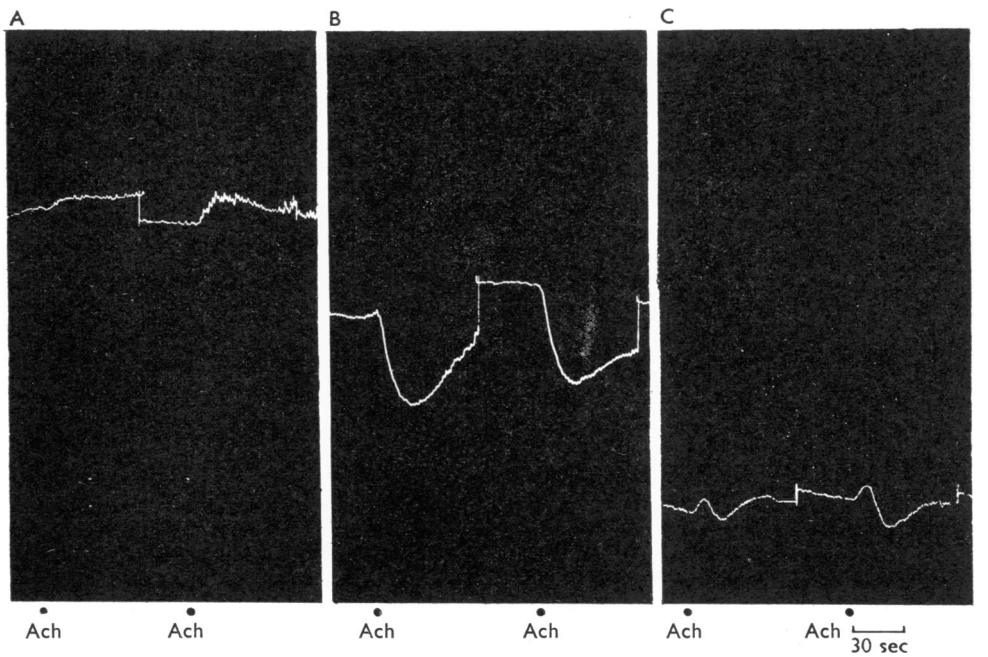

Fig. 4. Rat isolated ileum (suspended in Tyrode solution containing hyoscine, 1 μg/ml.) from reserpine treated rat. Responses at dots to acetylcholine (6 μg/ml.). Panel A shows control responses; panel B shows responses after the preparation was exposed to noradrenaline (2 μg/ml.) for 30 min and then repeatedly washed for 20 min; and panel C shows responses after xylocholine (20 μg/ml.) was placed in the bath for 20 min. Time mark, 30 sec.

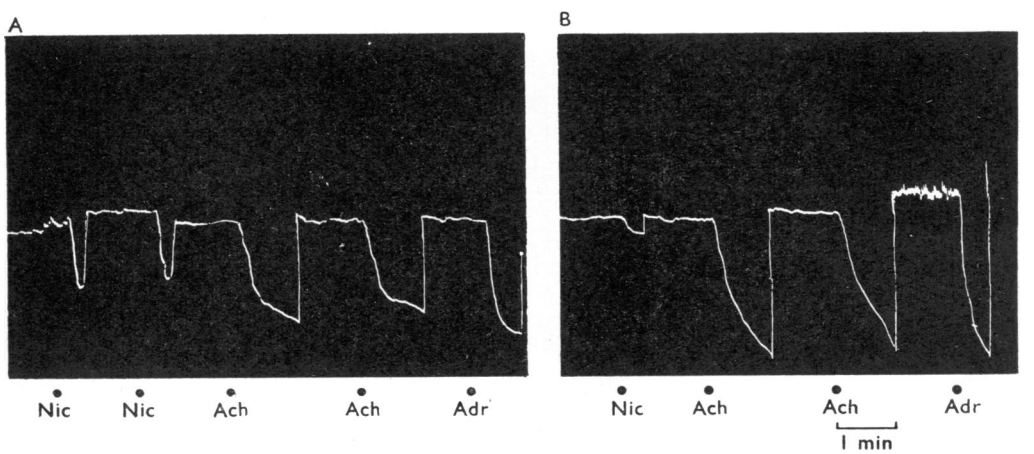

Fig. 5. Rat isolated ileum (suspended in Tyrode solution containing hyoscine, 1 μg/ml.). Responses to nicotine (2 μg/ml. at Nic); to acetylcholine (6 μg/ml. at Ach); and to adrenaline (.025 μg/ml. at Adr). Panel A shows control responses; and panel B shows responses after the preparation was exposed to hexamethonium (10 μg/ml.) for 10 min. Time mark, 1 min.

mecamylamine (four experiments) potentiated the responses to acetylcholine and adrenaline but totally blocked the responses to nicotine. In two experiments with hexamethonium (10 μg/ml.), the response to nicotine (2 μg/ml.) was almost totally blocked (Fig. 5).

Effect of cocaine

The ileum was exposed to cocaine (10 μg/ml.) for 5 min and responses to acetylcholine and adrenaline (0.025 μg/ml.) were elicited in the presence of cocaine. Cocaine (four experiments) did not modify the relaxant effect of acetylcholine but potentiated the effect of adrenaline.

DISCUSSION

Acetylcholine, in the presence of hyoscine, caused a relaxation of the rat ileum, which was partially blocked by phentolamine or pronethalol but was completely abolished following simultaneous exposure to both the blocking agents. This result would indicate that the relaxant action of acetylcholine on intestinal smooth muscle is mediated through an adrenergic mechanism involving both α and β types of adrenergic receptors.

Sympathomimetic effects of acetylcholine have been demonstrated in a number of other organs and this and many other considerations have led Burn & Rand (1965) to propose a cholinergic link between post-ganglionic sympathetic nerve impulses and the release of noradrenaline as the final transmitter. If post-ganglionic sympathetic nerve function indeed involves the liberation of noradrenaline by acetylcholine released within or in the vicinity of the nerve terminals by a nerve impulse then there must exist a close similarity between the pharmacological behaviour of the effects of sympathetic nerve stimulation and those of exogenous acetylcholine. A review of the pertinent literature reveals that such a similarity does exist in certain respects but is lacking in others. Thus a need for further investigation of the sympathomimetic effects of acetylcholine in different test organs definitely exists.

In the rat ileum the relaxant effects of acetylcholine were blocked by xylocholine, bretylium and guanethidine. Both bretylium and xylocholine have an obvious structural similarity to acetylcholine and it is understandable that they can act as antagonists of acetylcholine in certain situations. Even guanethidine, which appears to be structurally rather dissimilar to acetylcholine, contains a guanidine group which is functionally similar to the trimethylammonium group of acetylcholine. It is significant that these same drugs also block adrenergic nerve function, and it is conceivable that they might do so by interfering with the action of acetylcholine at post-ganglionic sympathetc nerve fibres as proposed by Burn & Rand (1965).

Block of acetylcholine responses by xylocholine could be easily removed by washing the preparation, whereas the action of bretylium often persisted for 10 to 15 min after washing. The block following guanethidine was even more persistent and lasted for as long as 90 min after repeated washing. Thus, we did not observe a total lack of persistence in the antagonism of acetylcholine by these drugs as reported by previous workers (Huković, 1960 ; de la Lande, Tyler & Pridmore, 1962). However, it must be conceded that the abolition of acetylcholine responses following adrenergic neurone blocking drugs

is much less persistent than the block of sympathetic impulses produced by them, as shown by the action of bretylium and guanethidine on the Finkleman preparation of the rabbit small intestine (Birmingham & Wilson, 1965).

Recently certain indirectly acting sympathomimetic amines like dexamphetamine and certain monoamine oxidase inhibitors like phenelzine have been shown to reverse the adrenergic neurone blocking action of bretylium, guanethidine and xylocholine (Day & Rand, 1962; Day, 1962; Gokhale, Gulati & Joshi, 1965; Gokhale, Gulati & Udwadia, 1966). Clinical observations also indicate that amphetamine and related drugs specifically antagonize the hypotensive action of bretylium and guanethidine (Wilson & Long, 1960; Laurence & Rosenheim, 1960; Gulati, Dave, Gokhale & Shah, 1966).

In the present experiments dexamphetamine and methylamphetamine were able to reverse the block of the relaxant effect of acetylcholine by xylocholine and bretylium but it was somewhat more difficult to overcome the block following guanethidine.

Though reversal by dexamphetamine and methylamphetamine of the block of the relaxant sympathomimetic effect of acetylcholine following adrenergic neurone blocking drugs, particularly guanethidine, is not quite as consistent as the reported reversal of the blockade of responses to sympathetic nerve stimulation, an overall similarity between the two phenomena appears to be securely established.

The relaxant effect of acetylcholine was absent in preparations obtained from rats treated with reserpine. Responses to acetylcholine could, however, be fully restored by exposure of the preparation to noradrenaline for 30 min. That the restituted responses could again be blocked by xylocholine, bretylium and guanethidine showed that they were pharmacologically similar to the effect of acetylcholine in control experiments.

Thus the relaxation of intestinal smooth muscle following acetylcholine is an indirect effect and depends upon intact noradrenaline stores in the post-ganglionic sympathetic fibre.

Ferry (1963) pointed out that, whereas hexamethonium blocked the action of acetylcholine and of nicotine, it did not block the response to sympathetic stimulation and since then this finding has constituted a nagging dissimilarity between the effect of post-ganglionic sympathetic stimulation and the effect of acetylcholine.

Burn & Gibbons (1964) using the rabbit isolated ileum, and stimulating the sympathetic nerves in the mesentery, found that the blocking of the inhibitory response to stimulation by bretylium was not seen when hexamethonium was present in the bath. Hexamethonium was thought to act by preventing the entry of bretylium into the sympathetic fibre and a similar explanation was offered to account for the block of acetylcholine responses by hexamethonium.

In the present experiments hexamethonium, pempidine or mecamylamine failed to block the relaxant effect of acetylcholine. Indeed they considerably potentiated the effect of acetylcholine, in concentrations which totally blocked the relaxant effect of nicotine. This result was observed in all the 20 experiments in which the ganglion blocking drugs were used. Our findings with ganglion blocking drugs, though at complete variance with the observations of previous workers with other organs, are of considerable significance in that they tend to establish a closer similarity between the effect of post-ganglionic sympathetic stimulation and the effect of acetylcholine.

Cocaine has been reported to potentiate the response to sympathetic nerve stimulation (Haefely, Hürlimann & Thoenen, 1964). However, cocaine had no significant effect on the responses of the rat ileum to acetylcholine.

The results discussed above demonstrate that the sympathomimetic effect of acetylcholine in the rat ileum closely resembles the response to sympathetic nerve stimulation in its pharmacological behaviour and the observations are generally consistent with the proposed (Burn & Rand, 1959) role of acetylcholine in sympathetic nerve function.

SUMMARY

1. Acetylcholine in the presence of hyoscine caused a relaxation of the rat ileum. Pronethalol or phentolamine only partially blocked the effect of acetylcholine but simultaneous exposure to both the blocking agents resulted in a total elimination of the response.

2. Bretylium, xylocholine and guanethidine blocked the relaxant effect of acetylcholine ; this block was many times reversed by dexamphetamine or methylamphetamine.

3. Pretreatment with reserpine blocked the effect of acetylcholine but this could be restored by exposing the ileum to noradrenaline. The restored response could again be blocked by bretylium, xylocholine or guanethidine.

4. Hexamethonium, pempidine and mecamylamine potentiated the effect of acetylcholine but blocked the effect of nicotine.

5. Cocaine did not modify the effect of acetylcholine.

6. The results demonstrate a close resemblance of the effect of aceylcholine to the response to sympathetic stimulation and are consistent with the concept of a cholinergic link between the sympathetic nerve impulse and the release of noradrenaline.

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