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EVIDENCE FOR THE RELEASE OF TWO ATROPINE-RESISTANT SPASMOGENS FROM AUERBACH'S PLEXUS

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SUMMARY

1. Two atropine-resistant response-components of nervous origin have been detected in plexus-containing preparations of the longitudinal muscle from the guinea-pig ileum, by alternate field stimulation with equal numbers of pulses at 50 Hz (response A) and at 5 Hz (response B). With trains of ten or more pulses, response A is always larger than B; the ratio of A/B (1·2–21·3) is subject to animal variation.

2. Both responses are abolished by tetrodotoxin and are absent from plexus-free preparations.

3. Neither response is reduced by ganglion-block with (+)-tubocurarine, dimethyltubocurarine or hexamethonium, or by ganglion-paralysing doses of nicotine; the contribution of excited preganglionic endings to these responses is therefore negligible.

4. Neither response is due to a release of histamine, 5-hydroxytryptamine (5-HT) or prostaglandins, since both A and B persist in the presence of mepyramine, methysergide and the prostaglandin-antagonist SC-19220 (1-acetyl-2(8-chloro-10,11-dihydrodibenz [b,f] [1,4]oxazepine-10-carbonyl) hydrazine).

5. The two response-components are affected differentially by a number of drugs.

6. Histamine, 0·1 μg/ml., reduces response A to the level of B; this selective inhibition of the histamine-sensitive component in A is specifically antagonized by nicotine, $1–2·5 \times 10^{-5}$ g/ml.

7. 5-HT, 0·1 μg/ml., and strychnine, 20–40 μg/ml., also reduce response A to the level of B, but these selective inhibitions are not antagonized by nicotine.

8. Diphenhydramine, 10 μg/ml., produces equality of the two responses by depressing A and potentiating B.

9. The inhibitory effects of the foregoing drugs are not due to catecholamine release, since they persist after $\alpha + \beta$ adrenoceptor blockade with phentolamine and pronethalol, and after previous reserpinization of the guinea-pigs.

10. In atropinized plexus-containing preparations of the longitudinal muscle from the guinea-pig descending colon, the responses elicited at 50 Hz and at 5 Hz are virtually equal and both appear to be of Type B since they are not inhibited by histamine, 5-HT or strychnine; diphenhydramine produces strong contractions.

INTRODUCTION

Strong evidence for the existence in Auerbach's plexus of non-cholinergic neurones, perhaps the Dogiel Type I and III cells(Dogiel, 1899; Hill, 1927), has been obtained in this laboratory (Ambache & Freeman, 1968*a*, *b*). The humoral operation of these neurones was detected by recording the atropine-resistant spasms which were evoked when plexus-containing preparations of the longitudinal muscle from the guinea-pig ileum were excited at regular intervals with 1 sec trains of 0·2 msec pulses at a set frequency of 50 Hz. On several occasions the spasms were seen to consist of three components, two of which were of nervous origin since they could be extinguished by tetrodotoxin.

In the present paper these atropine-resistant response-components have been distinguished more clearly in two ways: first, by comparing the response at the frequency used previously, 50 Hz, with the response to an equal number of pulses at a lower frequency, fixed at 5 Hz. These two responses have been termed A and B respectively. The ratio of A to B, though constant in any given experiment, has varied from 1·2 to 21·3 in different preparations, probably because of animal variation. Secondly, it has been found that a number of drugs exert differential effects upon these two responses. For instance, histamine, 5-HT and strychnine selectively inhibit a particular component in response A, and so reduce this response to the level of response B; whilst diphenhydramine has opposite effects on the A and B responses, also resulting in their final equality.

These results suggest that field stimulation releases, in addition to acetylcholine, not one, but two atropine-resistant spasmogens from Auerbach's plexus, possibly from the morphologically distinct Type I and Type III Dogiel cells in that plexus. The faster decay of one of these can be deduced from the comparison of the responses at high and at low frequency. The differential effects of the four drugs named also imply some biochemical distinction between the two spasmogens concerned.

A preliminary account of these results, which were demonstrated to the Physiological Society, has appeared previously (Ambache, Verney & Zar, 1970).

METHODS

In order to reduce biological variation, the guinea-pigs used were all male albinos; large animals were selected, as they yield more robust preparations.

The procedure described previously (Ambache & Freeman, 1968*b*) was used for stripping off the longitudinal muscle from the ileum with Auerbach's plexus, or without it (Paton & Zar, 1968). As before, the anomalous terminal portion of the ileum (Munro, 1953) was avoided and most of the preparations were taken at a distance of 20–40 cm from the ileocaecal valve. The design of the 2 ml. organ-bath with built-in vertical platinum electrodes was unchanged, as was the bath temperature and the composition of the bath fluid, Krebs–Henseleit solution, gassed with a mixture of 95 % O_2 and 5 % CO_2 in the reservoir and in the organ-bath. For better survival of the preparations a gentle throughflow, observable for regulation in a drop chamber below the reservoir, was maintained continuously except during drug contacts, when the tap was fully closed. Occasionally (see Fig. 7) slight, transient reduction of the response to electrical stimulation occurred just after drugs were washed out. It is unlikely that this was due to a temperature change during these washes (3–5 sec) since a large warming coil of 50 ml. capacity was interposed between reservoir and bath; but a small temporary change in pH, due to a possible fall in P_{CO_2} of the Krebs–Henseleit solution in transit from the reservoir and until its re-equilibration with the CO_2 bubbling through the organ-bath, might account for an effect of this kind.

Either of the two previously described electronic stimulators capable of delivering high currents (Bell, 1968) was used at outputs of either 27 V at 500 mA or 20–22 V at 800 mA; these settings were the highest possible without overload. The pulse width was fixed at 0·2 msec for all experiments. The atropine-resistant tetanic spasms were elicited at 1 min intervals by trains usually of ten pulses, either at 50 Hz to elicit 'response A', the duration of trains then being set at 0·2 sec, or at 5 Hz for 'response B', with a train duration of 2 sec. Each pulse was accompanied by an audible click from the microphone incorporated in the stimulators. Pulse delivery could thus be monitored acoustically in all the experiments, and in some the pulse totals could also be read, at the end of each train, on the numeral indicators of a TC-11 Timer Counter (Advance Electronics Ltd., Hainault, Essex). Allowance was made for the staircase phenomenon (Ambache & Freeman, 1968*b*) before initiating drug tests.

Plexus-containing preparations of the longitudinal muscle were obtained from the taenia-free guinea-pig descending colon at a distance of 70–80 cm below the ileocaecal valve, by the same procedure as for the ileum preparations.

Reserpinization. Two guinea-pigs were injected intraperitoneally with an aqueous solution of reserpine phosphate (5 mg/ml.) in a daily dose of 10 mg/kg for 3 days before the experiment.

Drugs. The dosages, given in the text, of the following drugs refer to their salts, namely: atropine sulphate; betazole, 2 HCl; dimethyltubocurarine bromide; diphenhydramine, HCl; (−)-ergothioneine, HCl.$2H_2O$; hexamethonium bromide; histamine, 2HCl; hordenine sulphate; 5-hydroxytryptamine creatinine sulphate; mepyramine maleate; methysergide bimaleate; nicotine hydrogen tartrate; (−)-noradrenaline bitartrate; phentolamine mesylate; pronethalol, HCl; semicarbazide, HCl; strychnine, HCl.$2H_2O$; (+)-tubocurarine chloride.$5H_2O$.

We are indebted to Dr J. H. Sanner, Searle & Co., Chicago, Illinois, U.S.A. for a supply of the prostaglandin-antagonist, 1-acetyl-2-(8-chloro-10,11-dihydrodibenz

[b,f] [1,4]oxazepine-10-carbonyl) hydrazine (SC-19220) and for the information that the limit of its solubility in water is *ca.* 5 μg/ml.

RESULTS

A. Experiments on preparations from the guinea-pig ileum

I. Influence of stimulation-frequency upon the height of atropine-resistant tetanic spasms

In previous experiments (Ambache & Freeman, 1968*a*, *b*) the atropine-resistant tetanic spasms were induced by field stimulation of plexus-containing preparations of the longitudinal muscle from the guinea-pig ileum with 1 sec trains of 0·2 msec pulses delivered over a wide range of frequencies between 10 and 100 Hz, but most commonly at 50 Hz, i.e. with 50 pulses for each response. In the present investigation tetanic spasms could be obtained, again in the presence of atropine, 10^{-8}–10^{-6} (usually 2×10^{-7}) g/ml., with 1 sec trains at even lower frequencies, 2–10 Hz, i.e. with as few as 2–10 pulses for each response. At any fixed frequency the responses grew bigger on increasing the number of pulses per train by lengthening the train duration.

In experiments in which the total number of pulses in each tetanic train was kept constant (usually either 10 or 20 pulses) but in which their frequency within the trains was varied between 5 and 50 Hz, it was found that the height of the tetanic spasms was greater at higher frequencies; the optimum frequency appeared to be 30–50 Hz. When the response-height obtained with 50 Hz was compared to that obtained with 5 Hz, the ratio was found to vary considerably from preparation to preparation (see the three experiments illustrated in Fig. 1) but was constant throughout the experiment in any preparation from a given animal. For instance, in Fig. 1 the two extremes of this variation, found rarely, are provided by Expts. 1 and 2; in both experiments the spasms were elicited at 1 min intervals by trains of ten 0·2 msec pulses, delivered at frequencies of, alternately, 50 Hz (responses marked A) and 5 Hz (responses marked B). The ratios of the response-heights A/B were 1·2 in Expt. 1, and 21·3 in Expt. 2. The more common type of result obtained in Expt. 3, in which trains of thirty 0·2 msec pulses were used throughout the experiment, occupies an intermediate position, with an A/B ratio of 2·9.

These variations were not correlated with the distance of the preparation from the ileocaecal valve. Whenever two preparations were set up from different regions of the ileum from the same guinea-pig, their behaviour was identical in this respect, which would suggest that the difference between preparations should be attributed to animal variation.

When only 5 pulses were delivered per train, the results sometimes became variable and the response to 5 Hz frequently matched or exceeded that to 50 Hz. However, even in these preparations, on reverting to 10 pulses per train, A was consistently greater than B.

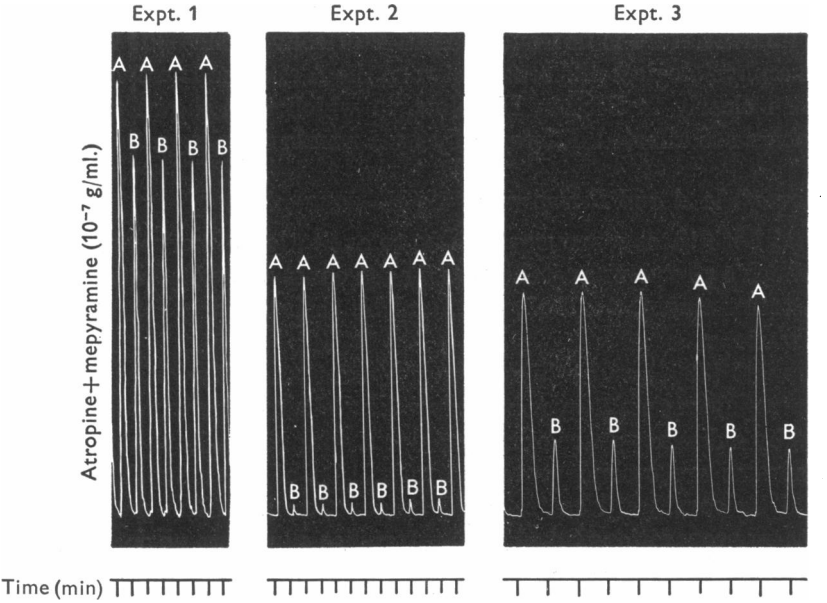

Fig. 1. Animal variation affecting the ratio of response-heights at high and at low stimulation frequency.

Plexus-containing longitudinal muscle preparations from the ileum of three guinea-pigs; suspended in 2 ml. baths containing atropine, 10^{-7} g/ml. and mepyramine, 10^{-7} g/ml. The contractions marked A and B were elicited alternately at 1 min intervals by trains of 0·2 msec pulses delivered at 50 Hz for those marked A and at 5 Hz for those marked B; the number of pulses per train was fixed in each experiment, i.e. ten in Expts. 1 and 2 and thirty in Expt. 3.

The greater efficacy of tetanic stimulation at higher frequencies was demonstrated again in a different type of experiment (Fig. 5). From the first five contractions at the beginning of the tracing, it can be seen that the tetanic spasms marked A, each elicited by a total of ten pulses delivered at the higher frequency (50 Hz), were still slightly greater than the alternate spasms marked B, which were here elicited by twice as many pulses (i.e. a total of twenty) delivered at the lower frequency of 5 Hz. Thus, in this experiment the two responses, A and B, initially dissimilar when evoked by an equal number of pulses (ten per train), were rendered almost equal by doubling the number of pulses per train delivered at the lower

frequency; but, again, the pulse-ratio to achieve this equality varied from one preparation to the next.

Throughout this paper all tetanic spasms elicited by stimulation with 10 or 20 pulses at the higher frequency (50 Hz) will henceforth be referred to as the 'A responses' in contradistinction to the 'B responses' elicited by stimulation with an equal number of pulses at the lower frequency (5 Hz). This nomenclature is not only a matter of convenience but is also based upon a physiological difference between the A and the B responses, as will be seen clearly in Section II below, in which it is shown that the two responses are affected differentially by a number of drugs.

Nature of the A and B responses

That both these responses are due to excitation of nervous elements in Auerbach's plexus was established by the complete extinction of A and B with tetrodotoxin, 2×10^{-7} g/ml., and by their absence from plexus-free preparations.

Bennett (1966) and others have noticed that in the guinea-pig taenia coli atropine-resistant spasms induced by electrical stimulation are preceded by a distinct phase of relaxation. These authors have suggested that the spasms are secondary to the relaxation of the smooth muscle and should be considered as 'rebound contractions'. In the present experiments on preparations taken from the guinea-pig ileum no such relaxation was seen to precede either the A or the B responses. It could be argued, however, that the lack of tone in these ileum preparations, when exposed to atropine, prevented the manifestation of such an initial relaxation phase; but, as shown in Fig. 2, even after the tone was raised considerably by the administration of bradykinin, 5 ng/ml., the A and B responses still occurred without any preceding relaxation and hence do not appear to be due to rebound.

Persistence after ganglion-block. Both the A and the B responses persist after ganglion-block by various means.

The two responses remained unaltered in the presence of nicotine, 25 μg/ml., allowing sufficiently long exposure of the preparation to this drug for the contraction arising from the initial ganglion-cell stimulation to subside and for the ganglion-cells to become fully paralysed and remain unresponsive to further doses of nicotine.

Neither A nor B responses were reduced by hexamethonium, 10^{-4} g/ml.; in fact B responses were, if anything, slightly potentiated by this drug.

In the presence of (+)-tubocurarine, 10–25 μg/ml., or dimethyltubocurarine, 20–50 μg/ml., the B responses were potentiated considerably and to a greater extent than the A responses.

These results suggest that, although the electrical field stimulation as

used in these experiments may excite preganglionic fibres and endings as well as the post-ganglionic elements in the preparations, the cholinergic contribution of any preganglionic excitation to the A or the B responses,

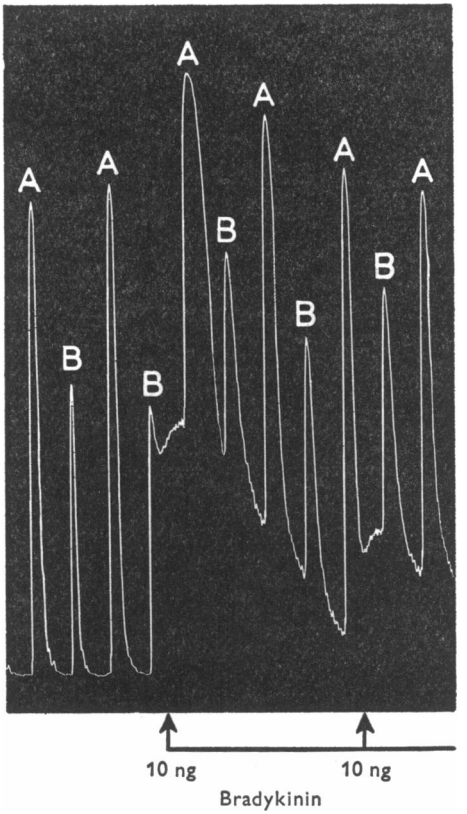

Fig. 2. Occurrence of atropine-resistant tetanic spasms without a preceding inhibitory phase.

Plexus-containing preparation from ileum in atropine and mepyramine, 2×10^{-7} g/ml.; 2 ml. bath. Alternate A and B responses were elicited at 1 min intervals by trains of ten 0·2 msec pulses delivered at 50 Hz for A and 5 Hz for B. After the first four contractions the tone of the preparation was raised by two successive 10 ng doses of bradykinin, at the arrows. Explanation in text.

either by way of additional stimulation of the ganglion cells humorally or by diffusion of the preganglionic transmitter from the preganglionic endings to the muscle fibres and its summation there with the post-ganglionic transmitter effect, is insignificant.

Exclusion of some known humoral agents as mediators of the A or B response. On several occasions the concentration of atropine was raised in steps of ten, first from 10^{-8} to 10^{-7} g/ml. and then to 10^{-6} g/ml., without producing any diminution in either the A or the B response. This type of experiment further excludes acetylcholine as a possible mediator of these responses.

Likewise, it is clear that the A and B responses cannot be due to an intermediate release of histamine, since they remained unaltered after histamine contractions of comparable height were abolished by mepyramine, 10^{-8} g/ml., and after further stepwise increases in the concentration of mepyramine, first from 10^{-8} to 10^{-7}, and then to 10^{-6} g/ml. Only when the concentration of mepyramine was raised still further to 10^{-5} g/ml. was an interesting action observed, affecting the A and B responses in opposite directions and indistinguishable from the dual effect of diphenhydramine, 10^{-5} g/ml., which is described in detail in the next section.

Next, the A and the B responses both persisted after the elimination of 5-HT contractions by block with methysergide, 10^{-8} g/ml. Thus, neither response is due to the excitation of tryptaminergic neurones.

Lastly, the A and the B responses both persisted after the elimination of prostaglandin contractions by means of a suitable antagonist. It has been reported by Sanner (1969) that the contractions elicited by prostaglandin E_2 in the guinea-pig ileum can be abolished by 1-acetyl-2-(8-chloro-10,11-dihydrodibenz [b,f][1,4]oxazepine-10-carbonyl) hydrazine (SC-19220); this block was specific to prostaglandins because the responses to acetylcholine and to bradykinin were not abolished, though both were slightly reduced by the SC-19220. In the present experiments on atropinized preparations it was found that the A and B responses persisted after the extinction of prostaglandin contractions by the presence of 1–5 μg/ml. of SC-19220 in the bath fluid. Such an experiment is illustrated in Fig. 3: after the introduction of the SC-19220 into the reservoir (at F) the A and B responses persisted to the end of the experiment but were slightly reduced; the response to PGE_2, 5 ng/ml., was completely abolished (at G; compare with B) and that to 7·5 ng/ml. was virtually extinguished (H; compare with C), whilst the control responses to bradykinin (I) and to histamine (J) remained unaltered (compare with D and E, respectively). In another experiment SC-19220, 1 μg/ml., again abolished the contractions elicited by PGE_2, 5–10 ng/ml., but allowed the A and the B responses to persist; these were again slightly reduced, but no more than bradykinin contractions of comparable height. When the SC-19220 was washed out the PGE_2 contractions recovered fully but the bradykinin contractions and the A and B responses failed to recover from this slight depression. These results exclude prostaglandins as possible mediators of the A or B responses.

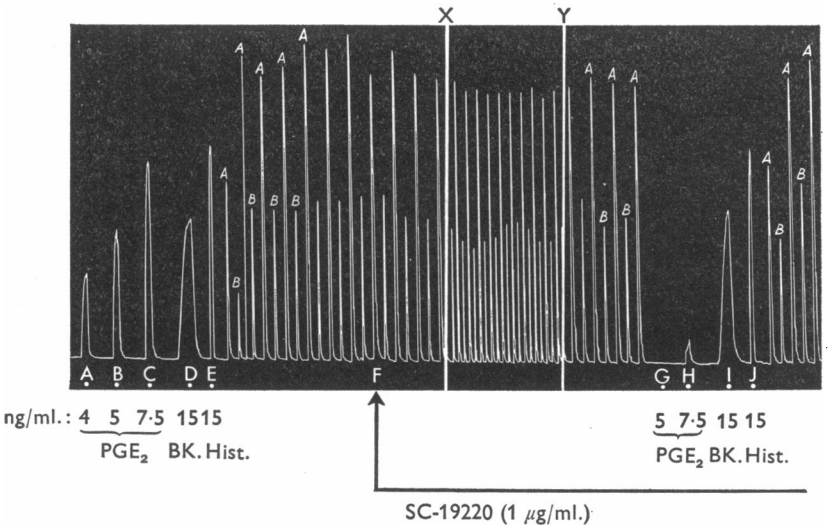

Fig. 3. Persistence of A and B responses after block of prostaglandin contractions by an antagonist. Plexus-containing preparation from ileum in atropine, 2×10^{-7} g/ml.; 2 ml. bath. At the dots: A–C and G–H, contractions elicited by PGE_2 4, 5 or 7·5 ng/ml., 30 sec contacts; D and I, bradykinin 15 ng/ml. (1 min); E and J, histamine 15 ng/ml. (20 sec). Between E and G and after J, alternate A and B responses elicited at 1 min intervals by trains of ten 0·2 msec pulses delivered at 50 Hz for A and 5 Hz for B; the first few responses after E and J display the staircase phenomenon (see Methods). Between X and Y, drum at half speed. From the arrow at F to the end of the experiment, the prostaglandin antagonist SC-19220 (1-acetyl-2-(8-chloro-10,11-dihydrodibenz[b,f][1,4]oxazepine-10-carbonyl) hydrazine), 1 μg/ml., present in the bath fluid.

II. Differential effects of drugs upon the atropine-resistant A and B responses

Histamine

The inhibition of atropine-resistant spasms by histamine in mepyramine-treated preparations, in which the contractile effect of histamine has been eliminated was described previously (Ambache & Zar, 1969, 1970). As the frequency of stimulation in most of those earlier experiments had been fixed at 50 Hz, the inhibitory effect of histamine has now been re-examined at both high and low frequencies of stimulation; the concentration of mepyramine has usually been 2×10^{-7} g/ml. but on several occasions even higher, 5×10^{-7}, 10^{-6} or even 10^{-5} g/ml. The results of the present experiments show clearly that histamine affects the A much more than the B response.

The inhibitory effect of histamine was first investigated on preparations in which the A and B responses were elicited at 1 min intervals by an

identical number of pulses, usually ten, per train. Under these conditions the effect of histamine, 0·1 μg/ml., has been to leave the B responses to 5 Hz almost unaffected, but to reduce the A responses (to 50 Hz) down to the level of the B responses; this is illustrated by Expt. 1 of Fig. 4. However, histamine may frequently also depress the B responses to a variable extent, though less than the A responses. Two extreme examples are illustrated in Fig. 4: in Expt. 1 the B response was reduced only slightly, but in Expt. 2 it was reduced to 55 % of its initial height. In both experiments, as a result of the much greater depression of A, the A and B responses became virtually equal.

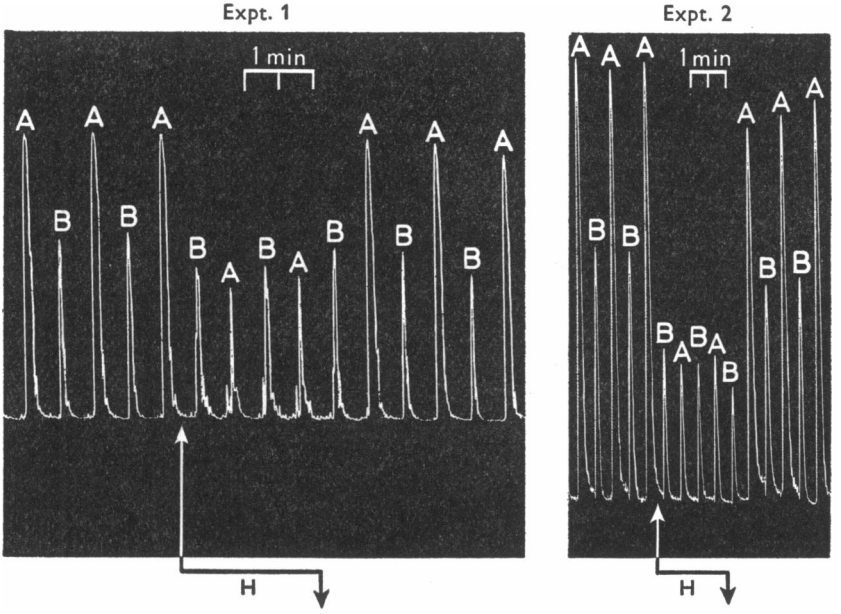

Fig. 4. Reduction by histamine of the A response to the level of the B response; the B response is either unaffected (Expt. 1) or also reduced by histamine (Expt. 2).

Plexus-containing preparations from the ileum of two guinea-pigs, suspended in 2 ml. baths containing atropine, 10^{-7} g/ml., and mepyramine (2×10^{-7} g/ml. in Expt. 1 and 5×10^{-7} g/ml. in Expt. 2). Alternate A and B responses were elicited at 1 min intervals by trains of ten 0·2 msec pulses delivered at 50 Hz for A and 5 Hz for B. At H, between the arrows, histamine, 0·1 μg/ml., left in the bath for 4 min.

When an equal number of pulses is used for eliciting the A and B responses there is usually a considerable disparity between the heights of these two responses, and it could be argued that the greater inhibition of the A responses just described was only due to the fact that they were initially larger than the B responses. However, the results illustrated in

Fig. 5 demonstrate the greater inherent susceptibility of A responses to the inhibitory action of histamine. In this case, the experiment was so designed as to approximate the height of the B responses to that of the A responses; this was accomplished by doubling the number of pulses per train to obtain the B responses. The administration of histamine, 0·1 μg/ml., reduced the A responses, again evoked by trains of ten pulses at 50 Hz, almost to a quarter of their original height; whilst the B responses, elicited alternately

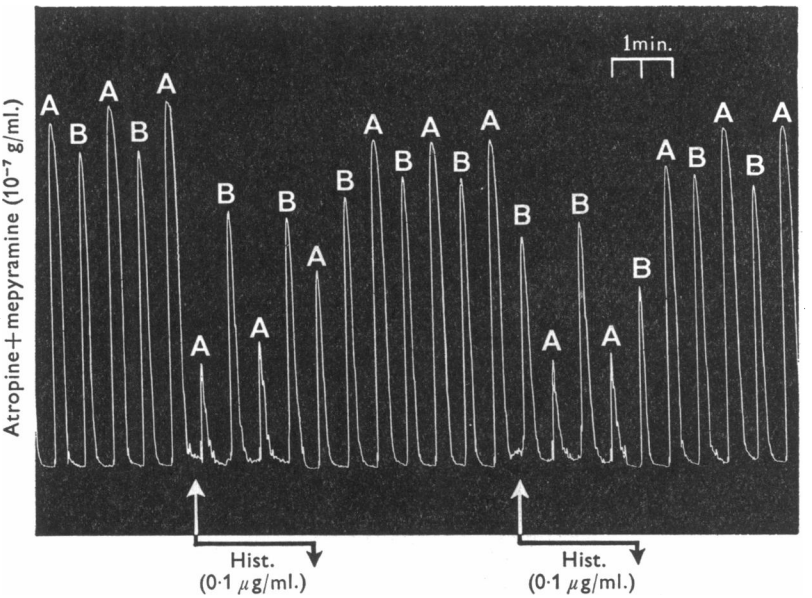

Fig. 5. The inhibitory effect of histamine is greater upon tetanic spasms elicited at 50 Hz (responses marked A) than at 5 Hz (responses marked B) even when these are approximated in height by doubling the number of pulses per train for the elicitation of B.

Plexus-containing preparation from ileum in atropine (10^{-7} g/ml.) and mepyramine (10^{-7} g/ml.). Alternate tetanic stimulation at 1 min intervals with trains of *ten* 0·2 msec pulses delivered at 50 Hz for responses marked A; or *twenty* 0·2 msec pulses at 5 Hz for those marked B. Between the arrows, histamine, 0·1 μg/ml., in the bath for 4 min.

at 1 min intervals by trains of 20 pulses at 5 Hz, were reduced only slightly and were now about twice as large as the A responses. This selective inhibition by histamine was quickly reversed on washing and was repeatable again 7 min later; as can be deduced from Fig. 5, these results were not influenced by the deliberate change of step in the sequence of stimulation at the start of the first exposure to histamine, since an identical effect was recorded during the second exposure to histamine, when the normal alternation of A and B responses was maintained.

A specific antagonism by nicotine towards histamine inhibition of tetanic spasms was reported previously (Ambache & Zar, 1969, 1970). In the present experiments nicotine, 10 μg/ml., administered 5 min before the histamine and left together with it in the bath for a further 4 min, again blocked the inhibitory effect of histamine, now exerted selectively upon the A responses. As previously, these tests were usually carried out after the motor effect of nicotine was abolished by the presence of hexamethonium, 10^{-4} g/ml. (Fig. 6), but the same result was obtained in the absence of hexamethonium, when the concentration of nicotine was raised to 25 μg/ml. and time was allowed for the nicotine contractions to subside before administering the histamine.

The effects of histamine could not be reproduced by its ring isomer, betazole, even in concentrations of 75 μg/ml.; A and B responses remained unaltered.

These results indicate that stimulation at both low and high frequencies elicits a histamine-insensitive component, which is common to both the A and the B responses and is predominant in the B response. The effect of stimulation at the higher frequency is to superimpose upon this a second, histamine-sensitive component in the A response. The contribution of this second component to the B response is sometimes, but not always, insignificant, whilst its contribution to the A response may be taken as the initial difference in height between the A and B responses. The variation in the A/B ratio described above and seen in Fig. 1 would be explicable on this assumption.

The histamine-liberator Compound 48/80 (Paton, 1951) in concentrations of 5–10 μg/ml. often reproduced partially the histamine inhibition of the A responses but, unlike histamine, it potentiated B responses; with higher concentrations, 50 μg/ml., this potentiation was greater and resulted in a final equality of A and B responses.

5-Hydroxytryptamine

In atropinized preparations the residual motor response to 5-HT is very feeble; it was completely eliminated by methysergide, 10^{-8} g/ml., which for these experiments was added to the reservoir together with the atropine and the mepyramine. Under these conditions, when 5-HT (0·1 μg/ml.) was injected into the organ-bath the B responses remained unaffected but the A responses were reduced, again to the level of the B responses (Fig. 6); if histamine, 0·1 μg/ml., was added at this stage there was no further reduction of the A responses. The obvious implication of this finding is that 5-HT and histamine could both be acting by suppressing the same component in the A response.

Although, superficially, this inhibitory effect of 5-HT appears to

resemble that of histamine, a difference was found: whereas the inhibitory action of histamine on the A responses was antagonized by nicotine,

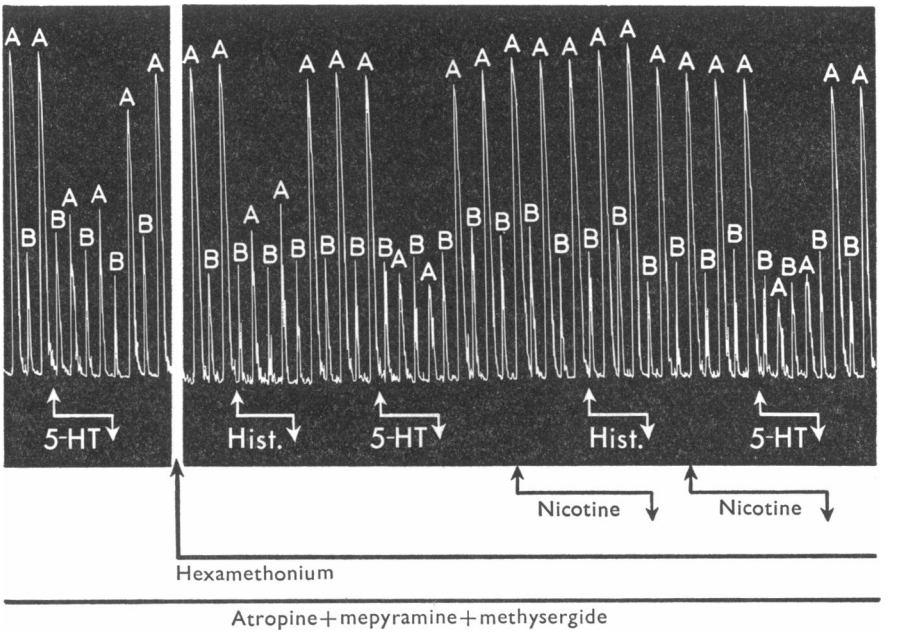

Fig. 6. The inhibitory effect of 5-HT upon the A responses is unaffected by hexamethonium and can be distinguished from the similar inhibitory effect of histamine by means of nicotine.

Plexus-containing preparation from ileum in atropine, 2×10^{-7} g/ml., mepyramine, 5×10^{-7} g/ml., and methysergide, 10^{-8} g/ml.; hexamethonium, 10^{-4} g/ml., added after Panel 1. The A and B responses were elicited alternately at 1 min intervals by trains of ten 0·2 msec pulses delivered at 50 Hz for A and at 5 Hz for B. Between the white arrows, 5-HT or histamine, 0·1 μg/ml., left in the bath for 4 min. Between the black arrows, the presence of nicotine, 10 μg/ml., in the bath, added 5 min earlier, abolishes the inhibitory effect of histamine but not that of 5-HT.

10 μg/ml., the inhibitory effect of 5-HT was not (Fig. 6); these tests were again carried out in the presence of hexamethonium, 10^{-4} g/ml.

The inhibitory effect of 5-HT could not be reproduced with tryptamine even in much higher concentration, 10 μg/ml.

Strychnine

Ambache & Freeman (1968*b*) reported an inhibition of atropine-resistant tetanic spasms, elicited at 50 Hz, by strychnine in doses which did not affect the cholinergic twitches in the same preparations when non-atropinized. The results of the present experiments indicate, further, a

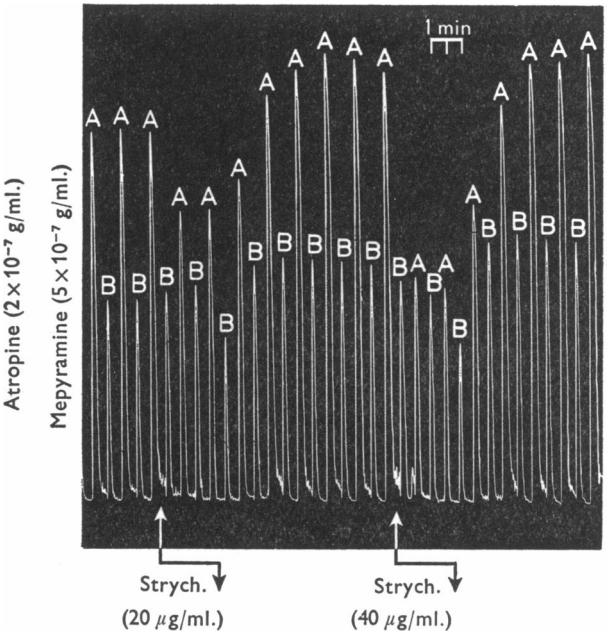

Fig. 7. Selective inhibition of A responses by strychnine.
Plexus-containing preparation from ileum in atropine, 2×10^{-7} g/ml., and mepyramine, 5×10^{-7} g/ml. A and B responses were elicited alternately at 1 min intervals by trains of ten 0·2 msec pulses delivered at 50 Hz for A and at 5 Hz for B. Between the arrows, strychnine left in the bath for 4 min; the 40 μg/ml. dose reduces the A responses to the level of the B responses. At the end of each exposure to strychnine there is, first, a slight depression of the next B response due to a wash-effect (see Methods) and then a slight potentiation of both A and B responses.

considerable specificity of strychnine inhibition towards the A responses. As with histamine, strychnine reduced A responses to the level of the B responses. Most commonly the effective dose of strychnine was 20 μg/ml., but occasionally it was necessary to raise it to 40 μg/ml. (Fig. 7). After the strychnine was washed out a potentiation of both A and B responses was noticeable, as can be seen in Fig. 7. In larger doses strychnine tended to inhibit the B responses as well.

The inhibitory effect of strychnine was not antagonized by nicotine, 10 μg/ml., administered 5 min earlier, in the presence of hexamethonium, 10^{-4} g/ml.

Diphenhydramine

The A and B responses were affected in opposite directions by this drug. In the experiment of Fig. 8 diphenhydramine, 10 μg/ml., was left in the bath for 12 min. During the first 6 min of this exposure not only was there a reduction of the A responses, as with the foregoing drugs, but there was also, concurrently, a distinct potentiation of the B responses, until finally A and B responses became equal for the remaining 6 min before the drug was washed out.

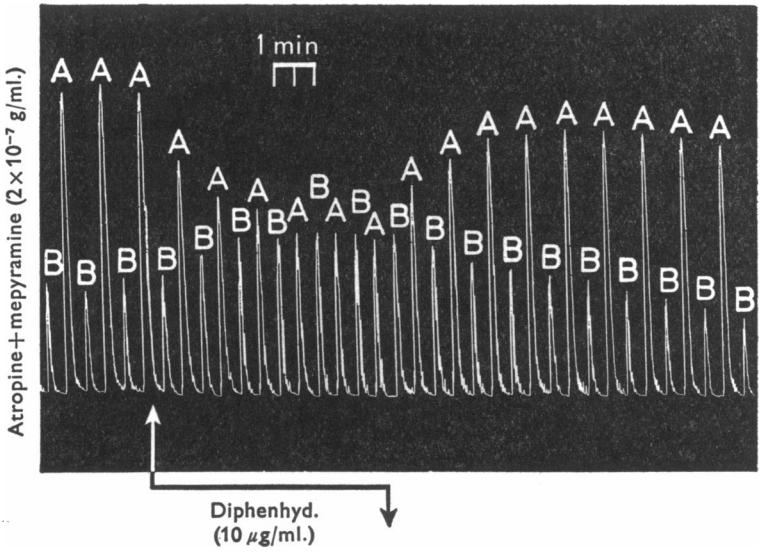

Fig. 8. Opposite effects of diphenhydramine upon the A and B responses. Plexus-containing preparation from ileum in atropine, 2×10^{-7} g/ml., and mepyramine (10^{-7} g/ml.). A and B responses were elicited alternately at 1 min intervals by trains of ten 0·2 msec pulses delivered at 50 Hz for A and at 5 Hz for B. Diphenhydramine, 10 μg/ml., left in the bath for 12 min (between the arrows) equalizes the height of the two responses by potentiating B and depressing A responses.

The inhibitory effect of diphenhydramine upon the A responses was not antagonized by nicotine, 10 μg/ml., administered 5 min earlier, in the presence of hexamethonium, 10^{-4} g/ml.

Exclusion of catecholamine release

The inhibitory effect of the above four drugs upon the A responses was not due to an intermediate release of catecholamines from adrenergic nerve-endings or from chromaffin cells, since it was not abolished by adrenoceptor-blocking agents. In preparations treated with atropine, mepyramine and methysergide both A and B responses were markedly inhibited by noradrenaline, 50 ng/ml. The addition to the bath of an α-blocker, phentolamine, 10^{-6} g/ml., together with a β-blocker, pronethalol, 5×10^{-7} g/ml., completely abolished the inhibitory action of noradrenaline, without altering that of histamine, 5-HT, strychnine or diphenhydramine.

The inhibitory effect of histamine was present as usual in preparations taken from the two guinea-pigs which had been pre-treated with reserpine, 10 mg/kg, intraperitoneally daily for 3 days.

Other drugs

Hordenine, 25 μg/ml., potentiated the B responses markedly but the A responses only slightly.

Prostaglandin E_2, 1 ng/ml., produced a 100 % increase in both the A and B responses; this potentiation was virtually abolished by SC-19220, 1 μg/ml.

Slight inhibitions of both A and B responses were produced by γ-aminobutyric acid, 1–10 μg/ml., and by ergothioneine, 2·5 μg/ml.

Neither A nor B responses were affected by the following: acetylsalicylic acid, 10 μg/ml.; capsaicin, 5 μg/ml.; reduced glutathione, 10 μg/ml.; picrotoxin, 25 μg/ml.; semicarbazide, 1 μg/ml.; and xanthine, 5 μg/ml.

B. Preparations from the guinea-pig descending colon

The descending colon of the guinea-pig differs from the ileum in several respects. In atropinized plexus-containing preparations of the longitudinal muscle from the descending colon there was hardly any difference between the heights of responses elicited at the high and at the low frequencies. In fact, the response to 5 Hz (what has been termed 'B' hitherto) may even be slightly greater (Fig. 9, Expt. 1) than the response to an equal number of pulses delivered at 50 Hz ('A'), whereas in the ileum B is always the smaller of the two responses. Furthermore, in the colon preparations neither response was inhibited by any of the drugs which depress the A response in preparations from the ileum. The implication of these findings is that one of the two response-components found in the ileum, namely the histamine-sensitive component of 'A', elicited at 50 Hz, is absent or weak

in the colon, and that the response of the descending colon preparation is constant at low and at high frequency because it always consists of the histamine-insensitive component which, in the ileum, was predominant in 'B'.

These differences are illustrated in the two experiments of Fig. 9. In both experiments the responses were elicited at 100 sec intervals by ten 0·2 msec pulses delivered alternately at 5 or at 50 Hz; atropine, mepyramine and methysergide were present in the bath fluid throughout. The tetanic spasms at both frequencies were preceded by a distinct phase of relaxation; this was much more pronounced in some experiments than in others, probably as the result of variation in the resting tone of the preparation. In the first experiment of Fig. 9 (panel *A*), the responses to 5 Hz were slightly greater than those to 50 Hz but in the second the reverse was found (panels *B*, *C*, *D*). There was no inhibition of either response by histamine, 0·1 μg/ml., in the presence of mepyramine (panel *A*), or by strychnine, 20 μg/ml. (panel *C*). In fact, the histamine slightly potentiated the response to 5 Hz and this concentration of strychnine potentiated the response to 50 Hz as well; a higher concentration of strychnine, 40 μg/ml., induced contractions of the colon preparation. There was also no inhibition by 5-HT, 0·1 μg/ml. (panel *D*), of the responses elicited at 5 or at 50 Hz; it should be mentioned that, although the preparations were treated with atropine, 2×10^{-7} g/ml., and methysergide, 10^{-8} g/ml., small contractions were still induced in them by the 5-HT, possibly due to stimulation of non-cholinergic ganglia. Finally, whereas diphenhydramine, 10 μg/ml., had no motor effect on the preparations from the ileum, this drug produced powerful contractions of the colon preparations (panel *B*), which made it impossible to test for an inhibitory action on the tetanic spasms.

DISCUSSION

Our results reinforce the conclusion (Ambache & Freeman, 1968*b*) that the occurrence of tetanic spasms in atropinized preparations of the longitudinal muscle is not due to an inadequacy of acetylcholine-receptor block. There is no evidence for the existence in this muscle of hypothetical 'muscarinic' receptors inaccessible to atropine. The cholinergic twitch evoked by single shocks in preparations from the ileum, when non-atropinized, is always easily abolished by atropine, 10^{-7} g/ml., or even 10^{-8} g/ml. It was also shown previously that atropine, 10^{-7} g/ml., effectively blocked doses of administered acetylcholine which were 200 times greater than the acetylcholine dose required to match this cholinergic twitch. It is therefore highly significant that in the present series of experiments tetanic spasms could be elicited in the presence of atropine, 10^{-7} g/ml., with trains of five,

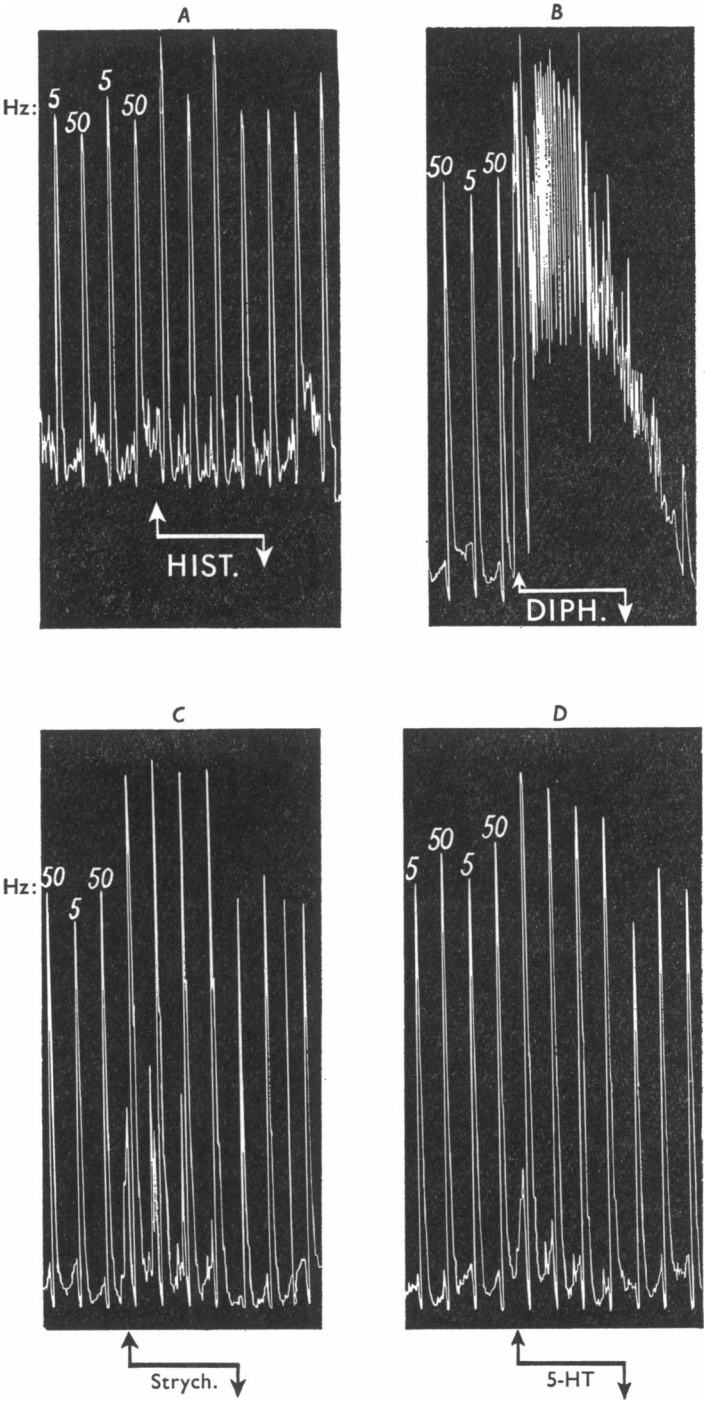

Fig. 9. For legend see opposite.

and often even with only two, pulses; in such cases the amount of acetylcholine released per train would be equivalent to only five or two 'twitch-matching doses' of acetylcholine administered to the bath, i.e. 40 or 100 times less acetylcholine, respectively, than could in fact be blocked completely by the atropine. Furthermore, the finding made in the present experiments that there was no diminution either in the A or in the B responses when the atropine concentration was stepped up 10 and then 100 times from 10^{-8} to 10^{-6} g/ml. is of great significance since, if either of these two responses were due to acetylcholine such a hundredfold rise in atropine concentration should have, at the least, produced some reduction in these contractions.

It is unlikely that the strong currents of 500 or 800 mA used for the field stimulation in our experiments failed to excite any of the neurones in Auerbach's plexus. Since the cells in that plexus have been classified histologically into three distinct types (Dogiel, 1899; Hill, 1927), it is tempting to assign to individual cell types one or other of the three main tetrodotoxin-sensitive motor responses of the longitudinal muscle in the ileum, which can be evoked by excitation of the plexus after ganglion-block by various means. There is, first, the immediate twitch elicited by single shocks in non-atropinized preparations, which we have already considered; this can be assigned to the post-ganglionic motor neurones, believed by Hill (1927) to be the Dogiel Type II cells. These cells send out their processes towards the longitudinal muscle and are considered to innervate it. They are presumably cholinergic like all other parasympathetic post-ganglionic neurones, since atropine and hyoscine abolish the twitch and reveal the other two, delayed, motor responses, here termed A and B, which appear to be due to two humorally elicited response-components with different rates of decay; these may, in turn, be termed components A and B. It is attractive to assign one of these two atropine-resistant components to the Dogiel Type I and the other to the Type III neurones. Both types are intercalated neurones which appear to have a

Fig. 9. In the descending colon the responses to 5 Hz and to 50 Hz both correspond to the B response of the ileum, because of their virtual equality and their insensitivity to the inhibitory effect of those drugs which depress the A response of ileum preparations (e.g. preceding Figures).

Plexus-containing longitudinal muscle preparations from descending colon of two guinea-pigs; suspended in 2 ml. baths containing atropine, 2×10^{-7} g/ml., mepyramine, 5×10^{-7} g/ml., and methysergide, 10^{-8} g/ml. The contractions were elicited at 100 sec intervals by trains of ten 0·2 msec pulses delivered alternately at 50 and at 5 Hz, as indicated. The responses to 5 Hz are slightly greater than those to 50 Hz in the first preparation (panel *A*) but smaller in the second (panels *B*, *C*, *D*). Between the arrows, 400 sec exposures to: in *A*, histamine 0·1 μg/ml.; in *B*, diphenhydramine 10 μg/ml.; in *C*, strychnine 20 μg/ml.; and in *D*, 5-HT 0·1 μg/ml. See text.

purely associative function in Auerbach's plexus and do not send their short processes towards the muscle; the reader is referred to Fig. 3 and Figs. 5 to 8 of Catherine Hill's (1927) paper and to her description of the striking differences between these two types of nerve cell, both in their morphology, particularly of the dendrites and their connexions, and especially in their staining properties (with methylene blue, the superficially placed Type I cells stain blue but the more granular Type III turn violet).

The somewhat delayed response-components A and B would then be incidental by-products of the excitation of these two cell types, after the overspill and relatively slow diffusion, outwards to the muscle, of their respective humoral transmitters, normally released for synaptic function within the plexus. In the ileum, when the frequency of stimulation is stepped up from 5 to 50 Hz, component B (assessed by the histamine-insensitive remnant in response B) is little affected, but the growth of component A (assessed by the histamine-sensitive portion of response A) is very obvious. Our results show that with stimulation at 5 Hz component A decays so rapidly during the 200 msec intervals between the pulses that its contribution to the response may become negligible compared with that of component B. This more rapid decay of component A, which makes it necessary to use a closer spacing of stimuli for its better detection, might be due to a greater lability, or to a faster inactivation, of the transmitter responsible for it. When the interval between pulses is reduced to 33 or 20 msec, by stimulation at 30 or 50 Hz, the transmitter-inactivating system concerned would become flooded, allowing substantial overspill to occur.

In the ileum the longitudinal muscle happens to be sensitive to both transmitters, but in the colon perhaps only to one, since component A is missing there; its absence accounts also for the lack of inhibitory effect with those drugs, e.g. histamine, which produce inhibitions in the ileum by suppressing component A. Thus the inhibitory effect, reported previously (Ambache & Freeman, 1968*b*), of strychnine upon tetanic spasms elicited by 50 Hz in preparations from the ileum can now be assigned to a suppression of component A; that would also explain the absence of this effect in the preparations from the colon.

Likewise, the existence of two components in the tetanic spasms of the ileum, only one of which is suppressed by histamine, now provides the explanation for the puzzling observation reported previously (Ambache & Zar, 1969, 1970) that the histamine inhibition of tetanic spasms elicited by 50 Hz was never total. This is now seen to be due to the persistence of component B in the response to 50 Hz after suppression of component A by histamine.

Mepyramine, 10^{-8}–10^{-6} g/ml., fails to antagonize the inhibitory effect of

histamine; in higher concentrations (10^{-5} g/ml.) it even mimics histamine. The possible mechanisms for the inhibitory action of histamine and the nature of the betazole-insensitive histamine receptors concerned have already been discussed at length in a previous paper (Ambache & Zar, 1970), to which the reader is referred also for the discussion of the antagonism of nicotine towards histamine, due perhaps to the ring resemblance between these two molecules. The specificity of this action of nicotine is reinforced by the new findings that it failed to antagonize the inhibition of component A produced by strychnine, 5-HT, and diphenhydramine.

The suppression of component A by all four of the above drugs is not mediated by a catecholamine release, since it persists after the preparations have been rendered insensitive to noradrenaline by treatment with the α and β adrenoceptor-blocking agents, phentolamine and pronethalol, and is found in preparations from reserpinized guinea-pigs. Moreover, if a catecholamine were released it would be expected to make the muscle fibres insensitive to all spasmogens, yet response B was often completely unaffected when component A was being suppressed by the four drugs. It should be stressed, however, that there is probably a small amount of component A also in response B, which would account for its slight reduction at times by histamine; with diphenhydramine and with mepyramine, 10^{-5} g/ml., any such effect was overshadowed by a concurrent potentiation of component B.

Little further light is thrown by our experiments upon the possible nature of these transmitters. It has been shown previously for response A (Ambache & Freeman, 1968*b*) and now for response B that they persist in the presence of mepyramine and methysergide; clearly, neither response is mediated by histaminergic or by tryptaminergic neurones. The results with the specific inhibitor SC-19220 also exclude mediation of either A or B by a release of prostaglandins.

In the guinea-pig ileum prostaglandins are known to potentiate subsequent contractions elicited by other spasmogens. Ambache & Freeman (1968*b*) therefore suggested that the staircase phenomenon which tetanic spasms display when periodic stimulation is resumed after rest periods might owe its origin to a release of prostaglandins. This is not borne out by the present findings that while the presence of SC-19220 antagonized and virtually abolished the potentiation of tetanic spasms by PGE_2, it did not affect the staircase phenomenon, as shown in Fig. 3. The staircase effect therefore appears to be due, rather, to some self-sensitizing property of one or both components herein described.

The fact that component A could not be obtained in the innervated preparations from the descending colon points to an important regional difference. We have already considered the possibility that this might be

due to an insensitivity of the longitudinal muscle fibres, in this part of the intestine, to the spasmogen responsible for component A. Alternatively, it is conceivable that the neurones which release this spasmogen in the ileum are absent from Auberbach's plexus in the descending colon.

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