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An inhibitory action of histamine on the guinea-pig ileum

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Summary

1. In atropinized, plexus-containing preparations of the longitudinal muscle from the guinea-pig ileum, in which histamine contractions were abolished by mepyramine or diphenhydramine, an inhibitory action of histamine was revealed on the " tetanic spasms " produced by field stimulation.

2. The inhibitory action of histamine on the atropine-resistant tetanic spasms, which are due to the excitation of non-cholinergic neurones in Auerbach's plexus (Ambache & Freeman, 1968a, b), was reversible. It is specific for the tetanic spasms, because histamine did not reduce contractions elicited by bradykinin, 5-hydroxytryptamine, prostaglandin E_2, nicotine or dimethylphenylpiperazinium.

3. L-Histidinol and 2-mercaptohistamine exerted a considerably weaker inhibitory effect upon the tetanic spasms than histamine. Four other imidazoles tested, L-histidine, murexine, dihydromurexine and imidazolecarboxylcholine, were ineffective ; so was the pyrazole ring isomer of histamine, betazole.

4. The inhibitory action of histamine persisted after adrenoceptor blockade by phentolamine and pronethalol and after prior reserpinization of the guinea-pigs.

5. The inhibitory action of histamine was also obtained after ganglionic paralysis by hexamethonium or dimethylphenylpiperazinium but was antagonized specifically by nicotine.

6. On atropinized preparations of the longitudinal muscle from the guinea-pig descending colon histamine exerted, at most, an insignificant inhibitory effect on the tetanic spasms.

Introduction

In plexus-containing preparations of the longitudinal muscle from the guinea-pig ileum tetanic field stimulation produces a contraction due to release of an atropine-resistant spasmogen from neurones in Auerbach's plexus that are non-cholinergic (Ambache & Freeman, 1968a, b). This " tetanic spasm " persists after mepyramine and is abolished by tetrodotoxin ; it cannot be elicited in plexus-free preparations. At least three histologically distinct types of nerve-cell are recognizable in Auerbach's plexus (Dogiel, 1899 ; Hill, 1927). As the Dogiel Type II cells are believed to represent the cholinergic postganglionic motor neurones of the vagus (Hill, 1927), the atropine-resistant tetanic spasm is attributed to excitation of the associative Dogiel Type I and/or Type III cells (Ambache & Freeman, 1968b).

In the present experiments the effect of histamine on these tetanic spasms has been examined. It was found that when the contractile response of the longitudinal muscle to histamine was abolished by mepyramine, an inhibitory effect was exerted by histamine upon the tetanic spasms. Although this inhibitory action of histamine persisted when the ganglion-cells were paralysed by hexamethonium or dimethylphenylpiperazinium, it was blocked by nicotine, owing to a specific antagonism of nicotine to histamine. A preliminary account of these results has appeared elsewhere (Ambache & Zar, 1969).

Methods

The procedure for obtaining and suspending plexus-containing preparations of the longitudinal muscle from the guinea-pig ileum, the experimental conditions for their electrical stimulation and the recording of tetanic spasms were identical with those described elsewhere (Ambache & Freeman, 1968b). To avoid the anomalous terminal portion of the ileum (Munro, 1953), the preparations were taken at a distance of between 20 and 70 cm (usually 20–40 cm) above the ileocaecal valve. In order to reduce biological variation the guinea-pigs used were all male albinos.

The preparations were suspended in a 2 ml. organ bath and the bath fluid was Krebs-Henseleit solution gassed with a 95% oxygen–5% carbon dioxide mixture. The tap of the organ bath was opened slightly to allow a gentle perfusion throughout the experiment, except during drug-contacts, when the tap was closed.

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 again the highest possible without overload. The pulse width was fixed at 0·2 ms for all experiments. Tetanic spasms were elicited at 1 min intervals by trains of such pulses, usually 50 Hz for a period of 0·2 s, but occasionally 20 Hz for 1 s. Before testing the effect of histamine or of other drugs time was allowed for the responses to reach a steady level after full development of the staircase phenomenon (Ambache & Freeman, 1968b). Some of the preparations were stimulated supramaximally with single pulses every 20 s.

In a few experiments plexus-containing preparations of the longitudinal muscle were obtained from the guinea-pig descending colon, some 70–90 cm below the ileocaecal valve, by the same procedure as described for the ileum preparations.

Reserpinization

Three guinea-pigs were used; the first two were injected intraperitoneally with a daily dose of 10 mg/kg for 2 days, and the third for 3 days, before the experiment. The first two received reserpine (Ciba) which was mixed with an equal weight of anhydrous citric acid and was solubilized with benzyl alcohol, "Polysorbate 80" and water, as described in Martindale (1967), and the third was injected with the more soluble reserpine phosphate (5 mg/ml. in distilled water), kindly supplied by Dr. A. J. Plummer, Ciba Laboratories, Summit, New Jersey.

Drugs tested on the longitudinal muscle preparations

These were atropine sulphate, betazole dihydrochloride, carbamylcholine chloride (carbachol), diphenhydramine hydrochloride, 1 : 1-dimethyl-4-phenylpiperazinium iodide (DMPP), hexamethonium bromide, histamine dihydrochloride, 5-hydroxy-

tryptamine creatinine sulphate, mepyramine maleate, morphine sulphate, nicotine hydrogen tartrate, phentolamine mesylate, pronethalol hydrochloride. All dosages refer to these salts. Pure bradykinin was kindly supplied by Dr. G. P. Lewis, Ciba Laboratories, Horsham, and prostaglandin E_2 by Dr. J. E. Pike, Upjohn Ltd., Kalamazoo, Michigan.

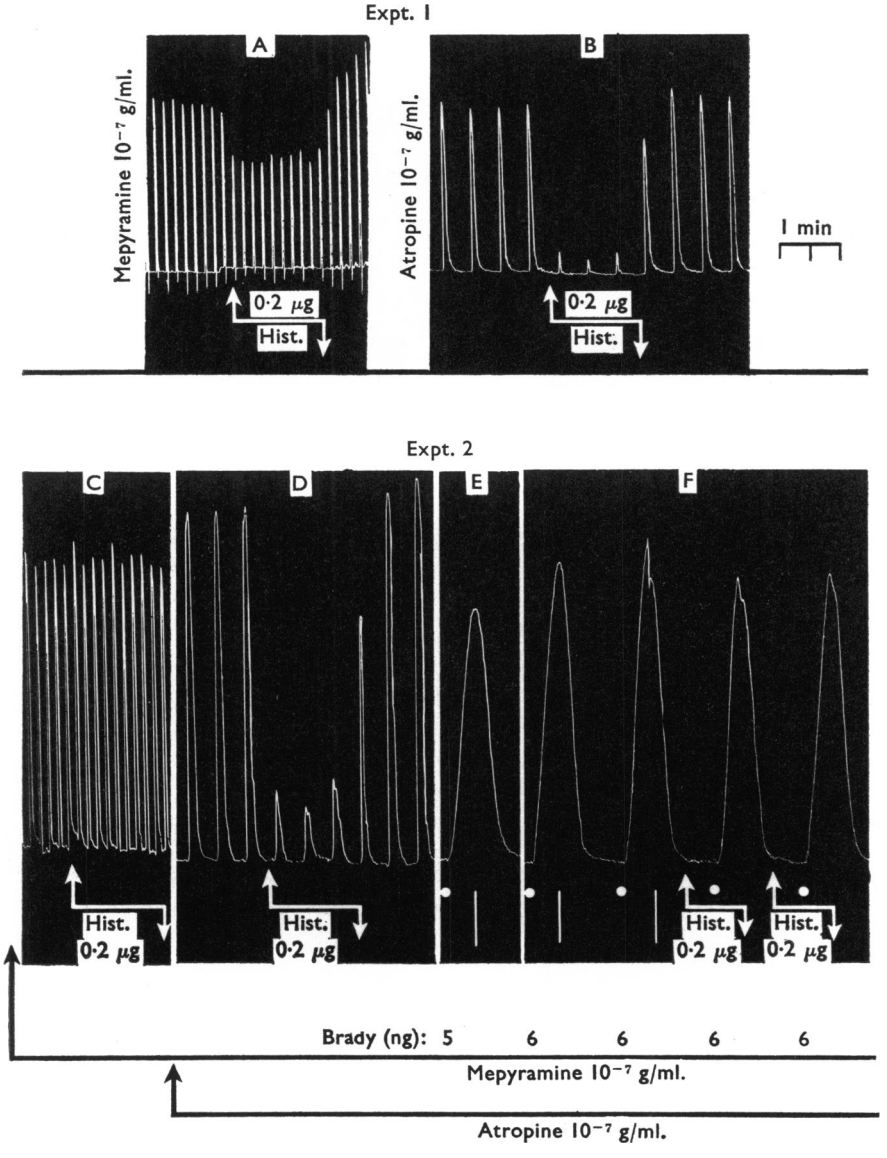


FIG. 1. Plexus-containing preparations of longitudinal muscle from the ileum of two guinea-pigs, suspended in 2 ml. baths in the presence of mepyramine 10^{-7} g/ml. The twitches in panels A and C, elicited in the absence of atropine, are due to single supramaximal shocks (0·2 ms), delivered every 20 s. The spasms in panels B and D are elicited in the presence of atropine 10^{-7} g/ml. at 1 min intervals by trains of 20 pulses at a frequency of 20 Hz and pulse width 0·2 ms. At the dots in E and F, bradykinin 5 or 6 ng for 1 min. Between the white arrows, histamine 0·1 μg/ml. in the bath for 3 min (in A–D) and for 2 min (in F).

Results

Experiments on preparations from the ileum

Effect of histamine, other imidazole derivatives and betazole upon atropine-resistant tetanic spasms

Histamine. After pre-treatment of the plexus-containing preparations with atropine $1\ 2 \times 10^{-7}$ g/ml. and with mepyramine 10^{-7} g/ml., which prevented the contractile response to histamine, the tetanic spasms of the longitudinal muscle produced by field stimulation were greatly reduced and sometimes nearly abolished by histamine 0·1 μg/ml. (Fig. 1, B and D). This inhibitory action was exerted within seconds of adding the histamine to the bath and recovery was rapid when it was washed out.

In a few preparations in which mepyramine at a concentration of 10^{-7} g/ml. did not fully block the contraction produced by histamine, its inhibitory action upon the tetanic spasms could still be demonstrated. For instance, in the experiment of

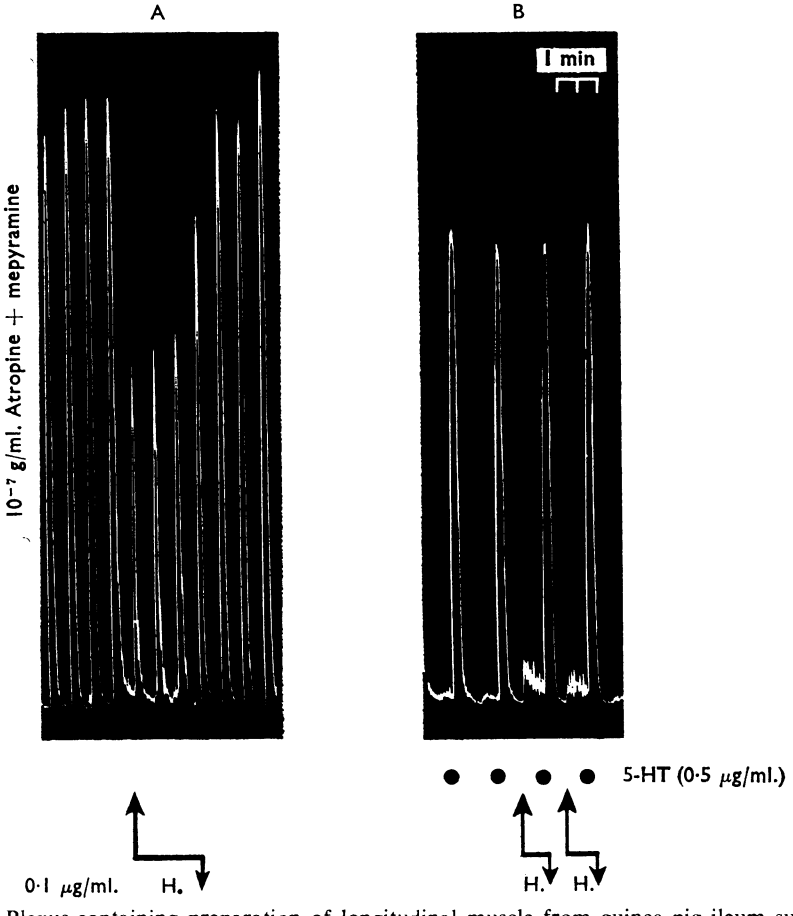


FIG. 2. Plexus-containing preparation of longitudinal muscle from guinea-pig ileum suspended in a 2 ml. bath in the presence of atropine and mepyramine 10^{-7} g/ml. Effect of histamine on: A, tetanic spasms elicited at 1 min intervals by trains of ten 0·2 ms pulses at 50 Hz; B, contractions elicited by 15 s exposures to 5-HT 0·5 μg/ml. At the arrows, histamine 0·1 μg/ml. in the bath for 3 min in A, and for 75 s in B.

Fig. 2 tetanic spasms were inhibited by histamine 0·1 μg/ml. (panel A), but in the absence of electrical stimulation the same dose of histamine still elicited minute contractions (panel B).

The inhibitory effect of histamine was obtained also with stronger concentrations of mepyramine, 2×10^{-7} to 10^{-5} g/ml., and with diphenhydramine (10^{-7} g/ml.), another antihistamine drug.

Inhibition by histamine was never complete; a small residual contraction in response to tetanic stimulation, such as shown in Fig. 1 B and D, remained even when the concentration of histamine was raised fivefold to 0·5 μg/ml.

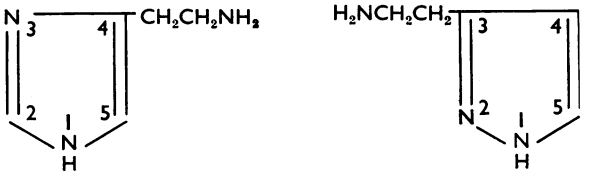
Maximal inhibition occurred with 0·1 μg/ml. of histamine, but the degree of maximal inhibition varied from preparation to preparation. With weaker concentrations of histamine the effect was less pronounced; some inhibition of tetanic spasms was obtained with as little as 0·005 μg/ml.

Unlike the contractions elicited by tetanic stimulation, those produced by bradykinin 3 ng/ml., 5-hydroxytryptamine (5-HT) 0·5 μg/ml., prostaglandin E_2 2·5–10 ng/ml., nicotine 2·5 μg/ml. and dimethylphenylpiperazinium (DMPP) 2·5 μg/ml. were not affected by histamine in preparations pre-treated with atropine and mepyramine. This is shown for bradykinin in Fig. 1 F and for 5-HT in Fig. 2 (panel B).

The tetanic spasms in Fig. 1 B and D were obtained in the presence of atropine and therefore cannot be due to release of acetylcholine. On the other hand, the twitch-responses to single shocks elicited in the absence of atropine are of cholinergic origin (Paton, 1955). They were still obtained after pre-treatment with mepyramine, but histamine either had no effect upon the twitch-response (Fig. 1C) or occasionally produced some inhibition (Fig. 1A). The difference, in one and the same preparation, between the effect of histamine on the twitch-response before, and on the tetanic spasm after, atropine is illustrated by the two experiments of Fig. 1.

In non-atropinized preparations pre-treated with mepyramine, histamine 0·1 μg/ml. also failed to inhibit the contractions elicited by acetylcholine 2·5 ng/ml., carbachol 5 ng/ml. or nicotine 1 μg/ml.; in some experiments these contractions were in fact slightly potentiated.

Other imidazole derivatives. Inhibition of the tetanic spasms in preparations pre-treated with atropine and mepyramine was obtained with L-histidinol and with 2-mercaptohistamine; L-histidinol was 4 times less active than histamine and 2-mercaptohistamine was even less active. Other imidazoles tested, L-histidine, murexine (β-(4-imidazolyl)acrylcholine), dihydromurexine and imidazolecarboxylcholine were ineffective in concentrations of 0·125–5 μg/ml.



Histamine Betazole

FIG. 3. Chemical structure of histamine and of betazole.

Betazole. As shown in Fig. 3, this ring isomer of histamine is a pyrazole derivative in which the two N atoms in the ring are adjacent as a result of the different location of the tertiary N, which now occupies position 2 instead of 3; likewise, the ethylamine side-chain now occupies position 3 instead of 4. The tetanic spasms were not inhibited by betazole even in a concentration 700 times greater than that of histamine. Thus, the ring change from imidazole to pyrazole is sufficient to abolish this inhibitory activity.

Effect of adrenoceptor-blocking agents, of reserpinization and of ganglion-blocking agents on the histamine inhibition

Adrenoceptor-blocking agents. The suppression of tetanic spasms by histamine is not due to an intermediate release of catecholamines from adrenergic nerve-endings or from chromaffin cells, for the addition to the bath of phentolamine, an α-blocker, together with pronethalol, a β-blocker, did not affect the inhibition. This is illustrated in Fig. 4. The initial inhibitory effects of noradrenaline and of histamine on the tetanic spasms before addition of the blockers are shown at A and B. The addition of pronethalol and phentolamine resulted in a slight lowering of the height of the tetanic spasms (at C) and abolished the inhibitory action of noradrenaline (at D) but not that of histamine (at E). After the blockers were washed out the tetanic spasms recovered (F and G).

Reserpinization. The inhibitory effect of histamine on the tetanic spasms was also obtained in preparations taken from the three guinea-pigs which had been pre-treated with reserpine 10 mg/kg intraperitoneally daily for 2 or 3 days.

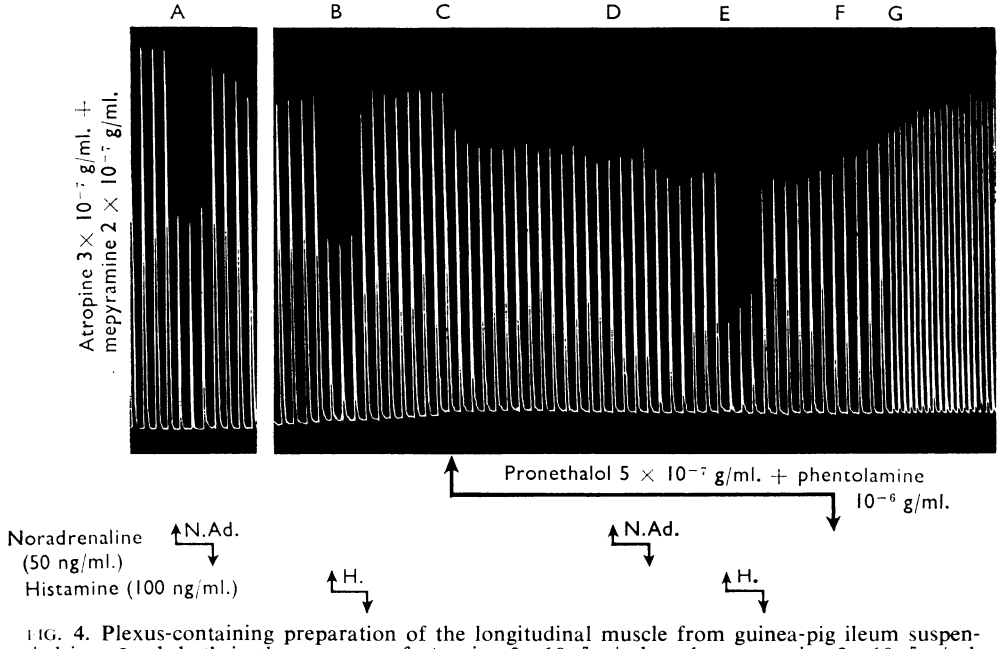


FIG. 4. Plexus-containing preparation of the longitudinal muscle from guinea-pig ileum suspended in a 2 ml. bath in the presence of atropine 3×10^{-7} g/ml. and mepyramine 2×10^{-7} g/ml. The contractions are due to stimulation at 1 min intervals with trains of ten 0·2 ms pulses at 50 Hz. Between the small arrows, at A and D, noradrenaline 50 ng/ml., and at B and E, histamine 0·1 μg/ml. added to the bath for 3 min. From C to F, pronethalol 5×10^{-7} g/ml. and phentolamine 10^{-6} g/ml. in the bath, as indicated by the large arrows. Drum at half-speed after G.

Ganglion-blockers. The three blocking agents used were hexamethonium, nicotine and dimethylphenylpiperazinium (DMPP).

After hexamethonium 10^{-4} g/ml., which did not interfere with the atropine-resistant tetanic spasms, the inhibitory effect of histamine was still present; as seen from a comparison of A and B in Fig. 5 it was, if anything, more pronounced. The ganglion-blocking efficacy of this concentration of hexamethonium is shown by the fact, illustrated in panel C, that there was no motor response to nicotine hydrogen tartrate 10 μg/ml. left in the bath for 8 min.

Unlike hexamethonium, nicotine, which did not itself affect the tetanic spasms, abolished the inhibitory action of histamine. This is illustrated in Fig. 5, panel C. The effect was obtained either with the hydrogen tartrate salt, as in this experiment,

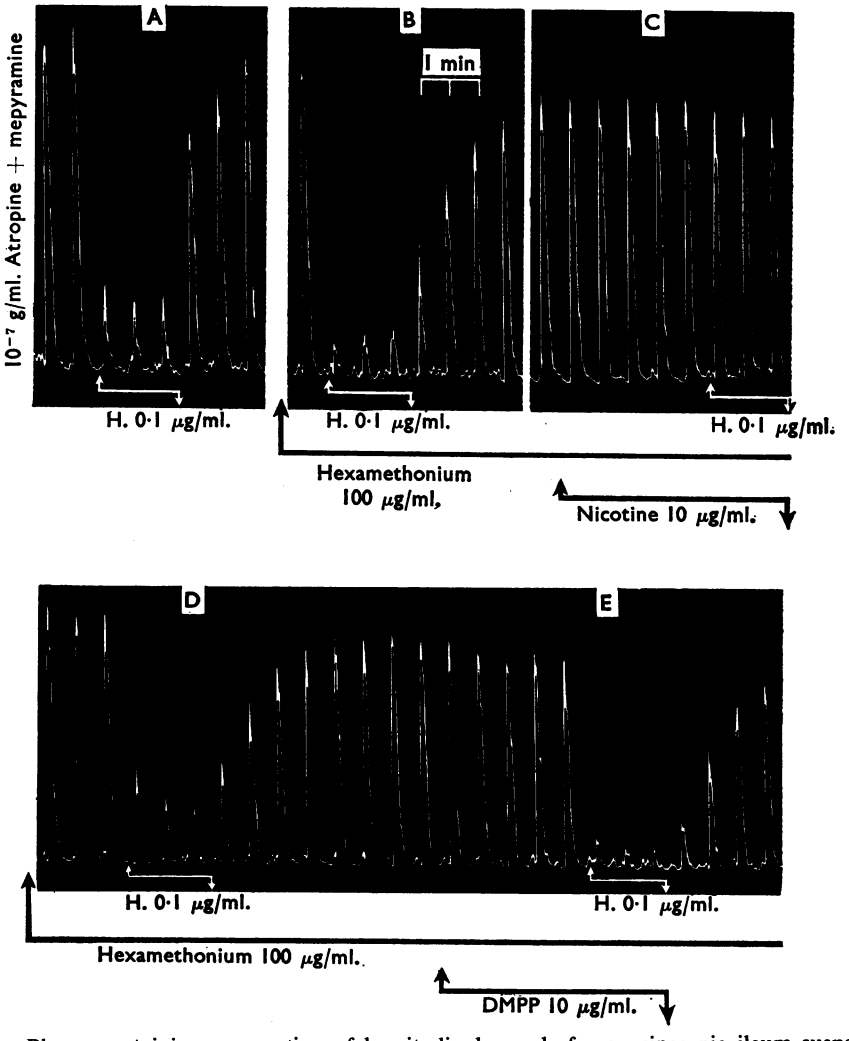


FIG. 5. Plexus-containing preparation of longitudinal muscle from guinea-pig ileum suspended in a 2 ml. bath in the presence of 10^{-7} g/ml. atropine and mepyramine. Tetanic spasms elicited at 1 min intervals by trains of ten 0·2 ms pulses at 50 Hz. Between the white arrows, histamine 0·1 μg/ml. in the bath for 3 min. After A, to the end of the experiment, hexamethonium 100 μg/ml. present in the bath. Between the black arrows, at C, nicotine 10 μg/ml., and at E, DMPP 10 μg/ml., in the bath for 8 min.

or with an equimolar dose of the base. The nicotine had to be left in the bath for some time (about 5 min) in order to abolish the inhibitory action of histamine. After shorter exposure, for example 2 min, some inhibition was still elicited by histamine. The antagonistic action of nicotine was reversible. As shown in Fig. 5 at D, histamine fully regained its inhibitory power 11 min after the nicotine was washed out. In this experiment nicotine exerted its effect in the presence of hexamethonium, but the effect was also obtained in the absence of hexamethonium. It was then necessary, however, to allow a sufficiently long exposure of the preparation to nicotine (25 μg/ml.) for the contraction due to the ganglion stimulation to subside and for the ganglion cells to become fully paralysed.

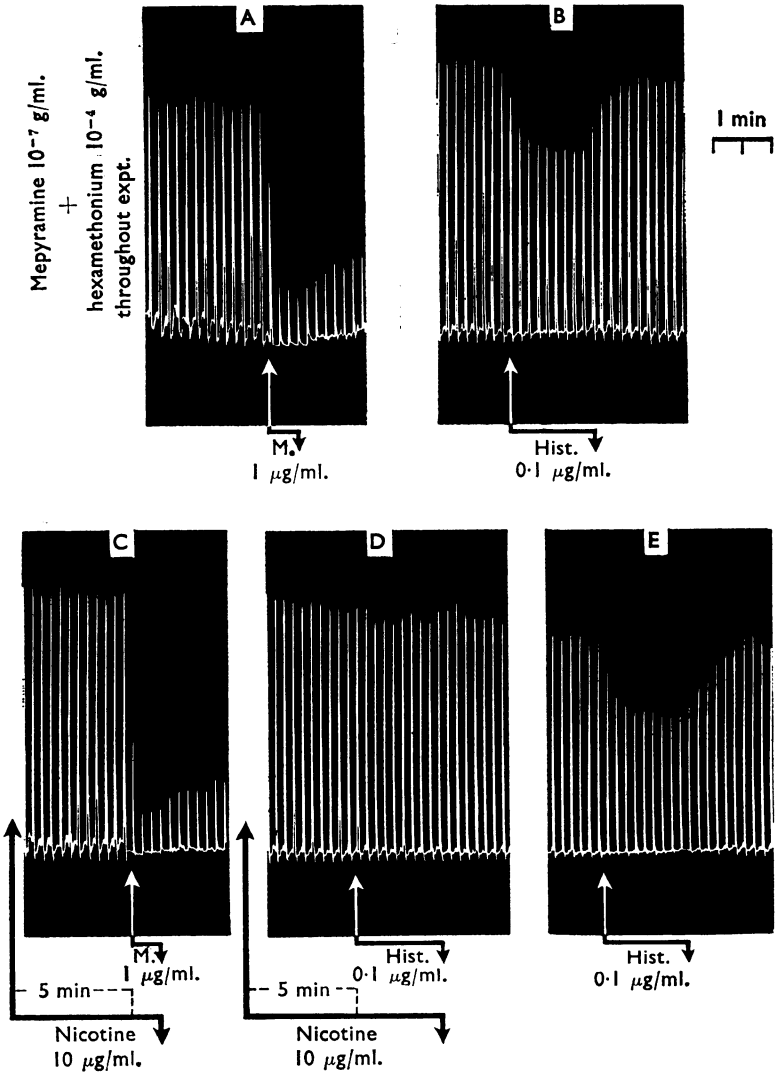


FIG. 6. Plexus-containing preparation of longitudinal muscle from guinea-pig ileum suspended in 2 ml. bath in the presence of mepyramine 10^{-7} g/ml. and hexamethonium 10^{-4} g/ml. Twitches elicited by single supramaximal shocks (0·2 ms) delivered every 20 s. At A and C, effect of morphine 1 μg/ml. and at B and D, of histamine 0·1 μg/ml., before and after the addition to the bath of nicotine 10 μg/ml. At E, effect of histamine 13 min after the nicotine was washed out.

The small inhibitory effect of histamine on the single-shock twitches, obtained in some non-atropinized preparations treated with mepyramine and hexamethonium, was also reversibly antagonized by nicotine. In the experiment illustrated in Fig. 6, histamine produced some inhibition of the twitch-response (at B), which no longer occurred in the presence of nicotine (at D) but reappeared after the nicotine was washed out (at E). In contrast, the much stronger inhibition produced by morphine 1 μg/ml. on the single-shock twitches (at A) was not affected by nicotine (at C).

Hexamethonium is a competitive, and nicotine a depolarizing, blocking agent, but this does not account for the difference between their actions with regard to the histamine inhibition, because DMPP, another depolarizing blocking agent, behaved like hexamethonium. As shown at E in Fig. 5, the inhibitory action of histamine persisted in the presence of DMPP 10 μg/ml. and was, in fact, accentuated. In this experiment DMPP was tested in the presence of hexamethonium, but the same result was obtained in its absence, when it was necessary to allow a sufficiently long exposure to the DMPP (25 μg/ml.) for its ganglion-stimulating action to subside.

Experiments on preparations from the descending colon

In the few experiments on the plexus-containing preparations of longitudinal muscle from the descending colon the inhibitory effect of histamine 0·1–0·5 μg/ml. on the tetanic spasms elicited in the presence of atropine and mepyramine was, at most, insignificant. Two such experiments, in which the tetanic spasms were elicited at different frequencies, are illustrated in Fig. 7.

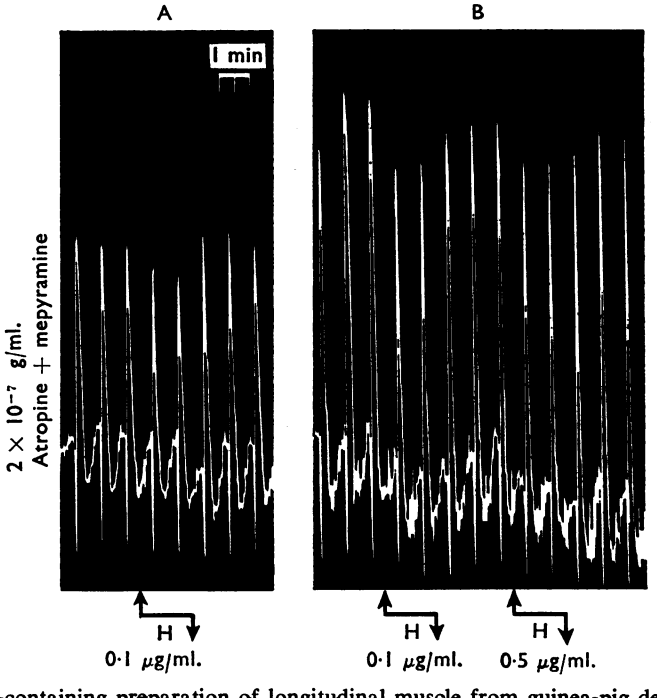


FIG. 7. Plexus-containing preparation of longitudinal muscle from guinea-pig descending colon suspended in 2 ml. bath in the presence of 2×10^{-7} g/ml. atropine and mepyramine. Tetanic spasms elicited at 100 s intervals by trains of five (panel A) or of ten (panel B) 0·2 ms pulses at 50 Hz. At H, between the arrows, histamine 0·1 or 0·5 μg/ml. in the bath for 200 s.

Discussion

The inhibitory effect of histamine, described in the present experiments, on the atropine-resistant " tetanic spasms " of the plexus-containing preparation of the longitudinal muscle from the guinea-pig ileum cannot be regarded as a weak local anaesthetic action, because of its specificity for the tetanic spasms in the ileum. If it were due to an anaesthetic action this would have also affected the contractions elicited by nicotine and DMPP (and the cholinergic twitches in the absence of atropine). Moreover, in the analogous preparations from the colon, which are also innervated, the inhibitory effect on tetanic spasms was virtually absent.

The inhibitory effect of histamine is of a type which is resistant to antihistamine drugs, for it is obtained when the contractile response to histamine has been eliminated by mepyramine or diphenhydramine. In this respect it resembles the secretory action of histamine on the oxyntic cells in the gastric mucosa and the inhibitory actions of histamine on the uterus of the rat (Fastier, 1949 ; Ash & Schild, 1966) and on the tracheobronchial muscle of the sheep (Eyre, 1969). In the present experiments the inhibitory action is exerted only on the tetanic spasms but not upon the contractions evoked by the usual smooth-muscle stimulants or by ganglion-stimulating drugs such as nicotine and DMPP, and thus displays a high specificity. Other characteristics of this inhibitory action are that it persists after ganglion paralysis produced by either hexamethonium, a competitive, or DMPP, a depolarizing, blocking agent but that it is abolished by nicotine. These findings have to be taken into account when trying to understand the mechanism of the inhibition produced by histamine.

Although the inhibitory effect of histamine on the plexus-containing longitudinal muscle of the ileum and the secretory action of histamine on the oxyntic cells are both resistant to antihistamine drugs, they differ in their susceptibility to betazole, which suggests that the receptors concerned are not identical. The difference in susceptibility is evident when a quantitative comparison is made between the action of histamine and of betazole on the following three types of receptor: (*a*) the mepyramine-sensitive histamine receptors ; (*b*) the oxyntic cell receptors ; and (*c*) the receptors affected by histamine in the present preparation. On the mepyramine-sensitive receptors betazole is 700 times weaker than histamine (Goodman & Gilman, 1965) ; on the oxyntic cells it is only 25 times weaker (Ash & Schild, 1966) ; but on the present preparation betazole was inactive even in a concentration 700 times higher than that of histamine.

The inhibitory action of histamine in the present experiments differs from that on the rat uterus and on sheep tracheobronchial muscle in the following respects. It is obtained with approximately 1,000 times lower concentrations of histamine —with 5–100 ng/ml. as against 20 μg/ml. for the rat uterus (Ash & Schild, 1966) and >3 μg/ml. for the tracheobronchial muscle (Eyre, 1969). A further difference is that in the rat uterus the inhibitory action of histamine is associated with a reduced sensitivity of the muscle fibres to acetylcholine and to carbachol, whereas in the present experiments contractions produced by these and other substances were not inhibited by histamine.

The finding that there was no decrease in the response of the smooth muscle to spasmogens which have widely different sites of action also rules out the possibility that the histamine inhibition is mediated by a muscle-relaxing neurohumoral

transmitter released from the nerve-endings of either of the two inhibitory innervations of the intestine: the extrinsic (sympathetic) or the intrinsic (non-adrenergic). Release of catecholamines by histamine is excluded by the finding that its inhibitory effect was obtained after exposure of the preparations to the α and β adrenoceptor-blocking agents, phentolamine and pronethalol, and in preparations from reserpinized guinea-pigs.

The tetanic spasms on which the histamine inhibition is exerted are due to release of an atropine-resistant spasmogen. Histamine might be acting either by competing with the spasmogen at the receptors in the smooth muscle or by inhibiting the release of the spasmogen. Either action would have to be antagonized by nicotine, because nicotine was found to abolish the histamine inhibition. If histamine were to compete with the spasmogen on the smooth muscle, this antagonism would be highly specific because contractions produced by other spasmogens were not inhibited by histamine. To explain the action of nicotine one would have to assume that nicotine, while preventing access of histamine to the receptors, still allows the spasmogen to reach, and act upon, the receptors. This would imply that histamine is acting as an antagonist to the spasmogen; and nicotine as an antagonist to this antagonist. Such a possibility cannot be excluded, because according to Ariëns (1966) the ring-bearing moieties of pharmacological antagonists may interact with so-called " accessory receptor areas " (probably non-polar) outside but adjacent to the (probably polar) main receptor of the agonist involved. Such a theory would open the way for the understanding of a possible competitive inhibition of antagonists by substances which do not themselves interfere with the effect of the agonist. On this theory a competitive inhibition of histamine by nicotine could arise from the molecular resemblance between their respective 5-membered rings (pyrrolidine in nicotine and imidazole in histamine); the additional pyridine ring of the nicotine molecule might increase its ability to bind to an accessory receptor area. Although it has been necessary to use in the present experiments a relatively high molar nicotine/histamine ratio of 33, this antagonism would appear nevertheless to be fairly structure-dependent because it was not obtained with DMPP, which shares many of the properties of nicotine, such as ganglion depolarization.

If histamine were to inhibit the release of the spasmogen, which may be the neuro-humoral transmitter of the Dogiel Type I or Type III cells (Ambache & Freeman, 1968b), nicotine would be acting as an antagonist to this action of histamine.

An interaction between nicotine and histamine on the guinea-pig ileum has been reported for the motor effect of histamine, which was reversibly inhibited by nicotine after ganglionic paralysis, without any reduction in bradykinin contractions (Ambache & Rocha e Silva, 1951).

The occasional slight inhibitory effect of histamine on the twitch-responses seen without atropine suggests that a small non-cholinergic component may contribute to these twitch-responses. This effect of histamine was also abolished specifically by nicotine, since the inhibition of twitches by morphine was unaffected by nicotine.

Finally, the virtual absence of the inhibitory effect of histamine in the innervated preparations from the descending colon points to an important regional difference, and suggests that the contribution of the histamine-sensitive component to the atropine-resistant tetanic spasms is insignificant in the colon.

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