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THE EFFECTS OF HISTAMINE AND OTHER NATURALLY OCCURRING IMIDAZOLES ON NEURONES OF *HELIX ASPERSA*

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There is at present but little evidence to suggest that histamine has the role of a transmitter substance in either the vertebrate or the invertebrate central nervous system. Few studies have, however, been made of the actions of histamine on the central nervous system and those results that have been reported have often been obtained by indirect methods. Furthermore there is still a great deal to be learnt about the nature of the central receptors for histamine.

The isolated brain of the snail *Helix aspersa* is a convenient central nervous system preparation for studying the actions of substances at the cellular level because it contains many large cells, from 80 to 150 μ in diameter, which can be penetrated with microelectrodes. Furthermore, the cells are placed superficially and so allow easy access for drug application.

Kerkut & Walker (1962) investigated the actions of a range of chemical substances on single neurones in the snail brain and found that some of the cells responded to histamine.

The experiments described in the present paper were undertaken to investigate the possibility that histamine is a transmitter substance in the invertebrate central nervous system and also to obtain information on the broader question of the general nature of histamine receptors in nervous tissue.

Some of these results were reported to the meeting of the British Pharmacological Society held at Nottingham in January, 1967.

METHODS

The experiments were performed on isolated circumoesophageal ganglionic masses from garden snails, *Helix aspersa*, which were collected locally. The shell was removed, the animal pinned out and the brain was exposed. The connective tissue covering the brain was removed and the brain was attached to a glass microscope slide with two rubber bands. The whole preparation was immersed in a 20 ml. bath containing snail Ringer solution. The snail Ringer contained (mM): KCl 4; NaCl, 80; $CaCl_2$, 7; $MgCl_2$, 5; Tris HCl, 5. The pH of the Ringer was 8.0.

Glass microelectrodes were pulled on a Palmer microelectrode puller and filled with M potassium acetate, or in some experiments, with M potassium chloride. The recording electrodes had a resistance of from 5 to 30 MΩ and a tip diameter of less than 0.5 μ.

The microelectrodes were inserted into cells in the visceral, left parietal and right parietal ganglia. The potentials were recorded using a Medistor negative capacity electrometer amplifier, Tektronix 502 and Tektronix 564 oscilloscopes, an A.E.I. pen oscilloscope and a loudspeaker.

Drugs were usually applied by direct addition to the bath, but in some experiments drug application was from a second electrode in close proximity to the cell whose activity was being recorded; in the latter case the chemical was applied by either iontophoretic injection, or by diffusion from a coarse electrode.

For the direct application experiments the following compounds, dissolved in snail Ringer, were used: acetylcholine chloride, L-histidine (B.D.H.); *N*-acetylhistamine, 1.4-methylhistamine (1-methyl-4-(β-aminoethyl)imidazole) dihydrochloride (Calbiochem); histamine acid phosphate (Koch-Light): mepyramine maleate (May & Baker): *N*-methylhistamine (4-(β-methylaminoethyl)imidazole) dihydrochloride and *N,N*-dimethylhistamine (4-(β-dimethylaminoethyl)imidazole) dihydrochloride (Smith, Kline & French). The drugs were added to the bath in a volume of not more than 1.0 ml. The preparation was washed with one or more 20 ml. quantities of Ringer between additions of drugs. The doses of histamine and its methyl analogues are expressed as micrograms of free base added to the bath. The doses of the other compounds are expressed as micrograms of the salt used.

For the iontophoresis experiments, a second electrode, filled with M acetylcholine chloride or with M histamine acid phosphate, was positioned near to the surface of the cell whose electrical activity was being recorded. A braking current was employed to prevent leakage of the drug from the pipette. Acetylcholine and histamine were passed as cations from solution at pH 4. Direct electrical effects were discounted by reversing the polarity of the ejecting current.

In the remaining experiments, electrodes were filled with M acetylcholine or with M histamine acid phosphate, both at pH 8. The electrodes were then broken down until the tip diameter was about 1–4 μ. The micropipette was mounted in a micromanipulator and brought up so that its tip was close to the cell being studied, allowing the drug to diffuse out on to the cell.

A total of ninety neurones in eighty different snail brain preparations was used in these experiments. The effects of histamine and of acetylcholine were investigated on each cell. The other imidazoles were only tested on some of the cells that responded to histamine.

A positive response was recorded to an agonist if the cell responded in the same way—that is, by excitation or by inhibition—to at least three separate applications of the agonist to that cell.

RESULTS

Most of the cells tested were spontaneously active. The resting potential was always in the range 30–50 mV. Action potentials were between 60 and 100 mV in height and from 2 to 15 msec in duration.

Acetylcholine

It has been shown that acetylcholine has either a depolarizing or a hyperpolarizing action on cells in the central nervous system of *Helix* (Tauc & Gerschenfeld, 1960 a, b, 1962; Gerschenfeld & Tauc, 1961; Kerkut & Walker, 1961, 1962).

In the present series of experiments acetylcholine was applied, by addition to the bath, to ninety cells in eighty different snail brain preparations. Acetylcholine caused an increase in the frequency of action potentials in fifty-six of the cells, twenty-nine cells were inhibited by acetylcholine and five cells failed to respond to doses of up to 100 μg added to the bath. Of the cells that did respond to acetylcholine, sixty-four were affected by doses of between 1 and 9 μg, twenty were affected by doses of 10 to 20 μg and one cell required a dose of 30 μg.

One of the difficulties associated with the technique of applying the drug directly to the ganglion is to establish that the drug is acting directly on the cell under investigation and not via interneurones. This problem was investigated in a series of experiments on eight neurones. Each of the eight cells was tested with acetylcholine applied in two different ways and the results were compared. One method of application was always by addition of the drug to the bath, and in the other method the drug was applied by either iontophoretic injection (three cells) or by diffusion from a coarse pipette (five cells).

Five of the cells were excited by acetylcholine, three were inhibited and the response was the same by both methods of application. In the experiments involving diffusion from a coarse pipette it is not possible completely to discard the effect on interneurones.

Histamine

The effects of histamine were also investigated on the ninety cells. Histamine was found to have both excitatory and inhibitory actions (Figs. 1 and 2); forty-one neurones were excited by histamine, fourteen were inhibited and thirty-five were unaffected by doses of up to 100 μg added to the bath.

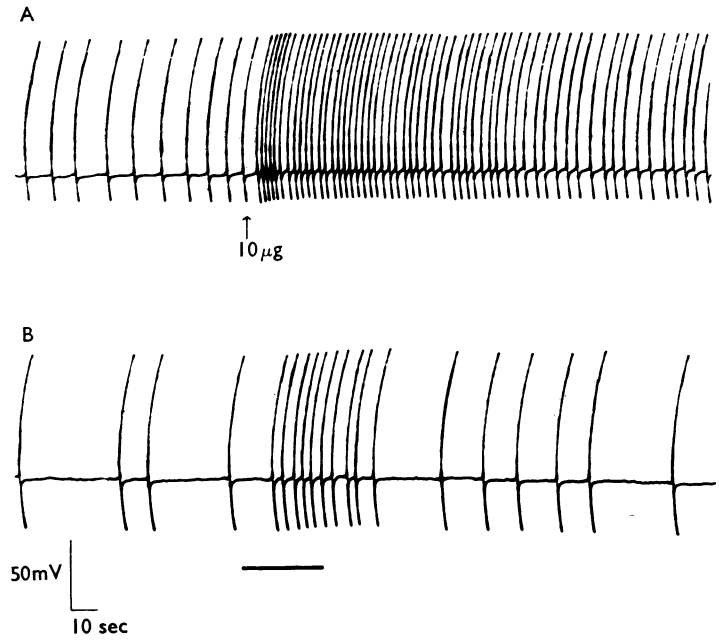

Fig. 1. Effect of histamine on the spontaneous activity of a single neurone in the brain of *Helix aspersa*. In A, histamine (10 μg) was added to the bath at the arrow. In B, the horizontal bar indicates application of histamine to the same cell by iontophoretic injection (100 nA).

The excitation produced by histamine differed from that produced by acetylcholine in three respects. First, the onset of the response to histamine was often delayed compared with that to acetylcholine; this could be associated with a difference in the diffusion rates for the two drugs. Secondly, the duration of the histamine response was usually

much longer than that to acetylcholine. Finally, histamine was less potent than acetylcholine in terms of both threshold amounts and maximal effect obtainable; these differences could be associated with different equilibrium potentials for acetylcholine and histamine. The histamine equilibrium potentials have not been determined.

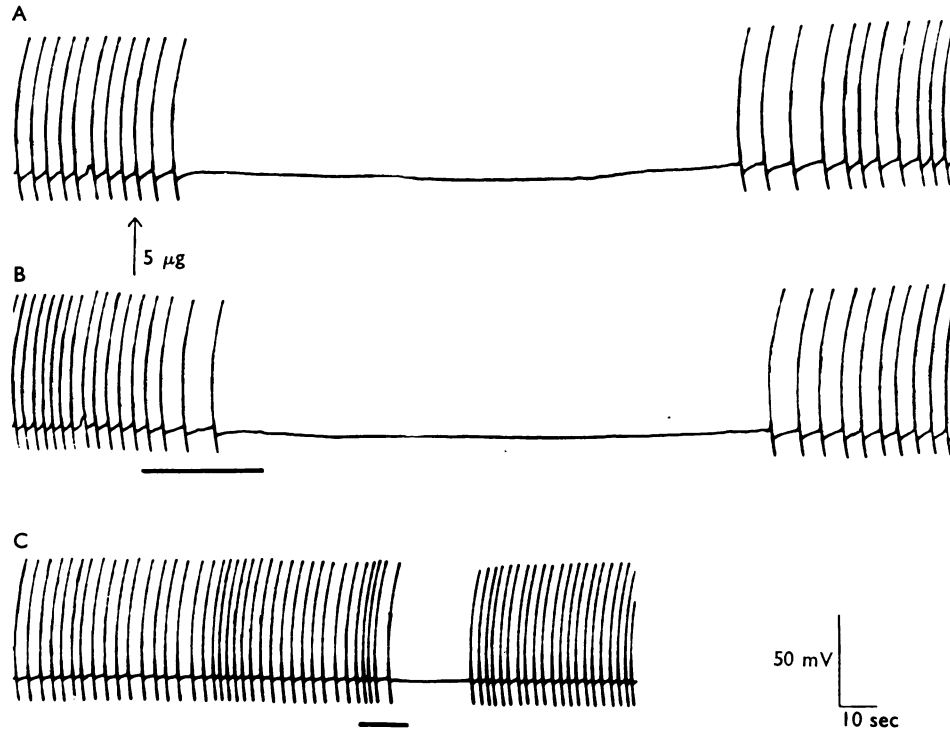

Fig. 2. Effect of histamine on the spontaneous activity of single neurones in the isolated brain of *Helix aspersa*. Records A and B are from the same cell. Record C is from a second cell. In A, histamine (5 μg) was added to the bath at the arrow. In B, the horizontal bar indicates application of histamine by diffusion from a coarse electrode in close proximity to the cell. In C, the horizontal bar indicates application of histamine by iontophoretic injection (100 nA).

Of the cells that were stimulated by histamine, thirteen were affected by a dose of from 1 to 9 μg, eighteen were activated by doses in the range 10–19 μg, seven required from 20 to 30 μg and the remaining three neurones were only affected by up to 50 μg of histamine. The doses refer to the amounts of histamine necessary to produce the first signs of excitation. All the cells inhibited by histamine were affected by doses of between 1 and 20 μg.

Of fourteen neurones inhibited by histamine, five were also inhibited by acetylcholine, eight were stimulated and one unaffected by acetylcholine. Of the forty-one neurones stimulated by histamine, twenty-seven were also stimulated by acetylcholine and fourteen were inhibited by acetylcholine.

There was thus no apparent relationship between the responses of a given cell to the different agonists. Further studies using iontophoretic application of the agonists are, however, necessary to eliminate interneurone effects.

The distribution, in the visceral, right parietal and left parietal ganglia, of located cells that responded to histamine is shown in Fig. 3.

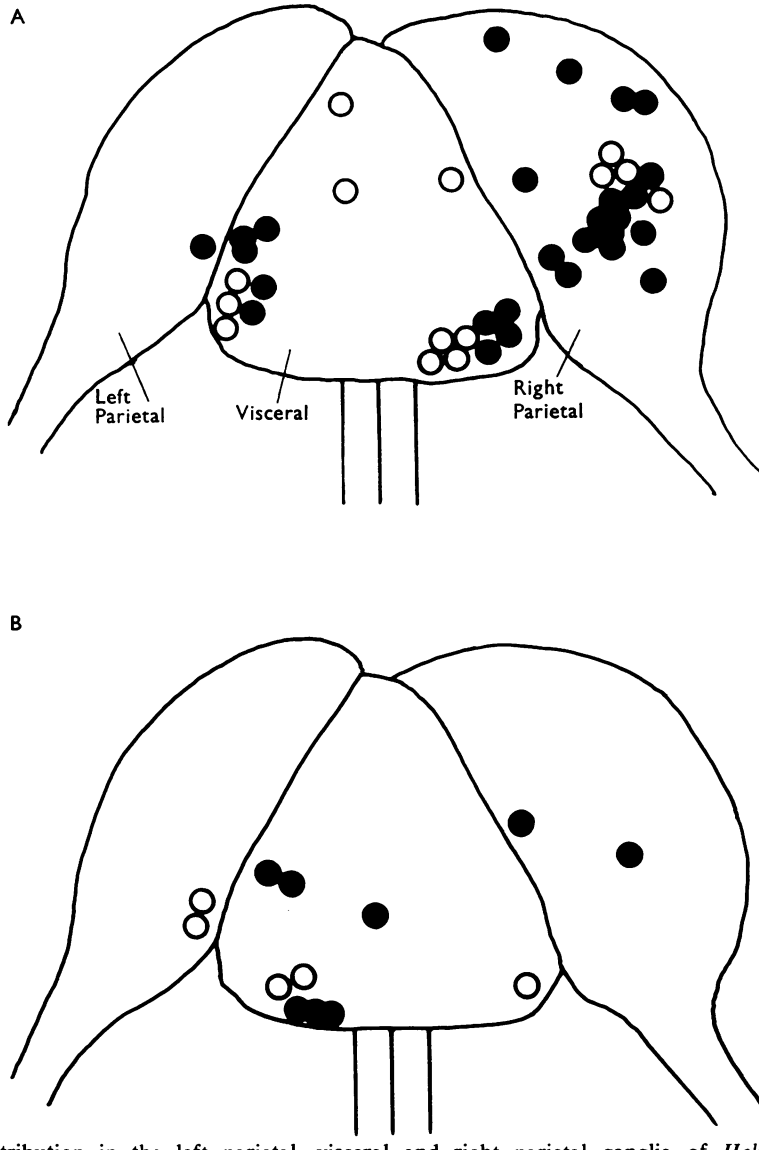

Fig. 3. Distribution in the left parietal, visceral and right parietal ganglia of *Helix aspersa* of neurones that respond to histamine and to acetylcholine. In A are marked the positions of cells that were excited by histamine (●, ○). In B are marked the positions of cells that were inhibited by histamine (●, ○). In both A and B cells that were stimulated by acetylcholine are indicated by ●, and cells that were inhibited by acetylcholine are marked ○.

The phenomenon of tachyphylaxis was investigated by selecting a dose of histamine which produced a good response in a neurone and then repeatedly adding the same amount of histamine to the bath at intervals, washing out the bath and allowing the activity of the cell to return to its resting level after each addition. Approximately two-thirds of the cells that responded to histamine exhibited tachyphylaxis, and it was particularly common in those neurones that required relatively high doses of histamine. Tachyphylaxis was more common to the excitatory action of histamine than to the inhibitory action. Thus most cells that were excited by the first application of histamine to the bath failed to respond after four or five additions of the same dose, whereas most cells that were inhibited by the first addition of histamine responded repeatedly, with similar degrees of inhibition, to up to six additions of the same dose of the drug. An example of a record obtained from a neurone that showed desensitization to histamine is shown in Fig. 4. Neurones did not show desensitization to acetylcholine.

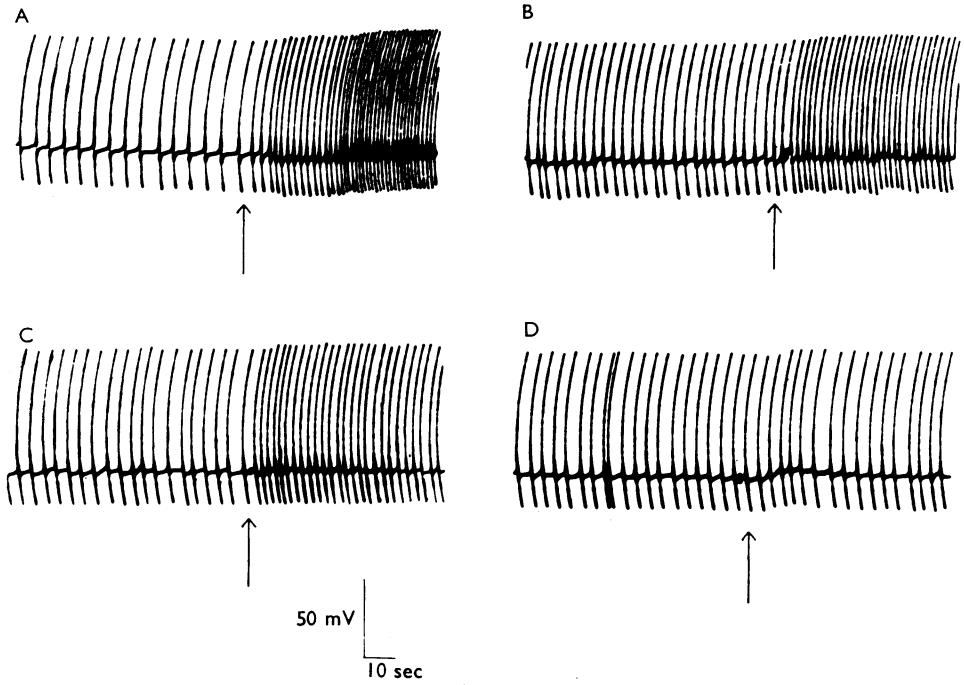

Fig. 4. Tachyphylaxis to the excitatory action of histamine on a neurone in the isolated snail brain. Record A is the response produced by the first application of histamine (15 μg). Records B, C and D are the responses produced by further 15 μg quantities of histamine, added at 5 min intervals in that order. The histamine was added to the bath at the arrows. The preparation was washed with snail Ringer between additions.

Mepyramine

The effect of mepyramine on the histamine response was investigated in seven cells. The mepyramine was added approximately 30 sec before a dose of histamine. In all cases the response produced by between 1.0 μg and 30.0 μg of histamine was completely abolished by the amounts of mepyramine (0.1–10.0 μg) added to the bath (approximately 0.005–0.5 μg/ml.). Five of the cells tested were excited by histamine and two were

inhibited. The mepyramine seemed to have a more long-lasting effect on cells that were inhibited by histamine than on those that were excited, but it was always possible to restore the response to histamine by washing out the bath with snail Ringer (Figs. 5 and 6). Mepyramine itself, in the doses used, had no effect on five of the neurones and caused a slight increase in the firing rate of the other two cells.

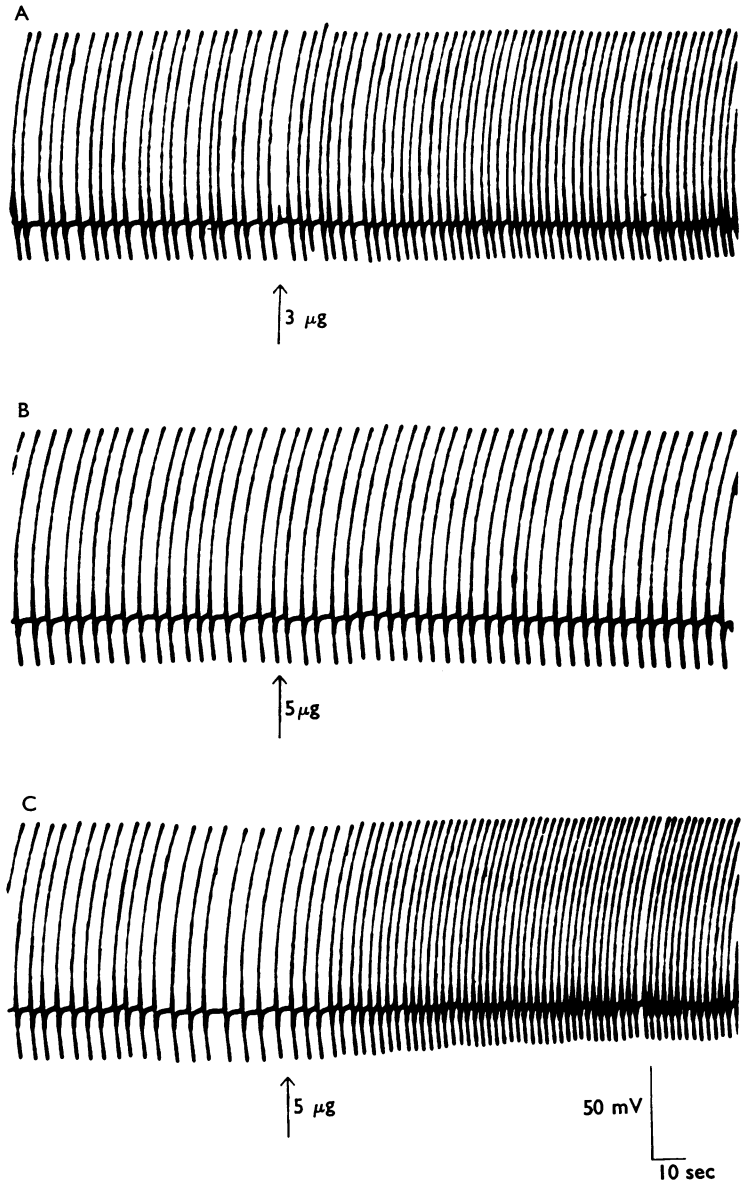

Fig. 5. Effect of mepyramine on the excitation produced by histamine in a single neurone in the isolated snail brain. Histamine was added at the arrows in the amounts indicated. Mepyramine (5 μg) was added between A and B. The preparation was washed with snail Ringer between additions of histamine.

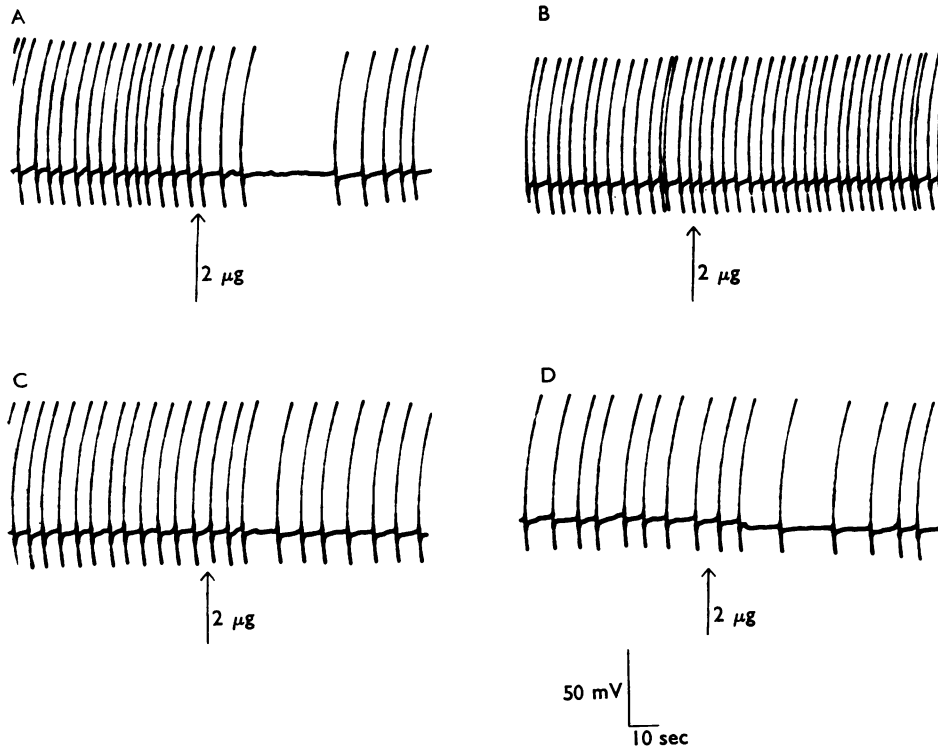

Fig. 6. Effect of mepyramine on the inhibition produced by histamine in a single neurone in the isolated snail brain. Histamine was added to the bath at the arrows in the amounts indicated. Mepyramine (0.1 μ3) was added between A and B. The preparation was washed with snail Ringer between additions of histamine.

The responses of the cells to acetylcholine were not affected by amounts of up to 20 μg of mepyramine, nor were the histamine responses affected by similar amounts of atropine.

Other imidazoles

Histidine, acetylhistamine and 1.4-methylhistamine were applied to a total of twelve neurones which responded to histamine, eight by excitation and four by inhibition. Histidine, in a dose of 500 μg, had a weakly depressant action on one cell. Acetylhistamine, in a dose of 200 μg, had a weakly excitatory action on two cells. The other neurones did not respond to amounts of up to 1,000 μg of these compounds. None of the neurones responded to up to 1,000 μg of 1.4-methylhistamine, nor was the response to histamine affected by any of these compounds.

N-methylhistamine and *N*,*N*-dimethylhistamine were applied to nine cells, five of which were stimulated by histamine and four of which were depressed. Neither of these compounds affected the activity of the neurones in doses of up to 500 μg.

DISCUSSION

The criteria that should be satisfied before a substance is accepted as a chemical transmitter have been discussed in review articles (Crossland, 1957; Werman, 1966). The evidence for a role of histamine in nervous tissue has been reviewed by Green (1964).

Work on the possible function of histamine in nervous tissue has been hampered by the lack of a simple, reliable method for its estimation. The biological method is time-consuming, and only recently has the fluorimetric method, developed by Shore, Burkhalter & Cohn (1959), been satisfactorily applied to the estimation of brain histamine levels (Medina & Shore, 1966). Histamine is, however, known to occur in mammalian brain (Adam, 1961; Carlini & Green, 1963; Crossland, Woodruff & Woodruff, 1966), together with enzymes for its synthesis and destruction (Brown, Tomchick & Axelrod, 1959; White, 1959, 1960). Histamine has recently been found in small nerve endings isolated from rat brain cortex (Kataoka & de Robertis, 1967).

In the invertebrate nervous system, histamine has been detected in the optic ganglia of *Eledone moschata* (Bertaccini, 1961).

There is, however, insufficient information on the central neuropharmocology of histamine. In the cat it has been shown that histamine inhibits transcallosally evoked potentials in the optic cortex (Gilfoil, Hart & Marrazzi, 1962), and that intraventricular injections of histamine produce behavioural changes in conscious animals (Feldberg & Sherwood, 1954) and stimulate higher sympathetic centres (Trendelenburg, 1957; White, 1965). Histamine has also been shown to affect the electrical activity of the cerebellum (Crossland & Mitchell, 1956; Fadiga, Gessi & Segata, 1962).

The results described here show that histamine fulfils one of the requirements demanded of a transmitter substance in the central nervous system of the snail—it affects the activity of individual cells. Other work in this laboratory has shown that histamine is present in snail brain. The snail also contains enzymes which metabolize histamine (Huggins & Woodruff, in preparation).

Because histamine excites some neurones and inhibits others, it can be regarded as a possible candidate for the roles of both excitatory and inhibitory transmitter.

In the present investigation, there was no relationship between the response of a cell to acetylcholine and its response to histamine. This could be because of a difference in the distribution of the two types of receptor in the central nervous system of *Helix* or the involvement of interneurones.

Histamine has been iontophoretically injected on to single neurones in the mammalian central nervous system and has been reported to be without effect on areas of the spinal cord (Curtis, Phillis & Watkins, 1961) and of the lateral geniculate nucleus (Curtis & Davis, 1962). Histamine and histidine applied iontophoretically both had a weakly inhibitory action on cortical neurones (Krnjević & Phillis, 1963).

It is not clear whether the differences between the results obtained in the mammalian experiments and those reported in this paper represent a difference in the reactivity of the two types of cell to histamine, or whether, in the mammalian experiments, the negative findings were the result of using an anaesthetic or the result of difficulties with the iontophoretic technique. Problems associated with the use of iontophoretic method for

some compounds have been reported (Bradley & Wolstencroft, 1965 ; Avanzino, Bradley, Comis & Wolstencroft, 1966). In the present investigation it was found that histamine applied iontophoretically was less effective than histamine added to the bath, which suggested the possibility that the iontophoretic method is not efficient for histamine, although the involvement of interneurones cannot be completely discounted when histamine is added to the bath. The neurones in the central nervous system of *Helix aspera* resemble those in the mammalian central nervous system in their response to acetylcholine, its analogues and antagonists (Walker & Hedges, 1967 a, b).

The final point is the problem of the nature of the histamine receptors in nervous tissue.

Some of the actions of histamine are specifically antagonized by low concentrations of antihistamines. Ash & Schild (1966) suggest the use of the term H_1 for histamine receptors that are blocked by antihistamines. Examples of histamine receptors of the H_1 type are those in the ileum and bronchi of the guinea-pig (Arunlakshana & Schild, 1959). Other actions of histamine are not antagonized by antihistamines: for example, the actions on isolated atria (Trendelenburg, 1960) and on gastric secretion (Ashford, Heller & Smart, 1949).

In the experiments reported in this paper the action of histamine was blocked by low concentrations of mepyramine, a specific antihistamine. Mepyramine blocked both the excitatory and the inhibitory actions of histamine on snail neurones. The possibility that histamine was exerting either of its two types of action via interneurones was excluded by showing that histamine, applied directly to the surface of different cells, would produce excitation or inhibition. It can be concluded, therefore, that in the central nervous system of *Helix aspera* the histamine receptors mediating both the excitatory and the inhibitory actions of histamine are both of the H_1 type.

One rather surprising finding in these experiments was the lack of activity of both *N*-methylhistamine and *N,N*-dimethylhistamine. In nine experiments on cells that responded to histamine, the side chain methylated analogues had no effect in doses of up to 250 times the dose of histamine that would affect the cell In the mammalian peripheral system, *N*-methylhistamine is approximately equipotent with histamine on H_1 receptors and has about 50% of the activity of histamine on gastric secretion and on the isolated rat uterus (Ash & Schild, 1966).

It may be that although the histamine receptors in *Helix* central nervous system can be classified as H_1 in terms of their behaviour towards antagonists, further studies with agonists will reveal differences between H_1 receptors in *Helix aspersa* and those in the mammalian system. In any event it is felt that the isolated snail brain preparation is a useful preparation for the study of drug-receptor interactions in single neurones.

SUMMARY

1. The effects of histamine and related compounds were investigated on the spontaneous electrical activity of neurones in the isolated brain of the snail, *Helix aspersa*.

2. Histamine stimulated the activity of forty-one of the ninety cells tested, inhibited the activity of fourteen of the cells, and had no effect in the remaining thirty-five neurones.

3. There was no correlation between the response of a cell to histamine and its response to acetylcholine.

4. Both the excitatory and the inhibitory actions of histamine were blocked by low concentrations of mepyramine.

5. Acetylhistamine, L-histidine, 1.4-methylhistamine, *N*-methylhistamine and *N,N*-dimethylhistamine were all tested on neurones which responded to histamine and found to have no effect.

6. It is suggested that histamine could be a possible transmitter substance in the central nervous system of the snail, *Helix aspersa.*

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