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HISTAMINE AND SOME ANTIHISTAMINES: THEIR ACTIONS ON CEREBRAL CORTICAL NEURONES

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The presence of histamine in various areas of the feline central nervous system was originally described by Kwiatkowski (1943) and most of these findings have been confirmed by subsequent investigators (Harris, Jacobsohn & Kahlson, 1952 ; McGeer, 1964 ; White, 1966). Although its distribution in mammalian brain is comparable in many respects with that of the biogenic monoamines, noradrenaline and 5-hydroxytryptamine, histamine has not, until recently, been as favourably regarded as a potential neurotransmitter (Crossland, 1960). This situation arose from the discovery that, in many parts of the body, histamine is primarily located in mast cells (Riley, 1959), and the possibility that it was similarly located in the nervous system tended to confuse interpretations of the significance of its presence in brain (Crossland, 1960).

The grey and white matter of the brain does not contain mast cells (Riley, 1959 ; Adam, 1961), however, and it has recently been demonstrated that histamine is present in the nerve terminal and synaptic vesicle fractions of homogenates of dog thalamus and hypothalamus (Michaelson & Coffman, 1967) and rat cerebral cortex (Kataoka & de Robertis, 1967).

Histamine synthesis from its amino-acid precursor, histidine, occurs rapidly in the feline cerebral cortex (White, 1959). Degradation of histamine to methylhistamine by the enzyme histamine *N*-methyltransferase also occurs in the cerebral cortex (White, 1959). An alternative pathway for histamine metabolism to imidazolacetic acid by the enzyme histaminase (Schayer, 1959) does not seem to be of significance in the brain (Burkard, Gey & Pletscher, 1963 ; White, 1959, 1960).

Histamine has potent effects on neuronal activity when administered intracarotidally (Crossland & Mitchell, 1956), into the cerebral ventricles (Kohn & Millichap, 1958) or by direct injection into discrete areas of the brain (Heath & de Balbian Verster, 1961).

These findings indicate that histamine deserves consideration as a possible neurotransmitter in the brain. A depressant action of histamine on neurones in the cerebral cortex has been described (Krnjević & Phillis, 1963). These observations have been confirmed in the present investigation, which has also revealed excitant effects of histamine. In addition, a survey has been made of the actions and specificity of several histamine antagonists.

METHODS

Thirteen adult cats were anaesthetized with intravenous thiopentone sodium (Intraval Sodium, May & Baker), supplementary doses of which were administered as required until preparation of the animal had been completed. Anaesthesia was then maintained with nitrous oxide, supplemented with either halothane (Fluothane, I.C.I.) or methoxyflurane (Penthrane, Abbott).

The head of the animal was fixed in a stereotaxic frame and the abdomen rested on an automatically controlled heating pad which maintained the preparation at 37° ±0.5° C.

A wide exposure of the left cortical hemisphere was made to allow recording from the pre-cruciate cortex and insertion of stereotaxically placed coaxial stimulating electrodes into the hypothalamus (A 11.5, L4.5, D-3). The medullary pyramidal tracts were exposed by removal of the overlying basioccipital bone and stimulated with a bipolar concentric electrode.

The exposed cortical surface was covered with a 4–5 mm thick layer of 4% agar in physiological saline which prevented cooling and drying of the cortical surface as well as minimizing the respiratory and vascular pulsations. This protective layer was removed after each penetration to allow repositioning of the electrode and replaced with a fresh layer.

The compounds were applied from five and nine barrelled micropipettes which were filled by centrifugation with aqueous drug solutions of pH not less than 3.5 immediately before use.

Spike potentials of single neurones in the pre-cruciate cortex were recorded through a sodium chloride (2M) filled barrel of the micropipettes. This recording barrel was connected to a negative capacitance probe and after amplification the responses were displayed on a CRO. An electronic counter (Hewlett Packard 5214L) was used to analyse the discharge rates of the cells tested and the output of this counter was displayed on an ink recorder. Full details of the recording apparatus have been published elsewhere (Phillis & Tebēcis, 1967; Phillis, Tebēcis & York, 1967).

The following drugs were tested during this investigation: acetylcholine chloride (British Drug Houses Ltd. (B.D.H.)); atropine sulphate (Parke Davis & Co.); betazole hydrochloride (E. Lilly & Co.); chlorcyclizine hydrochloride (Burroughs Wellcome & Co., (B.W.*)); chlorpromazine hydrochloride (May & Baker Ltd., (M. & B.*)); cyclizine hydrochloride (B.W.*); diphenhydramine hydrochloride (Parke Davis & Co.*); histamine acid phosphate (B. D. H.); (+)(−)-histidine hydrochloride (Sigma Chemical Co.); 5-hydroxytryptamine creatinine sulphate (M. & B.); imidazoleacetic acid hydrochloride (Calbiochem); (−)-glutamic acid sodium salt (B.D.H.); mepyramine maleate (M. & B.); (−)-noradrenaline bitartrate (Light & Co. Ltd.), phenindamine tartrate (Roche Products Ltd.*); tripelennamine hydrochloride (CIBA Pty. Ltd.*); strychnine hydrochloride (B.D.H.).

Firms marked with an asterisk donated the substances mentioned and their generosity is appreciated.

RESULTS

Histamine and related compounds

Histamine was tested on 276 neurones in the pre-cruciate motor cortex of thirteen cats. Most of these cells were located by ejecting (−)-glutamate continuously as the electrode tip passed through cortical tissue. Others were identified by their synaptically evoked responses to stimulation in the lateral hypothalamus or by the invasion of an antidromically propagating spike after stimulation of the ipsilateral medullary pyramidal tract. The approximate depth of neurones from the surface of the cortex was ascertained by estimating the distance travelled by the electrode tip, from a dial on the micromanipulator.

The predominant action of histamine on cortical neurones was inhibitory, 181 of 276 (65.5%) neurones tested being depressed by histamine. This depression was manifested by a reduction or abolition of the excitant effects of lateral hypothalamic stimulation, by

a failure of an antidromically propagating spike to invade the cell soma, by a depression of spontaneous, or (−)-glutamate and acetylcholine (ACh) induced, firing. Examples of the depressant effects of histamine are presented in Fig. 1.

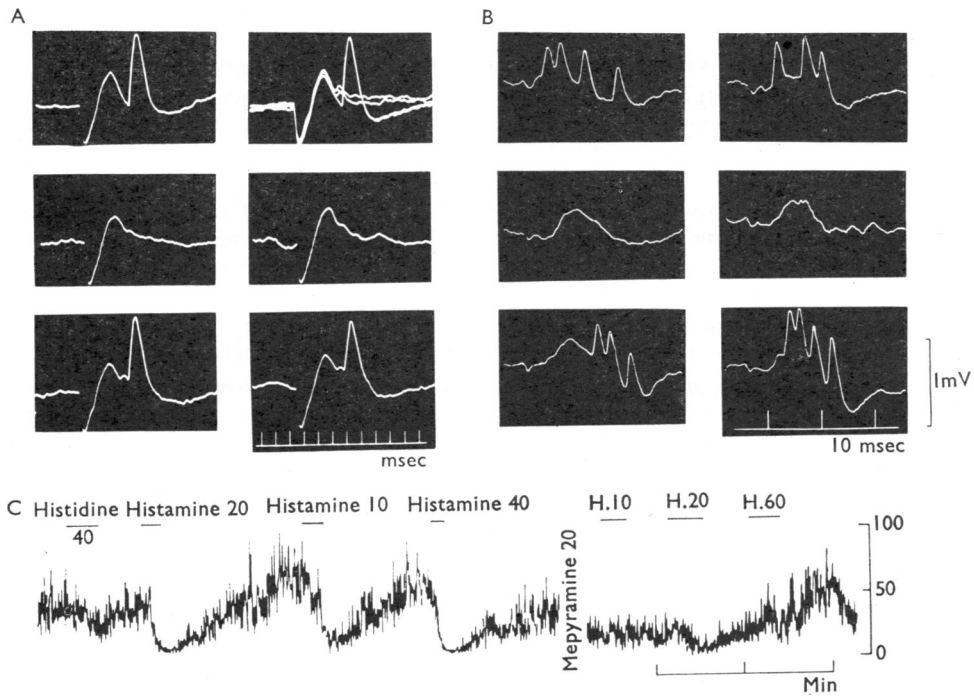

Fig. 1. Histamine depression of antidromic (A), synaptic (B) and spontaneous (C) firing of three cortical neurones. (A) Pairs of consecutive records of antidromic spikes evoked by PT stimulation. At threshold stimulus intensity the spike appeared in an all-or-none manner (top records). 10 sec after the application of histamine (80 nA) antidromic invasion had failed (middle records). Recovery occurred within 12 sec (lower records). (B) A similar series of synaptic responses evoked by lateral hypothalamic stimulation. Histamine (30 nA) abolished the response. Recovery followed within 15 sec of the termination of histamine application. (C) Depression of spontaneous firing by histamine (10 nA, 20 nA and 40 nA). (+)(−)-histidine (40 nA) had a weakly depressant action. After the application of mepyramine (20 nA), histamine depression was reduced and larger amounts (60 nA) now had an excitant action. Record (C) and the subsequent records in this paper were obtained with an electronic counter and ink recorder. The vertical scale on the right of each of these records indicates actual discharge rates. Drug applications are indicated by horizontal solid lines above and below the traces.

Eighteen cortical neurones tested in this survey were identifiable as pyramidal tract (PT) cells. These units responded to stimulation of the medullary pyramidal tracts with constant short (<4 msec) latencies, followed PT stimulation at frequencies in excess of 100/sec and had refractory periods of less than 2 msec. Pyramidal tract stimulation evoked cortical potentials which frequently showed two distinct components with latencies of 0.7–0.8 msec and approximately 2.5 msec, respectively. The longer latency potentials appeared at higher stimulus strengths than the initial response. The two

potentials probably reflect the initiation of action potentials in the different sized groups of fibres in the bulbar pyramidal tracts (Bishop, Jeremy & Lance, 1953). The latencies of antidromically evoked spikes in PT neurones frequently correlated with those of the two evoked potentials and as expected the threshold for inducing activity in the longer latency cells was usually higher than that for short latency units. Although histamine depressed the glutamate evoked firing of ten PT neurones, it blocked the invasion of an antidromic spike in only two of these. In both instances the failure occurred in a longer latency PT cell.

One of these neurones is shown in Fig. 1A. The invasion of an antidromic spike into this neurone occurred after a latency of 3.2 msec and applications of histamine (80 nA) rapidly abolished this response. Recovery occurred within a few seconds of the termination of histamine application. The ability of histamine to abolish the antidromic response of some PT cells and not of others could be interpreted as an indication of a difference in its actions on the sensitive cells. Histamine depressed glutamate firing of both short and longer latency cells, however, so it is unlikely that its mode of action differed. A more plausible explanation for our observations would be that the safety factor for propagation of antidromic spikes into the PT neurone somas is lower for the longer latency PT cells. For cell somas of comparable size, a failure of the invasion of an action potential in a small (higher threshold) nerve fibre would be expected to occur more readily than that from a larger (lower threshold) axon.

Histamine also depressed synaptically evoked responses. The neurone in Fig. 1B responded synaptically to stimulation of the lateral hypothalamus. During the application of histamine by a current of 30 nA, this response failed, uncovering an evoked field potential generated by the activity of more distant neurones. Recovery occurred within a few seconds of the termination of histamine application.

Histamine also depressed spontaneous firing of cortical neurones. When applied by currents of 10, 20 and 40 nA, histamine depressed spontaneous firing of the neurone illustrated in Fig. 1C (+)(−)-Histidine (40 nA) had a very weak depressant action on this cell. After a 2 min application of the histamine antagonist, mepyramine, by a current of 20 nA, the effects of histamine (10 and 20 nA) were greatly reduced and a larger application of histamine (60 nA) now excited the cell, increasing its rate of firing even though, before the application of mepyramine, histamine (60 nA) had depressed spontaneous firing.

An excitant action was observed on many neurones tested with large applications of histamine and often complicated attempts to assess the depressant effects of this compound. Histamine excitation was most frequently observed as an enhancement of (−)-glutamate evoked firing. A sequence of events observed with many cells was that small applications of histamine (20–30 nA) caused a marked reduction in glutamate evoked firing during the period of application, followed by a 30–60 sec period during which (−)-glutamate firing was enhanced. Larger applications of histamine to such cells often caused progressively less of a reduction in the (−)-glutamate response and with histamine applications of 60–80 nA excitability was often enhanced even during the period of histamine application.

In effect, this phenomenon required each neurone to be tested with histamine applied by currents of different magnitudes in order to determine the effects. Although excitation

was usually observed on neurones which were depressed by smaller amounts of histamine, excitation was recorded as the primary effect on only 5% of the neurones tested. An example of one of these cells is shown in Fig. 2A. This neurone responded to histamine with an enhanced excitability, indicated by the increased frequency of firing evoked by (−)-glutamate. Smaller applications of histamine were either without effect or increased excitability to a lesser extent.

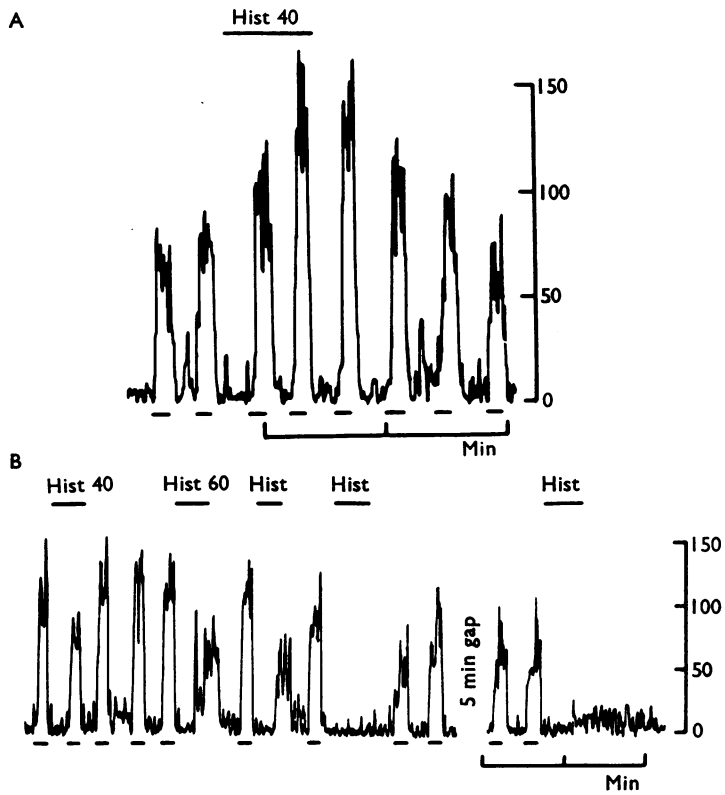

Fig. 2. A, Facilitation of (−)-glutamate (60 nA) firing by histamine. B, Inhibitory and excitatory effects of histamine on a single cortical neurone.

The dual effects of histamine observed on many neurones are clearly illustrated in Fig. 2B. When applied by a current of 40 nA, histamine depressed the excitability of this neurone. A larger application (60 nA) made the cell discharge, indicating that histamine had caused either a marked increase in excitability or depolarized the cell. A second application by a current of 60 nA induced less excitation and a third application failed to initiate an excitant response. Immediately after the third application, the response to (−)-glutamate was depressed, indicating that the inhibitory effects of histamine were still present. After a period of 5 min during which no histamine applications were made, a further application by a current of 60 nA evoked a small excitant response from the cell. The reduction in excitant responses observed when histamine was applied

repeatedly suggests that the membrane receptor responsible for this effect can be desensitized. As a depressant effect was still present after excitation had failed it can be assumed that the inhibitory effects of histamine are mediated by a separate receptor which is more resistant to desensitization. Further evidence for the presence of two receptors was forthcoming from experiments with histamine antagonists which blocked the receptor for histamine inhibition more readily than that for excitation.

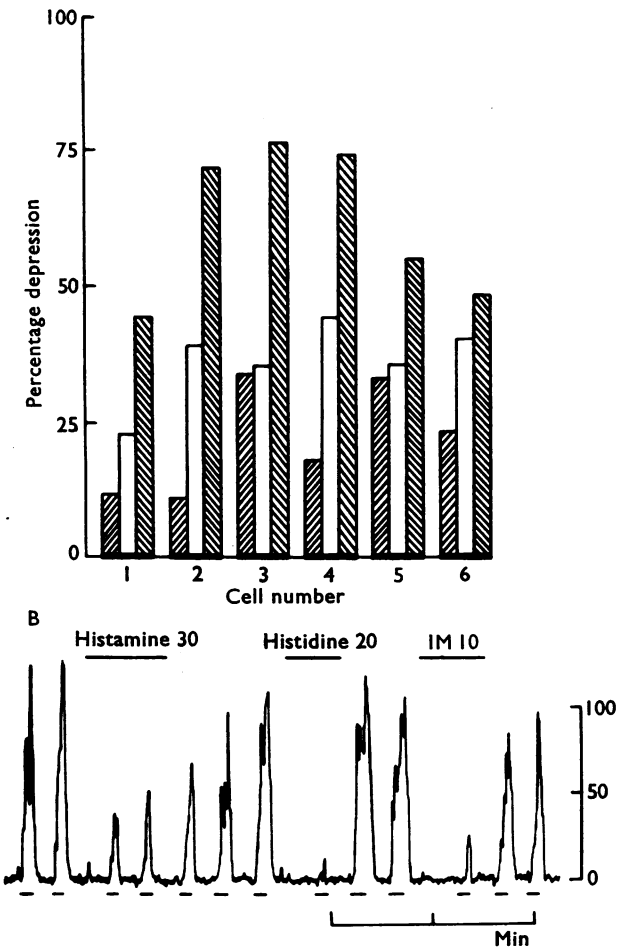

Fig. 3. A, Histogram comparing the relative percentage depression of (−)-glutamate (40 nA) evoked firing by histamine (▨), histidine (□), and imidazoleacetic acid (▧) (all applied at 40 nA) on each of six cortical neurones. The size of each vertical bar was determined by measuring the area under the response evoked by (−)-glutamate immediately before and after application of histamine. (−)-glutamate was applied for a constant period of 10 sec in each case. Areas were measured by a polar planimeter (Allbrit). B, Comparison of the relative potencies of histamine (30 nA), histidine (20 nA) and imidazoleacetic acid (10 nA) on the same cortical neurone.

The dual effects of histamine may have contributed to the relative weakness of its depressant actions in comparison with those of various related compounds. The relatively greater potencies of the histamine precursor, histidine, and its metabolic product, imidazoleacetic acid, are clearly illustrated in Fig. 3A. This figure illustrates the percentage depressions of (−)-glutamate evoked firing of six neurones by similar currents passed through the histamine, histidine and imidazoleacetic acid barrels. The magnitudes of currents required to produce comparable levels of depression of (−)-glutamate firing of another neurone are shown in Fig. 3B. Histidine and imidazoleacetic acid depressions typically had rapid onsets and ceased within a few seconds of the termination of application. Histamine, however, had a slower onset of action and continued to depress some neurones for up to 1 min after its application had ceased.

TABLE 1

SUBSTANCES TESTED ON CORTICAL NEURONES

−, Depression; +, excitation.

Classification	Substance	Alternative name	Action on cell	Antihistamine action
Histamine and related compounds	Histamine		− and +	
	(+)(−)-Histidine		−	
	Betazole	Histalog	−	
	Imidazoleacetic acid		−	
	1-Methylhistamine		− and +	
Histamine antagonists	Chlorcyclizine	Di-paralene	−	Yes
	Chlorpromazine	Largactil	−	Yes
	Cyclizine	Marezine	−	Yes
	Diphenhydramine	Benadryl	−	Yes
	Mepyramine	Neo-antergan	−	Yes
	Phenindamine	Thephorin	−	Yes
	Tripelennamine	Pyribenzamine	−	Yes
Other compounds	Acetylcholine		− and +	
	(−)-Glutamic acid		+	
	5-Hydroxytryptamine		−	
	Noradrenaline		− and +	
	Strychnine		−	Yes

The relative depressant potencies of imidazoleacetic acid, histidine and histamine firing evoked by (−)-glutamate were similar to those described in an earlier report (Krnjević & Phillis, 1963). Imidazoleacetic acid is comparable in its potency and time course of action with the monocarboxylic amino-acid γ-amino-butyric acid. When tested on spontaneously firing cells and synaptically evoked responses, however, histamine frequently appeared to be a more powerful depressant than histidine (see Fig. 1C).

The histamine isomer, betazole (histalog) is especially interesting in that it shares with histamine the property of stimulating the secretion of gastric acid without contracting the guinea-pig ileum or lowering cat blood pressure (Lee & Jones, 1949; Rosiere & Grossman, 1951). Betazole was frequently more effective as an inhibitor of cortical neurones than histamine (see Fig. 4B). It did not seem to have any of the excitant actions of histamine.

Histamine antagonists and histamine

A series of compounds which are known to antagonize the peripheral actions of histamine was tested on cortical neurones (see Table 1). All these compounds had depressant effects on the cortical neurones tested. Examples of the various actions of histamine antagonists are presented in Figs. 4–7.

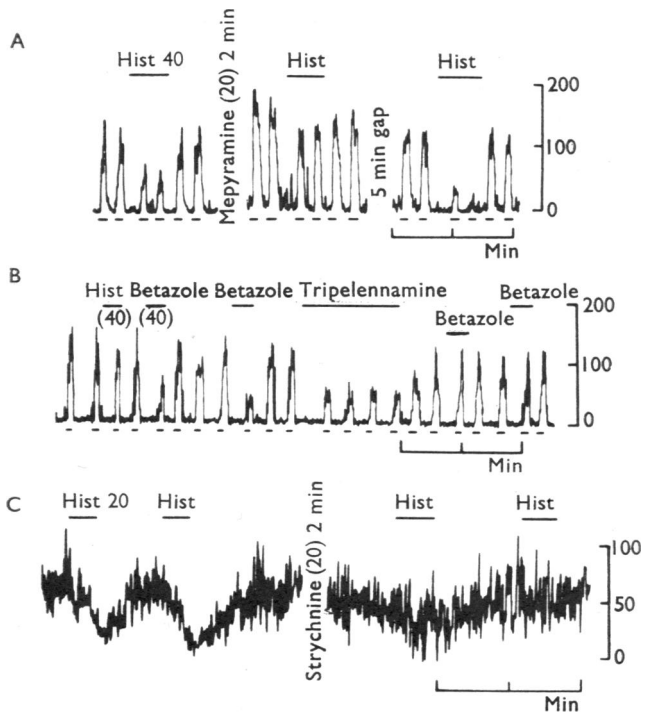

Fig. 4. Examples of histamine antagonism by mepyramine, tripelennamine and strychnine. A, Histamine (40 nA) depression of (−)-glutamate (50 nA) firing was greatly reduced by mepyramine. The effect of mepyramine had worn off 6 min later. B, Betazole (40 nA) was a more effective depressant of (−)-glutamate (40 nA) firing than histamine. Tripelennamine (20 nA for 100 sec) antagonized betazole. C, Histamine (20 nA) depression of this spontaneously firing neurone was abolished by strychnine (20 nA for 2 min).

Each compound was tested as a histamine antagonist on at least five histamine depressed cells and anti-histaminergic activity was detected for all compounds. The antihistamines all depressed cell excitability to a greater or lesser extent, and recovery from this depression frequently took several minutes.

Mepyramine, tripelennamine and chlorcyclizine have been tested more extensively than the other antihistamines in this survey. Mepyramine had a powerful depressant action on cortical neurones, depressing synaptic and (−)-glutamate induced firing. This action, which was probably related to the local anaesthetic effect that has been observed with this substance (Reuse, 1948), had a duration of several minutes and excitability frequently failed to return to control levels. An example of the antihistaminic actions of mepyramine is shown in Fig. 4A. After an application of mepyramine (20 nA) for 2 min, the cell excitability was depressed for a further 3 min. When the responses to (−)-glutamate had returned to control level, histamine depression was markedly reduced in comparison with the pre-mepyramine levels. After a further 5 min period, histamine depression had returned to its control level. Long applications of mepyramine with low currents were attempted on several cells to ascertain whether a histamine antagonism could be induced in the absence of a prior reduction in cell excitability, but this was never possible.

Tripelennamine and chlorcyclizine were more satisfactory antagonists because they frequently abolished the depressant effects of histamine without first reducing cell excitability. Even when these agents depressed the response to (−)-glutamate, this effect had a short duration, frequently wearing off during the period of application of the antagonist. Examples of the anti-histaminic actions of tripelennamine are shown in Figs. 4B, 6A and B, and 7A. After an application of tripelennamine or chlorcyclizine, the depressant effects of histamine were reduced for a period of 10–20 min.

Strychnine was also tested as a histamine antagonist. The ability of this compound to abolish post-synaptic inhibition of spinal cord motoneurones is well known (Eccles, 1957) and it has recently been shown that it depresses some synaptic inhibitions as well as the inhibitory effects of ACh, noradrenaline and 5-hydroxytryptamine on cortical neurones (Phillis & York, 1967b). A similar effect was observed on the histamine depression of cortical neurones (Fig. 4C). The inhibitory actions of histamine, but not of imidazoleacetic acid, were abolished by intravenous picrotoxin (0.5 mg/kg).

A block of histamine depression by antagonists frequently revealed an underlying excitation if the histamine applying currents were in excess of 40 nA. This excitant action of histamine was also susceptible to the antagonists and an example of this is shown in Fig. 5. Histamine (60 NA) potentiated the effect of (−)-glutamate on this neurone. During an application of the histamine antagonist, phenindamine, the excitability of the cell was depressed and recovery took approximately 2 min. Subsequent applications of histamine failed to potentiate the effects of (−)-glutamate.

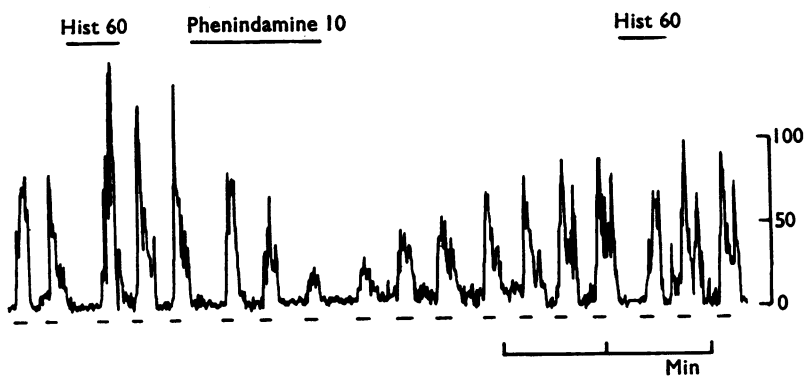

Fig. 5. Histamine (60 nA) facilitation of (−)-glutamate (40 nA) excitation was abolished by phenindamine (10 nA for 80 sec). The depression of (−)-glutamine firing by phenindamine is also illustrated by this record.

The results described above demonstrate that histamine has profound effects on some cortical neurones and that both its inhibitory and excitatory effects are blocked by histamine antagonists. These findings, together with those of other workers, suggest that histamine may be a synaptic transmitter. Attempts were therefore made to find a histaminergic pathway.

Histamine, like noradrenaline and 5-hydroxytryptamine, is found in large amounts in the hypothalamus. It has recently been shown that destruction of tissue in the lateral hypothalamic nucleus is associated with a marked fall in the levels of noradrenaline and

5-hydroxytryptamine in the pre-cruciate neocortex of cats (Moore, Shiu-Loong Wong & Heller, 1965 ; Heller, Seiden & Moore, 1966) and it seems that a monoaminergic pathway from the hypothalamus to the cortex may exist in cats (Phillis & York, 1967b). It is also possible that hypothalamico-cortical histaminergic pathways are present.

Histamine antagonists—Synaptic inhibition and other drugs

Stimulation in the lateral hypothalamus evoked short latency negative focal potentials in the feline pre-cruciate neocortex, with monosynaptic firing of some cortical neurones and polysynaptic excitatory responses from others. Associated with hypothalamic stimulation were short latency inhibitions with a duration of 100–150 msec and, less frequently, a long lasting inhibition with a duration of up to 1 min. The short duration inhibition is resistant to strychnine which, however, abolishes the long duration inhibitory effects (Phillis & York, 1967b).

The antihistamines, tripelennamine, mepyramine and chlorcyclizine were tested on long duration cortical inhibitions evoked by lateral hypothalamic stimulation. These compounds blocked the depressant effects of both lateral hypothalamic stimulation and histamine, tested on the same cells—a finding which suggested the existence of a histaminergic pathway.

To confirm this suggestion, the specificity of the histamine antagonists mepyramine, tripelennamine and chlorcyclizine was determined by testing them on depressions evoked by ACh, noradrenaline and 5-hydroxytryptamine. Most of the antihistamines used have been reported to have some ACh-antagonist actions (Reuse, 1948 ; Lands, Hoppe, Siegmund & Luduena, 1949).

The present results show that iontophoretically applied antihistamines are not specific in their actions. The neurone in Fig. 6A was depressed by histamine (40 nA), ACh (40 nA) and by lateral hypothalamic stimulation. After a 2 min application of tripelennamine the depressant effects of both substances and electrical stimulation were abolished. Tripelennamine also depressed the excitant effects of ACh on cortical neurones. The spontaneously firing cell in Fig. 6B was excited by (−)-glutamate and ACh, and depressed by histamine. After an application of tripelennamine, (−)-glutamate continued to excite this neurone whilst the effects of ACh and histamine were largely abolished.

Further evidence that the depressant effects of lateral hypothalamic stimulation on cortical neurones was the result of a release of ACh is presented in Fig. 6C. This neurone was also depressed by histamine and ACh. After an application of atropine, which abolished the inhibitory effects of ACh, the effects of lateral hypothalamic stimulation but not of histamine were abolished. Similar effects on ACh depression were observed with mepyramine.

Tripelennamine, mepyramine and chlorcyclizine also antagonized the effects of noradrenaline and 5-hydroxytryptamine on cortical neurones. Histamine (40 nA) and 5-hydroxytryptamine (40 nA) depressed the cortical neurone in Fig. 7A. Tripelennamine (20 nA) was applied for 80 sec and although this substance had little effect on cell excitability it greatly reduced the effects of histamine and 5-hydroxytryptamine.

Another example of the antagonism between tripelennamine and the monoamines is shown in Fig. 7B. The depression of this cell by noradrenaline and 5-hydroxytryptamine

was greatly reduced by a 2 min application of tripelennamine (20 nA). Similar effects were observed with mepyramine and chlorcyclizine; the amount of reduction in the depressant effects of noradrenaline and 5-hydroxytryptamine being comparable with the degree of antagonism of histamine.

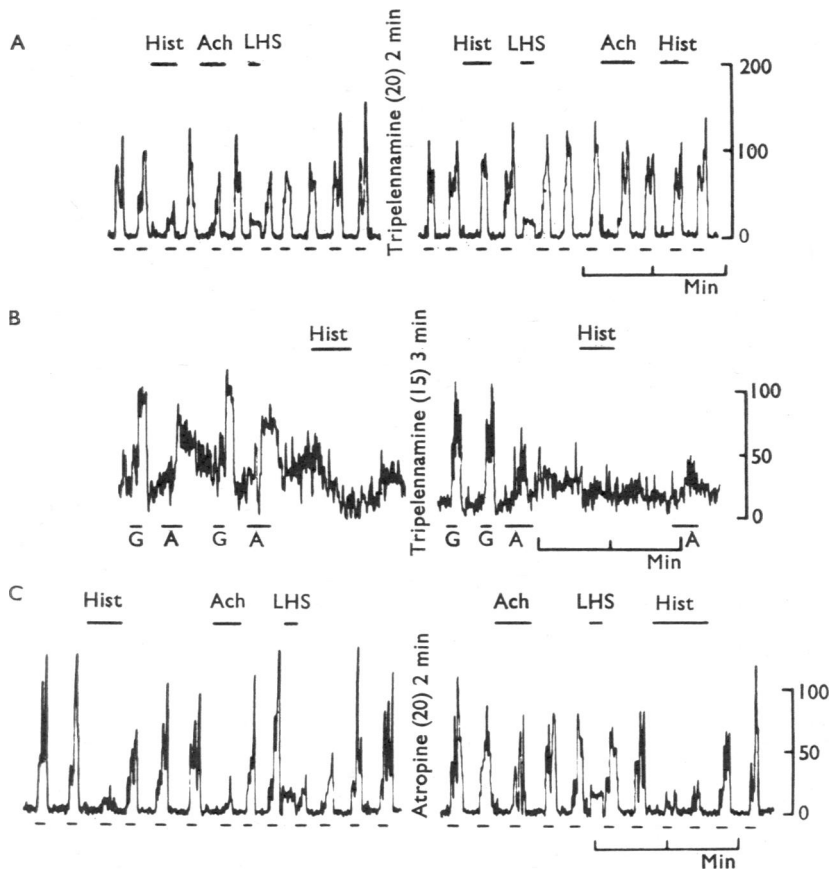

Fig. 6. A, Histamine (40 nA), ACh (40 nA) and lateral hypothalamic stimulation (LHS, 15/sec for 10 sec) induced depressions of this cortical neurone were abolished by tripelennamine (20 nA for 2 min). B, ACh (40 nA) excitations and histamine (50 nA) depressions of this neurone were antagonized by tripelennamine (15 nA for 3 min) whereas (−)-glutamate excitation was not affected. C, Atropine (20 nA for 2 min) antagonized the inhibition of this cell by ACh (60 nA) and lateral hypothalamic stimulation (LHS, 15/sec for 10 sec) but did not reduce the histamine (50 nA) depression.

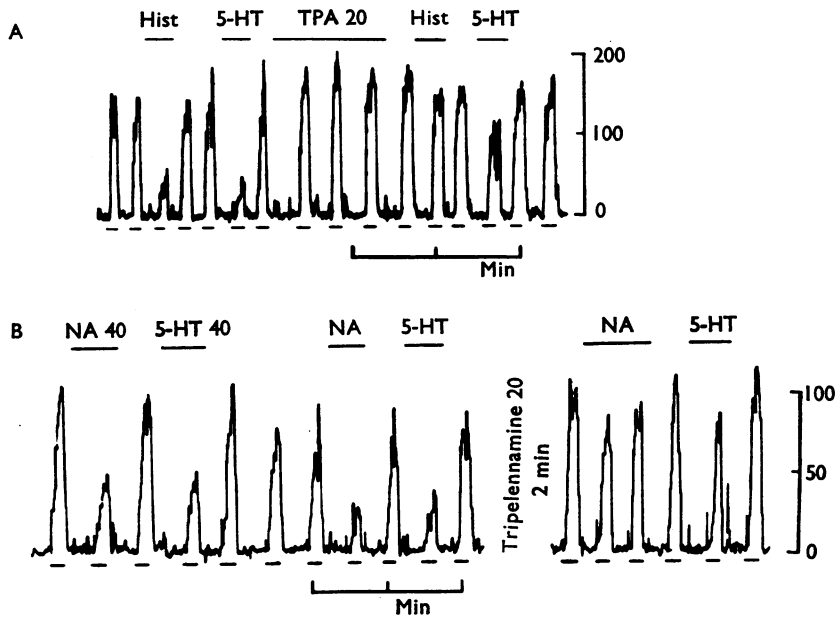

Fig. 7. A, Tripelennamine (TPA, 20 nA for 80 sec) antagonized the depressant effects of histamine (40 nA) and 5-hydroxytryptamine (5-HT, 40 nA) on (−)-glutamate (60 nA) induced firing of this neurone. B, Tripelennamine (20 nA for 2 min) antagonized the depressant effects of 5-hydroxytryptamine (40 nA) and noradrenaline (NA, 40 nA) on (−)-glutamate (50 nA) firing.

DISCUSSION

The excitability of many neurones in the pre-cruciate cerebral cortex was depressed by relatively small amounts of histamine. This depression was manifested by a reduction or abolition of the excitant effects of lateral hypothalamic stimulation, by a failure in some instances of an antidromically propagating spike to invade the cell soma, by a depression of spontaneous firing and the excitant effects of (−)-glutamate or ACh. Larger amounts of histamine frequently excited cortical neurones, potentiating (−)-glutamate firing and initiating a discharge of spikes in some neurones. Both depressant and excitant effects of histamine were observed on many neurones, and the interaction of these conflicting actions frequently obscured the fact that histamine had a powerful effect on such cells. The application of smaller amounts of histamine, however, usually revealed a depressant action on these cells. Although antihistamines antagonized both actions, the application of one of these compounds to neurones that were initially depressed by large amounts of histamine frequently resulted in the uncovering of an excitant action. Because excitation was rarely observed as a primary phenomenon and because larger applying currents were necessary to evoke excitation than inhibition, it seems that the membrane receptors mediating inhibition have a lower threshold for histamine.

Dual effects of biogenic amines on single neurones in the central nervous system have previously been encountered. Noradrenaline had both excitant and depressant actions on some thalamic neurones (Phillis & Tebēcis, 1967). The noradrenaline receptors

mediating this excitation were also prone to desensitization when the drug was applied repeatedly. Excitant and depressant effects of choline esters upon the same thalamic neurone (McCance, Phillis, Tebēcis & Westerman, 1968) and Renshaw cells (Curtis & Ryall, 1966) have been described. This phenomenon has recently been given considerable significance with the discovery of a neurone in *Aplysia* that is both excited and inhibited by synaptically released ACh (Wachtel & Kandel, 1967). In this instance, low frequency stimulation of the presynaptic cell has an excitant action on the postsynaptic neurone, whereas at higher frequencies of stimulation the excitatory receptor desensitizes and an inhibitory action predominates.

It has been assumed, in the above discussion, that histamine is acting on the post-synaptic membrane of cortical neurones. The presence of histamine in presynaptic nerve terminals and synaptic vescicles in the cortex (Kataoka & de Robertis, 1967) raises the possibility that histamine acts as a synaptic transmitter. Alternatively, it is possible that it acts presynaptically, postsynaptically on non-synaptic receptors, or even on glial cells around the neurone. Although actions mediated through pre-synaptic terminals or glial cells are difficult to eliminate, it is more likely that the observed effects of histamine are a result of an action on the post-synaptic membrane, though not necessarily on sub-synaptic receptors. Evidence for a synaptic transmitter role of histamine will, however, have to await the availability of more specific antagonists.

The non-specificity of action by histamine antagonists on cortical neurones may possibly be explained by the so-called "local anaesthetic," anti-ACh and anti-catecholamine actions of some of these compounds (Reuse, 1948; Lands *et al.*, 1949; Fleckenstein, 1952; Sethi, Gulati, Gokhale & Joseph, 1967). The iontophoretic method of application of drugs seems to discriminate between the specificity of various types of antagonist compound. This may be a result of the application of relatively large amounts of substance to a very limited area of neuronal membrane for a brief period of time with the iontophoretic technique. The situation presents a considerable contrast to that obtaining in an organ bath, where preparations can be exposed to low concentrations of drugs for long periods. Thus when tested on guinea-pig ileum, mepyramine is a highly specific histamine antagonist, being 40,000 times as active against histamine as it is against acetylcholine, whereas atropine is only 1,000 times as active against acetylcholine as it against histamine (Schild, 1947).

The preferred degradation pathway of brain histamine is by methylation. Both methylhistamine and the enzyme responsible, histamine *N*-methyl-transferase, are present in the feline cerebral cortex (White, 1959, 1966). Little is known about specific inhibitors for this enzyme. An alternative degradation pathway of histamine is deamination to imidazoleacetic acid, catalysed by the enzyme histaminase (Schayer, 1959). This enzyme has not been found in brain (Burkard *et al.*, 1963), however, and imidazoleacetic acid was not detected in cortical homogenates after administration of ^{14}C-labelled histamine (White, 1959). Thus it is possible that the potent depressant action of imidazoleacetic acid on cortical neurones is unrelated to synaptic transmission.

Betazole was more potent than histamine at depressing cortical neurones. This compound is about 1/700 as potent as histamine in depressing blood pressure and does not possess its contractile activity on the ileum (Lee & Jones, 1949; Rosiere & Grossman, 1951). Like histamine, however, it possesses the property of stimulating gastric acid

secretion and there is some evidence to suggest that the histamine receptor for this action is different from those elsewhere because (*a*) antihistaminic drugs fail to prevent histamine induced secretion of gastric acid and (*b*) xanthine alkaloids inhibit the actions of histamine at other sites but potentiate its gastric secretory action (Rosiere & Grossman, 1951).

Acetylcholine has been implicated as an inhibitory transmitter in the cerebral cortex, released during stimulation of the medullary pyramidal tract and brain stem reticular formation (Phillis & York, 1967a). The results presented in this paper add further support to the concept of a cholinergic inhibitory synapse in the cortex. Similar atropine-sensitive inhibitions can be evoked by surface stimulation of the adjacent cerebral cortex, so it is likely that an intra-cortical cholinergic neurone is involved. Further details of the results of this investigation will be described in a subsequent paper (Phillis & York, in preparation).

SUMMARY

1. Histamine, some related compounds and various histamine antagonists were administered iontophoretically to neurones in the pre-cruciate cerebral cortex.

2. Histamine had a dual effect on many neurones, small amounts causing a depression of cell excitability and larger amounts a mixture of excitation and depression. Imidazole-acetic acid, histidine and betazole also depressed cortical neurones.

3. Antihistamines antagonized the depressant and excitant effects of histamine. They also antagonized the effects of acetylcholine, noradrenaline and 5-hydroxytryptamine.

4. Evidence is presented that the inhibitory effect of lateral hypothalamic stimulation on some cortical neurones, which was abolished by antihistamines, may be due to a cholinergic synapse.

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Note added in proof

A sample of 1-methylhistamine (Calbiochem), the metabolite of histamine in brain, was obtained only after the submission of this paper. 1-Methylhistamine was generally comparable with histamine in its actions, causing either excitation, inhibition or a combination of these effects. Applications with low currents (10–15 nA) frequently excited cortical neurones which were depressed by histamine. The possibility arises that termination of histamine inhibition may be effected by its conversion into an excitant product.

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