J. exp. Biol. **124**, 203–224 (1986)
Printed in Great Britain © *The Company of Biologists Limited 1986*

PROPERTIES AND ROLES OF THE THREE SUBCLASSES OF HISTAMINE RECEPTORS IN BRAIN

BY JEAN-CHARLES SCHWARTZ, JEAN-MICHEL ARRANG, MONIQUE GARBARG AND MARIE KORNER

Unité 109 de Neurobiologie, Centre Paul Broca de l'INSERM, 2 ter rue d'Alésia, 75014 Paris, France

SUMMARY

In brain, histamine (HA) is the transmitter of a neuronal system resembling other monoaminergic systems. It is also present in mast cells from which it may control vascular and inflammatory processes. Its various actions are mediated not only by the two well known H_1- and H_2-receptor subclasses but also by the recently discovered H_3-receptors, with distinct localization and pharmacology. H_1-receptors mediate a series of biochemical responses which have several features in common: they require intact cells to be observed and largely depend upon the availability of Ca^{2+}.

H_1-receptor-mediated responses include glycogenolysis, stimulation of cyclic GMP formation, potentiation of cyclic AMP formation. Recent studies indicate that H_1-receptors are linked with phosphatidylinositol breakdown and generation of two intracellular signals which both contribute to the final response (e.g. in the cyclic AMP generation).

H_2-receptors seem to be directly linked with an adenylate cyclase and their stimulation results in enhanced electrophysiologically recorded responses to excitatory agents.

Finally whereas H_1- and H_2-receptors appear to be postsynaptically located, a novel subclass (H_3) of HA receptors was recently revealed with a presynaptic localization. H_3-receptors are autoreceptors mediating inhibition of HA release from and biosynthesis in histaminergic nerve terminals in the CNS.

The physiological and pharmacological implications of three distinct receptor subclasses for HA will be discussed.

INTRODUCTION

Historically, drugs interacting with histamine (HA) receptors have been, by chance, at the origin of the two major classes of agents presently used in psychiatry and which were introduced before the realization that HA receptors were present in brain.

HA does not readily cross the 'blood–brain barrier' and, therefore, the function of HA receptors in the CNS is to mediate the actions of the locally synthesized, stored and released amine. The two types of brain cells (neurones and mast cells) in which these processes occur were identified more than 10 years ago, mainly from indirect

Key words: histamine receptors, brain, autoreceptors, H_1-receptor, H_2-receptor, H_3-receptor, phosphatidylinositol, cyclic AMP, calcium.

approaches, i.e neurochemical and lesion studies (Schwartz, 1975). However, it is only in the last few years that immunohistochemical methods, developed in several laboratories, have allowed them to be visualized, largely confirming and extending these previous studies (reviewed by Watanabe *et al.* 1985; Pollard *et al.* 1985*a*; Steinbusch & Mulder, 1985).

Most histaminergic neurones arise from several magnocellular mamillary nuclei in the ventral part of the posterior hypothalamus. Their varicose axons constitute long, ascending fibre tracts passing through the lateral hypothalamic area, projecting, mainly in an ipsilateral fashion, to almost all regions of di- and tele-encephalon. A minor descending pathway, also emanating from the hypothalamus, projects to mesencephalic and, presumably, more posterior structures. Hence, the general disposition of the histaminergic neuronal system (i.e. a compact cell group and a widespread distribution of long fibres) resembles that of catecholaminergic and serotoninergic systems in brain. As described below, these similarities extend to receptor-mediated intracellular events and, most probably, to functional roles (reviewed by Schwartz *et al.* 1980*a*, 1982; Schwartz, Pollard & Quach, 1980*b*; Schwartz, Garbarg & Pollard, 1986; Hough & Green, 1984).

However, an original feature of cerebral HA is the existence of a non-neuronal, slowly turning over pool, which contributes about 50% of the total amine content, mainly held in mast cells. These connective tissue cells are scarce in brain (but their HA content is relatively high) and seem closely associated with vascular elements; in addition, cerebral microvessels themselves may also store HA. HA might be released from these non-neuronal stores, for example during inflammatory processes, and thereby may control blood flow and vascular permeability in brain. In any case, the multiple cellular localizations of HA in brain suggest multiple messenger actions, thus accounting for the presence of specific receptors.

PHARMACOLOGICAL TOOLS FOR THE DISTINCTION OF HISTAMINE RECEPTOR SUBCLASSES

Two major breakthroughs have dominated the history of HA pharmacology: the discovery of 'antihistamines' (now designated H_1-receptor antagonists) by Bovet & Staub (1937) and that of H_2-receptor antagonists by Black *et al.* (1972). These two classes of compounds, as well as more or less selective HA receptor agonists (Ganellin, 1982), have not only established that HA actions are mediated by two distinct molecular entities but also provided physiologists with invaluable tools to delineate the functions of the amine: for example the presence of both H_1- and H_2-receptors in brain and their participation in the stimulation of cyclic AMP formation was shown in 1975 (Baudry, Martres & Schwartz, 1975). Furthermore, in the same way as selective H_1-receptor antagonists, like mepyramine, have paved the way for the identification of the H_2-receptor, the utilization of various HA agonists, or antagonists, has facilitated the characterization of the H_3-receptor when their potencies at H_1- and H_2-receptors (expressed by relative potencies of agonists

and apparent dissociation constants of antagonists) were taken into consideration (Arrang, Garbarg & Schwartz, 1983).

Fig. 1 shows the structures of a few characteristic agents acting at HA receptors (see also reviews by Ganellin, 1982, 1985; Durant *et al.* 1985) and their potencies on reference systems (i.e. guinea-pig ileum contractions, atrium rate and inhibition of HA release from brain slices for H_1-, H_2- and H_3-receptors, respectively). It is apparent that no highly selective H_1-receptor agonist, and H_3-receptor agonist or antagonist is as yet available. From a practical point of view it should be emphasized that H_1-receptor antagonists are, in general, highly lipophilic molecules which easily penetrate the blood–brain barrier. However, this is not the case for any other agents which act at HA receptors. Hence pharmacological tools which modify cerebral histaminergic transmission *in vivo*, are still largely lacking for either experimental or therapeutic purposes.

HISTAMINE RECEPTORS STUDIED WITH RADIOLIGANDS

Binding techniques (which have been so fruitful in recent years in investigating the properties of a variety of central nervous receptors) have also been applied to HA receptors.

Hill, Young & Marrian (1977) demonstrated that [^{3}H]mepyramine can be used as a selective ligand of H_1-receptors in homogenates of smooth muscle of the guinea-pig ileum. In this preparation, there is very good agreement between the affinity constants, determined for a range of ligands from the inhibition of [^{3}H]mepyramine binding, and the values derived from competitive antagonism of the contractile response to HA.

Specific binding (defined as that inhibited by a micromolar concentration of a non-radioactive H_1 antihistamine, promethazine or triprolidine), occurs with the expected high affinity (K_D less than 1 nmol l^{-1}) in guinea-pig brain but with a lower affinity (K_D more than 5 nmol l^{-1}) in rat brain (Chang, Tran & Snyder, 1978*a*; Hill & Young, 1980). The pharmacological specificity of the binding sites (assessed by establishing the inhibitory potencies of a variety of histaminergic and non-histaminergic antagonists) leaves little doubt that they represent the recognition moiety of H_1-receptors in the guinea-pig. There are, however, some discrepancies for rat brain. Stereospecificity is shown by the large difference in inhibitory potency of the two chlorpheniramine enantiomers, although the active one is an order of magnitude less potent in the rat than in the guinea-pig. It is likely therefore, that [^{3}H]mepyramine labels, with high affinity, a heterogenous population of sites in rat, which may mainly, but not only, represent H_1-receptors. HA itself presents a relatively low inhibitory potency towards [^{3}H]mepyramine binding. However, in the micromolar range, its estimated affinity is roughly consistent with its median effective concentration (EC_{50}) for H_1-receptor-mediated stimulation of either cyclic AMP accumulation (Palacios, Garbarg, Barbin & Schwartz, 1978*a*) or [^{3}H]glycogen hydrolysis in brain slices (Quach, Duchemin, Rose & Schwartz, 1980*a*).

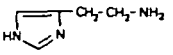
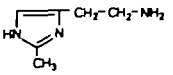
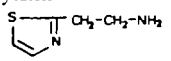
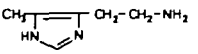
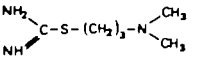
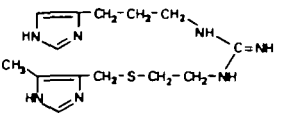
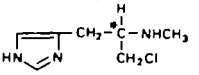
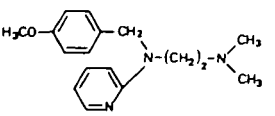
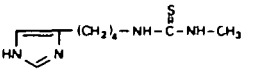
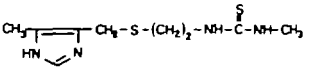
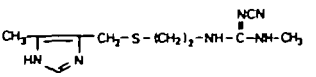
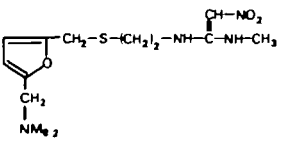
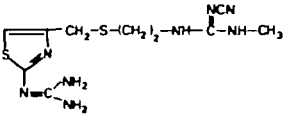
AGENTS		ACTIVITY ON BIOLOGICAL SYSTEMS		
		Ileum contraction (H_1-receptor)	Atrium rate (H_2-receptor)	Brain histamine release (H_3-receptor)
AGONISTS		Relative agonist potency		
Histamine 		100	100	100
2-Methylhistamine 		16·5	4·4	<0·08
2-Thiazolylethylamine 		26	2·2	<0·008
4-Methylhistamine 		0·2	43	<0·008
Dimaprit 		<0·0001	71	<0·008
Impromidine 		<0·001	4800	Antagonist ($K_i = 6{\cdot}5\times10^{-8}$ mol l^{-1})
N$^{\alpha}$-methyl-α-chloromethylhistamine 	S(+)	0·3	7	1·1
	R(−)	0·3	51	0·006
ANTAGONISTS		Antagonist activity, K_i (mol l^{-1})		
Mepyramine 		$0{\cdot}4\times10^{-9}$	—	$>3\times10^{-6}$
Burimamide 		$2{\cdot}9\times10^{-4}$	$7{\cdot}8\times10^{-6}$	$7{\cdot}0\times10^{-8}$
Metiamide 		$>10^{-4}$	$0{\cdot}9\times10^{-6}$	$2{\cdot}5\times10^{-6}$
Cimetidine 		$4{\cdot}5\times10^{-4}$	$0{\cdot}8\times10^{-6}$	$3{\cdot}3\times10^{-5}$
Ranitidine 			$6{\cdot}3\times10^{-8}$	$>10^{-6}$
Tiotidine 			$1{\cdot}5\times10^{-8}$	$1{\cdot}7\times10^{-5}$

Fig. 1. Pharmacology of the three subclasses of histamine receptors. The agonist and antagonist potencies of the various agents are shown at typical biological systems on which responses to histamine are mediated by a single receptor subclass.

The mean density of [^{3}H]mepyramine binding sites in the brain of several species is about 100 fmol mg^{-1} protein, but there are considerable differences in their regional distribution: for instance, although the cerebellum shows the highest density in the guinea-pig brain (Hill, Emson & Young, 1978), it exhibits the lowest in the rat brain (Chang *et al.* 1978*b*). This rather unusual situation remains to be clarified.

Studies of receptors at the molecular level commenced with the advent of selective radioligands. These not only facilitate purification of macromolecules but even allow biochemical studies to be carried out when they are still in their membrane-bound state. For example, covalent chemical modifications of receptor proteins have been useful in obtaining information about functional groups. The most widely used are reagents modifying sulphhydryl groups and disulphide bonds. Agonist and antagonist binding to receptors are often affected differentially, suggesting a role of thiol groups or disulphide bonds in changes of conformation of the receptor protein elicited only by agonists (see review by Strauss, 1984). The reactive groups may play a critical role for ligand binding as well as in transduction. In the case of HA, receptor alkylation of sulphhydryl groups by *N*-ethylmaleimide (NEM) inhibits the H_1-receptor-mediated contractions of rabbit aorta, whereas reduction of disulphide bonds by dithiothreitol potentiates both this response (Fleisch, Krzan & Titus, 1973, 1974) and the guinea-pig ileum contraction (Glover, 1979). However, the specificity of the dithiothreitol effects was recently questioned (Fontaine, Famaey & Reuse, 1984) and the effects of these reagents on intact cell preparations cannot be easily interpreted.

The effect of the thiol alkylating agent NEM on HA H_1-receptors from guinea-pig cerebellum, labelled with [^{3}H]mepyramine, has been investigated (Yeramian, Garbarg & Schwartz, 1985). The properties of [^{3}H]mepyramine binding (apparent dissociation constant and maximal number of sites) were not modified by prior treatment of the membranes with 2 or 5 mmol l^{-1} NEM. This treatment did not change the inhibition curves of *d*-chlorpheniramine or mianserin, two H_1-receptor antagonists. In contrast, treatments of membranes with NEM significantly decreased the IC_{50} values (concentrations at which binding is inhibited by 50 %) of HA and the slope indexes (pseudo-Hill coefficients) of HA inhibition curves, which became less than unity. These effects were irreversible; their extent was related to the duration of NEM treatment and to NEM concentration. A computer analysis of the data indicated that some of the H_1-receptors were converted from a state of low affinity for the amine (IC_{50} value of 75 μmol l^{-1}) to a high affinity state (IC_{50} value of 2 μmol l^{-1}). The change was less marked for partial agonists than for HA. The NEM-induced change was observed in the presence and absence of Na^+ ions which are known to decrease the affinity of HA for H_1-receptors. Agonists or antagonists did not protect against the modification of HA affinity induced by NEM.

NEM treatment might alkylate a critical thiol group located outside the ligand binding domain of the H_1-receptor and thereby stabilize the latter in a different conformation from that of the activated state.

Some progress has already been reported towards purification of the H_1-receptor: it is easily solubilized and can be retained on a lectin affinity column, indicating its glycoprotein nature (Garbarg, Yeramian, Korner & Schwartz, 1985). Sucrose gradient and gel filtration experiments indicated an M_r of approximately 430 000 for the receptor–digitonin complex and the isoelectric point of the receptor is 4·8 (Toll & Snyder, 1982).

In view of the low abundance of neurotransmitter receptors in the mammalian CNS, sensitive probes are important tools for biochemical and localization studies. For this purpose an iodinated, highly sensitive, probe for the H_1-receptor was recently developed (Korner *et al.* 1986). [^{125}I]iodobolpyramine is a mepyramine derivative with increased affinity for H_1-receptors of guinea-pig cerebellum (K_D 0·15 nmol l^{-1}) and a 100-fold higher specific radioactivity. Together with a rather low non-specific binding, these features ensure a 50- to 100-fold increase in sensitivity of assays of H_1-receptors in membranes or in solubilized form.

Furthermore, [^{125}I]iodobolpyramine allows a detailed autoradiographic mapping of H_1-receptors in guinea-pig brain to be established (Fig. 2). Well-contrasted autoradiographic pictures, with low non-specific binding, were obtained after only 2 days' exposure. The highest grain densities were found in the molecular layer of the cerebellum. This agrees with both autoradiographic (Palacios, Young & Kuhar, 1979; Palacios, Schwartz & Garbarg, 1981*a*) and biochemical (Hill *et al.* 1978) data with [^{3}H]mepyramine. Functional H_1-receptors seem to be present in the cerebellum (Daum, Downes & Young, 1984) which, otherwise, contains a low L-histidine decarboxylase activity (Schwartz, Lampart & Rose, 1970). In the hippocampal formation, a projection field for histaminergic neurones (Barbin, Garbarg, Schwartz & Storm-Mathisen, 1976; Haas *et al.* 1978), the distribution of [^{125}I]iodobolpyramine autoradiographic grains was apparently similar to that of [^{3}H]mepyramine binding sites (Palacios, Wamsley & Kuhar, 1981*b*).

Other areas of very high H_1-receptor densities are the nucleus accumbens (not previously reported) and the whole thalamus (Hill *et al.* 1978). There was more or less uniform labelling of the whole cerebral cortex with some higher densities in superficial layers (laminae II–III). In rat brain, on the other hand, [^{3}H]mepyramine binding sites were mainly located in lamina IV of the temporal cortex (Palacios *et al.* 1981*b*). The widespread occurrence of H_1-receptors in the whole cerebral cortex could conceivably account for the sedating ('mental clouding') properties of H_1-antihistamines (Quach *et al.* 1979). Moderate to rather high labelling was found in the colliculi, mesencephalic central grey and ventral tegmental area, as well as in the bed nucleus of the stria terminalis. Very high levels of L-histidine decarboxylase activity have been reported in the three last areas (Pollard *et al.* 1978; Ben Ari *et al.* 1977). In the hypothalamus, several mamillary nuclei were distinctly labelled with [^{3}H]mepyramine (Palacios *et al.* 1981*b*). Localization of H_1-receptors in this region, where HA cell bodies of the ascending pathway have been localized by lesion (Schwartz *et al.* 1982) and immunohistochemical studies (Watanabe *et al.* 1984; Steinbusch & Mulder, 1984; Panula, Yang & Costa, 1984; Pollard *et al.* 1985*a*;

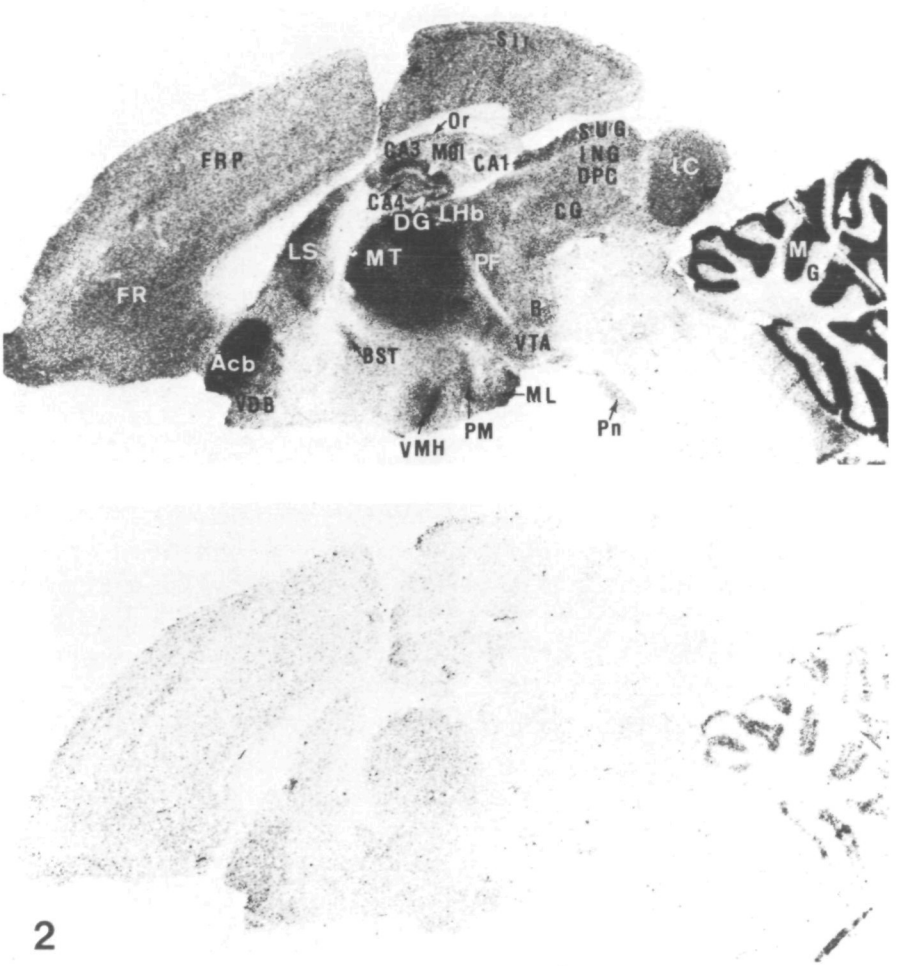


Fig. 2. Autoradiograms of H_1-receptors labelled with [^{125}I]iodobolpyramine on sagittal sections from guinea-pig brain. The structures with the highest density of H_1-receptors are the cerebellum (molecular layer, M), the thalamus (medial nucleus, MT) and the nucleus accumbens (Acb). H_1-receptors are also present in various other structures such as cerebral cortex (frontal cortex, FR, and frontoparietal cortex, FRP), hypothalamus (medial mamillary nucleus, ML, premamillary nucleus, PM, and ventromedial nucleus, VMH). Top, total binding; bottom, non-specific binding obtained in the presence of 0·2 μmol l^{-1} mianserin. BST, bed nucleus of stria terminalis; CA1, field CA1 of Ammon's horn; CA3, field CA3 of Ammon's horn; CA4, field CA4 of Ammon's horn; CG, central grey; DG, dentate gyrus; DPG, deep grey layer of superior colliculus; G, granular cell layer of cerebellum; IC, inferior colliculus; ING, intermediate grey layer of superior colliculus; LHb, lateral habenular nucleus; LS, lateral septal nucleus; MOl, molecular layer of dentate gyrus; Or, oriens layer of hippocampus; PF, parafascicular thalamic nucleus; Pn, pontine nuclei; R, red nucleus; Str, striate cortex; SUG, superficial grey layer of superior colliculus; VDB, nucleus of the vertical limb of the diagonal band; VTA, ventral tegmental area. From Korner *et al.* (1986). Reprinted by permission of the *European Journal of Pharmacology*.

Pollard, Pachot & Schwartz, 1985*b*), raises the possibility (but does not prove) that H_1-receptors may be present on HA cell bodies or dendrites.

In general, the localization of H_1-receptors (visualized with [^{125}I]iodobolpyramine in the guinea-pig brain) is consistent with the known histaminergic projections and HA cell bodies or dendrites and this agrees well with studies using [^{3}H]mepyramine in rat brain. However, there is also a species difference in regional distribution. The regions with the highest density of H_1-receptors in guinea-pig brain, namely cerebellum, thalamus and nucleus accumbens, were hardly labelled with [^{3}H]mepyramine in rat brain (Palacios *et al.* 1981*b*).

Furthermore, mepyramine and several other antagonists display a higher affinity for H_1-receptors in the guinea-pig than in rat or mouse brain (Chang *et al.* 1978*b*; Hill & Young, 1980; Quach *et al.* 1980*a*). We consistently found bolpyramine to be about four times more potent in guinea-pig than in rat cerebral membranes and there was no evidence for significant [^{125}I]iodobolpyramine-specific binding to rat brain membranes. This negative result can be tentatively explained by the combination of a lower affinity of [^{125}I]iodobolpyramine, a relatively high level of non-specific binding and a limited number of binding sites in rat brain membrane preparations. Specific binding was also not seen in tissue sections.

These observations strongly confirm the species differences in H_1-receptor pharmacological specificities while, at the same time, indicating that the new ligand will not be usable for rat tissues.

[^{3}H]Mepyramine can also be used to label H_1-receptors in the brain of the mouse *in vivo* (Quach *et al.* 1980*b*; Schwartz *et al.* 1980*a*). Thus, a few minutes following intravenous administration of [^{3}H]mepyramine in low doses, a saturable binding occurs *in vivo* which presents characteristics of regional heterogeneity and pharmacological specificity paralleling those observed in binding studies *in vitro*. The density of [^{3}H]mepyramine binding sites *in vivo* agrees well with their density estimated *in vitro*, indicating that no substantial loss of H_1-receptors occurs during the preparation of membranes used for the *in vitro* test. A major interest in the *in vivo* test is that it shows that systemic administration of most H_1-antihistamines in doses currently used in therapeutics to alleviate allergic symptoms results in the occupation of a major fraction of H_1-receptors in the brain. This observation strongly suggests that the well-known central effects of H_1-antihistamines (such as sedation 'mental clouding', increased sleep duration, EEG changes resembling those elicited by barbiturates) are, indeed, mediated by blockade of the actions of endogenous HA at H_1-receptors. Interestingly, mequitazine, an H_1-antihistamine that a double-blind study has shown to be devoid of sedative properties (Gervais, Gervais, De Beule & Van Der Bijl, 1975), does not occupy H_1-receptors in brain, probably because it does not cross the blood–brain barrier. It should also be noted that several psychotropic compounds, classified as either antidepressants (doxepin, amitriptyline) or neuroleptics (levomepromazine, droperidol), but which are all characterized by sedative properties in patients, significantly inhibit [^{3}H]mepyramine binding (in therapeutic doses) in the brain of the mouse *in vivo*.

With H_2-receptor labelling by radioligands, the situation seems much less favourable than in the case of H_1-receptors.

A saturable binding of [^{3}H]cimetidine to homogenates from guinea-pig or rat brain has been described, but it is unlikely that this ligand labels H_2-receptors (Warrander, Norris, Rising & Wood, 1983). This conclusion is based on several pieces of evidence: the K_D of cimetidine differs according to the preparation and to the authors (range 40–400 nmol l^{-1}) and was 10–100 times lower than its K_i regarding HA antagonism at well-defined H_2-receptor systems; the inhibitory potency of various histaminergic agents did not reflect their pharmacological potency: ranitidine, for example, gave no detectable inhibition even at 1 mmol l^{-1}; there was no clear-cut regional distribution of the binding sites. Other attempts to label H_2-receptors have been performed with [^{3}H]ranitidine (Bristow, Hare, Hearn & Martin, 1981), [^{3}H]impromidine or [^{3}H]tiotidine (unpublished observations from our laboratory), also apparently without success. More recently, a successful binding of [^{3}H]tiotidine to H_2-receptors was reported, a difference attributed by the authors to the availability of a chemically more stable batch of the ligand (Gajtkowski, Norris, Rising & Wood, 1983). Although specific [^{3}H]tiotidine binding was relatively low as compared to non-specific binding, its pharmacology was well correlated with that of reference H_2-receptor-mediated responses. The distribution of binding sites between regions of guinea-pig brain (striatum ≫ cerebral cortex > hippocampus, no binding in cerebellum or brainstem) was markedly different from that of H_2-linked adenylate cyclase and inconsistencies were reported in peripheral tissues (Norris, Gajtkowski & Rising, 1984; Rising & Norris, 1985).

Labelling the H_2-receptors is likely to be more successfully undertaken with the availability of new [^{3}H]-labelled ligands that present higher selectivity and affinity than those currently available.

Another approach to HA receptors in brain has used [^{3}H]HA itself as a ligand. In general [^{3}H]-agonists cannot be utilized as easily as [^{3}H]-antagonists for receptor labelling because, in most cases, the antagonists display much higher affinities. However, high-affinity binding sites for catecholamine or serotonin agonists, which are likely to represent true receptors, have been described during the past few years. [^{3}H]HA associates with rather high affinity (K_D 10 nmol l^{-1}) to membrane fractions from rat brain (Palacios *et al.* 1978*b*; Barbin *et al.* 1980). The binding sites have several characteristics which suggest that they might represent HA receptors. For example, many other putative neurotransmitters or HA metabolites fail to displace [^{3}H]HA, while various agonists (N^{α},N^{α}-dimethylHA; dimaprit) are reasonably potent inhibitors of binding. In addition, several other correlative features also suggest that these sites represent postsynaptic receptors: the marked regional heterogeneity, the localization into subcellular fractions enriched in synaptosomal membranes, the developmental pattern during ontogenesis paralleling development of putative histaminergic synapses and the decrease following administration of the neurotoxin kainic acid (Barbin *et al.* 1980).

However, the [^{3}H]HA binding sites might well correspond to a class of 'desensitized state' HA receptors as suggested by the decrease of the maximal capacity of

[^{3}H]HA binding sites in the presence of guanyl nucleotides; this effect occurs in particulate fractions as well as in a solubilized preparation.

The pharmacological specificity of [^{3}H]HA binding sites differs from that of typical H_1- and H_2-receptors. This is clearly shown by the relative potencies of agonists: impromidine is 10 times less potent than HA on these sites, although it is 10–50 times more potent on H_2-receptors; 2-thiazolylethylamine is 1000-fold less potent than HA, although it is only four times less potent on H_1-receptors. Moreover, the inhibition of [^{3}H]HA binding by H_1- or H_2-antagonists such as mepyramine or metiamide yielded data indicating that [^{3}H]HA may bind to a unique site with cooperativity, or to more than one site. The latter hypothesis is supported by the observation that [^{3}H]HA binding is only partially decreased in the presence of guanyl nucleotides (Barbin *et al.* 1980). Recently, a heterogeneous binding has also been suggested, with a fraction occurring at H_2-receptors (Wells *et al.* 1985), but the identity of the [^{3}H]HA binding sites largely remains to be elucidated.

HISTAMINE H_1-RECEPTOR AND CALCIUM TRANSLOCATION

The occurrence of H_1-receptors in nervous tissues was first demonstrated by pharmacological analysis of a series of cellular responses induced by HA. These responses require intact cells (or vesicular sacs). Whereas activation of the H_1-receptor alone does not stimulate cyclic AMP formation, it potentiates the action of direct activators of adenylate cyclase at either adenosine (Daum, Hill & Young, 1982) or HA H_2-receptors (Palacios *et al.* 1978*a*) by a complex mechanism that will be discussed below. H_1-receptors also mediate more directly other effects of HA, like the activation of cyclic GMP formation, as mainly studied in neuroblastoma cells (Richelson, 1978) and lysis of [^{3}H]glycogen in slices from cerebral cortex (Quach *et al.* 1980*a*). Although these responses seem quite diverse, they all depend upon the availability of Ca^{2+}, suggesting that the primary action of H_1-agonists on all cell types (including those of peripheral smooth muscles that they contract) is to induce increases in the intracellular concentration of Ca^{2+}, by which other intracellular events, like activation of guanylate cyclase or glycogen phosphorylase, are triggered.

However, changes in intracellular Ca^{2+} elicited by H_1-receptor stimulation, although likely, have never been directly shown, even when monitored in neuroblastoma cells with the luminescent protein aequorin (Richelson, 1985).

Nevertheless, the theory of a link between H_1-receptor activation and Ca^{2+} mobilization has recently received strong support. Following initial observations that HA stimulates the incorporation of inorganic [^{32}P] into cerebral phospholipids *in vivo*, H_1-receptor-mediated stimulation of [^{3}H]phosphatidylinositol 4,5-bisphosphate breakdown was shown in several laboratories (Daum, Downes & Young, 1983; Brown, Kendall & Nahorski, 1984; Donaldson & Hill, 1985) by monitoring the accumulation of [^{3}H]inositol phosphate in the presence of Li^+, and inositol phosphatase inhibitor.

HISTAMINE H_2-RECEPTORS AND ADENYLATE CYCLASE

The HA-sensitive adenylate cyclase which, in a variety of tissues, appears to be uniformly coupled to H_2-receptors, was first characterized in P. Greengard's laboratory on membranes from guinea-pig hippocampus (reviewed by Schwartz *et al.* 1982; Johnson, 1982). Both subcellular fractionation (Kanof, Hegstrand & Greengard, 1977) and lesion studies (Garbarg, Barbin, Palacios & Schwartz, 1978) are consistent with a postsynaptic localization of the HA-sensitive, cyclic-AMP-generating system in brain. This suggests that at least some synaptic actions of HA are mediated by a rise in the intracellular level of the cyclic nucleotide and the relationship between the two series of cellular events has started to be clarified with the electrophysiological studies of H. Haas in Zurich (reviewed in Haas, 1985).

Stimulation of H_2-receptors only slightly depolarizes pyramidal cells of slices of rat hippocampus, but profoundly potentiates a variety of excitatory signals like depolarizations induced by excitatory amino acids, synaptically-evoked spikes, epileptiform bursts. These potentiations result from a disinhibitory effect of HA, consisting of a blockade of long-lasting hyperpolarizations or of accommodations of firing which, otherwise, restrict the amplitude and duration of excitatory signals (Haas & Konnerth, 1983).

This modulatory effect, attributable to a decrease of calcium-activated potassium conductance, is potentiated by a phosphodiesterase inhibitor and mimicked by intracellular application of cyclic AMP. Similar modulatory effects occur following stimulation of β-adrenoreceptors, also known to be coupled with adenylate cyclase. All these observations strongly suggest that the rise in nucleotide level elicited by HA is responsible for the change in conductance. However the link between the two processes remains unknown since HA-induced protein phosphorylation, *via* a cyclic-AMP-dependent protein kinase, has never been described.

This mode of action, together with the highly divergent topography of HA neurones, may indicate that the function of the latter is not to transmit discrete information but to increase the sensitivity of large cerebral areas to excitatory inputs. This is consistent with the hypothesis that HA might be a 'waking amine' through the activation of H_2-receptors (as well as of H_1-receptors mediating the potentiation of the cyclic AMP response).

An H_2-receptor-mediated stimulation of cyclic AMP formation also occurs in cerebral microvessels (Karnushina *et al.* 1980) and might be related to the increased capillary permeability and brain oedema elicited by HA (Gross, 1985).

INTERACTION OF H_1- AND H_2-RECEPTOR-MEDIATED EVENTS IN THE GENERATION OF CYCLIC AMP

Although HA maximally stimulates (*via* H_2-receptors linked to adenylate cyclase) cyclic AMP formation by only 2- to 3-fold in a cell-free system, the corresponding response in brain slices (which involves both H_1- and H_2-receptors) can be as high as a 10- to 20-fold stimulation (Palacios *et al.* 1978*a*). The participation of H_1-receptors

in cyclic AMP accumulation in intact cells thus results in a striking amplification of the intracellular message generated by HA.

The H_1-receptor-mediated amplification seems to involve a dual mechanism. This is first shown by the influence of Ca^{2+} ions in the H_1-receptor-mediated response in hippocampal slices. In the absence of extracellular Ca^{2+} the H_2-receptor-mediated stimulation of cyclic AMP accumulation (evaluated by the response to dimaprit, a selective H_2-agonist) is not affected, whereas the H_1-receptor-mediated stimulation (e.g. the response to 2-thiazolylethylamine) is reduced by about 50% (Fig. 3). This suggests that Ca^{2+} translocation is partly involved in the H_1-receptor-mediated amplification.

The dual mechanism is also suggested by the effects of phorbol esters (i.e. protein kinase C activators). As previously discussed, H_1-receptor agonists stimulate phosphatidylinositol hydrolysis (reviewed by Carswell, Daum & Young, 1985), an effect which is known to result in the release of two distinct intracellular messengers (Fig. 4). The first is inositol 1,4,5-trisphosphate (IP_3), a messenger mobilizing Ca^{2+} from its intracellular stores, for example in the endoplasmic reticulum (ER). The

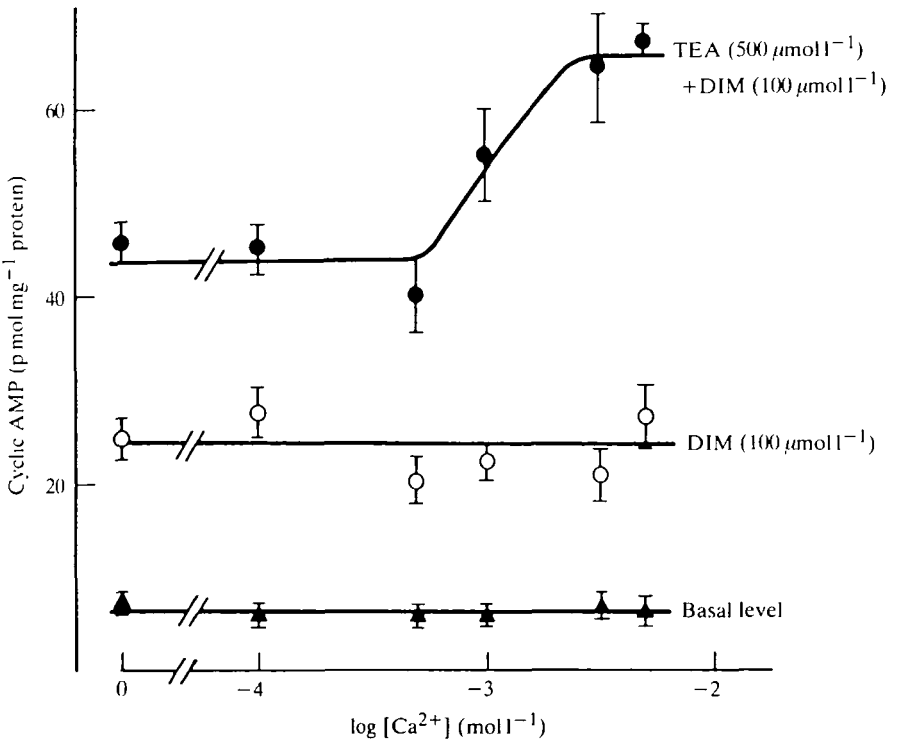


Fig. 3. Influence of calcium on the stimulation of cyclic AMP accumulation in slices from guinea-pig hippocampus elicited by activation of histamine H_1- and H_2-receptors. Pooled slices were prepared and preincubated 30 min at 37 °C in a normal Krebs–Ringer solution (containing 2·6 mmol l^{-1} $CaCl_2$). After four washes with a calcium-free Krebs–Ringer solution, aliquots were incubated for 15 min with increasing concentrations of calcium. A further 15 min incubation was performed in the presence of dimaprit (DIM) and 2-thiazolylethylamine (TEA).

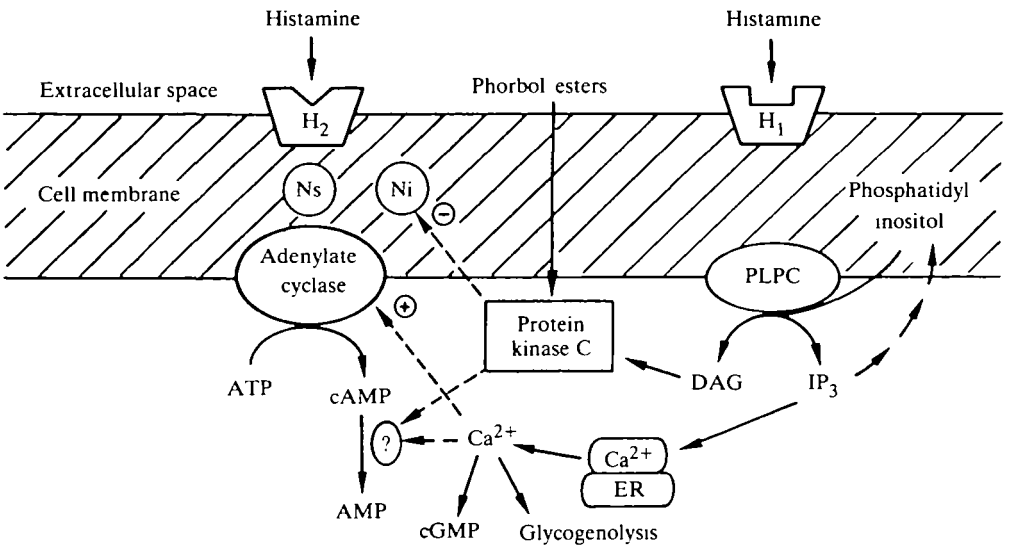


Fig. 4. Diagrammatic representation of biochemical events triggered by stimulation of H_1- and H_2-receptors and their interaction leading to the enhanced cyclic AMP response in brain slices. Stimulation of H_1-receptors (H_1) could activate phospholipase C (PLPC) which hydrolyses phosphatidyl inositol to generate two intracellular signals: (1) inositol 1,4,5 trisphosphate (IP_3), a Ca^{2+}-mobilizing messenger; (2) diacylglycerol (DAG), a protein kinase C activating messenger. The mechanisms through which Ca^{2+} and protein kinase C could enhance the cyclic AMP (cAMP) response elicited by stimulation of the cyclase-linked H_2-receptors are hypothetical. Ns, stimulatory guanine nucleotide regulatory protein; Ni, inhibitory guanine nucleotide regulatory protein; H_2, H_2-receptors.

second is diacylglycerol (DAG). This activates protein kinase C and thereby promotes the phosphorylation of intracellular regulatory proteins. Phorbol esters like 4β-phorbol 12,13-dibutyrate (PDB) readily enter intact cells and mimic the effect of DAG on protein kinase C. PDB strongly potentiated, in a concentration-dependent manner, the effects of dimaprit (Table 1) or HA (Hollingsworth, Sears & Daly, 1985). This suggests that protein kinase C activation results in the phosphorylation of a protein regulating the H_2-receptor-linked adenylate cyclase. The latter has not yet been identified but, by analogy with other systems, could be a guanylnucleotide-binding protein.

It is important to emphasize, however, that, even in the presence of PDB at maximally activating concentration, 2-thiazolylethylamine (or HA) was still effective in inducing its H_1-receptor-mediated amplification (Table 1). This suggests that IP_3 (the other messenger released by H_1-receptor-activated phosphatidyl inositol hydrolysis) may also play a role in the amplification process. Alternatively, H_1-receptors might mediate an increased influx of extracellular Ca^{2+}, although this effect is not directly documented. In this case the amplification of the cyclic AMP response could result from an action of the Ca^{2+}–calmodulin complex at the level of the catalytic unit of cerebral adenylate cyclase (Coussen, Haiech, D'Alayer & Monneron, 1985).

Table 1. *Effect of a phorbol ester (4β-phorbol 12,13-dibutyrate; PDB) on the stimulation of cyclic AMP accumulation in slices from guinea-pig hippocampus elicited by activation of histamine H_1- and H_2-receptors*

	Cyclic AMP accumulation (pmol mg^{-1} protein)	
	Without PDB	With PDB (10 μmol l^{-1})
Control	3·4 ± 0·3	8·4 ± 0·7
Dimaprit (300 μmol l^{-1})	13·7 ± 1·8	42·2 ± 5·8
Dimaprit (300 μmol l^{-1}) + TEA (1 mmol l^{-1})	37·6 ± 7·9	61·2 ± 8·0
Histamine (400 μmol l^{-1})	59·5 ± 10·4	107·2 ± 17·2

The phorbol ester was preincubated for 15 min before the addition of histamine or agonists. Other conditions as described in legend of Fig. 3, except that only a normal Krebs–Ringer solution containing 2·6 mmol l^{-1} $CaCl_2$ was used throughout the experiment.

TEA, 2-thiazolylethylamine.

In summary, although the molecular details of the interaction are not yet completely unravelled it appears that the amplification of the H_2-receptor-mediated response involves a dual mechanism triggered by H_1-receptor stimulation: protein kinase C activation and Ca^{2+} mobilization.

AUTOINHIBITION OF BRAIN HISTAMINE RELEASE MEDIATED BY H_3-RECEPTORS

Elevated extracellular K^+ concentration has been largely used as a depolarizing stimulus for release of various neurotransmitters and, in the case of either endogenous HA (Taylor & Snyder, 1973) or endogenously synthesized [^{3}H]HA (Verdière, Rose & Schwartz, 1975), results in a Ca^{2+}-dependent HA release. It is well established that several neurotransmitters may control their own release from cerebral neurones by interacting with presynaptic autoreceptors (Langer, 1977; Starke, 1981). To assess the existence of such a self-regulation process of HA release in brain, we developed a model in which slices of rat cerebral cortex were labelled by preincubation with [^{3}H]-L-histidine (^{3}H-L-His), to allow for [^{3}H]HA synthesis, and subsequently depolarized in a non-superfused system. The cerebral cortex was selected for this study as various approaches have shown that HA synthesis in this region occurs in terminals of histaminergic extrinsic neurones (Baudry *et al.* 1973; Garbarg, Barbin, Feger & Schwartz, 1974; Barbin, Hirsch, Garbarg & Schwartz, 1975).

Depolarization by 30 mmol l^{-1} K^+ of cortical slices in the absence of any exogenous HA added, resulted in the release of about 15 % of tissue [^{3}H]HA. However, when non-radioactive exogenous HA was present in the medium, an inhibition of the K^+-induced [^{3}H]HA release was observed (Arrang *et al.* 1983). The inhibitory action of HA was concentration-dependent and saturable with a maximal inhibition of 60 % (Fig. 5A).

The EC_{50} value of HA was of 0·13 ± 0·02 μmol l^{-1}, indicating that HA in this response is about 100 times more potent than in H_1- or H_2-receptor-mediated responses.

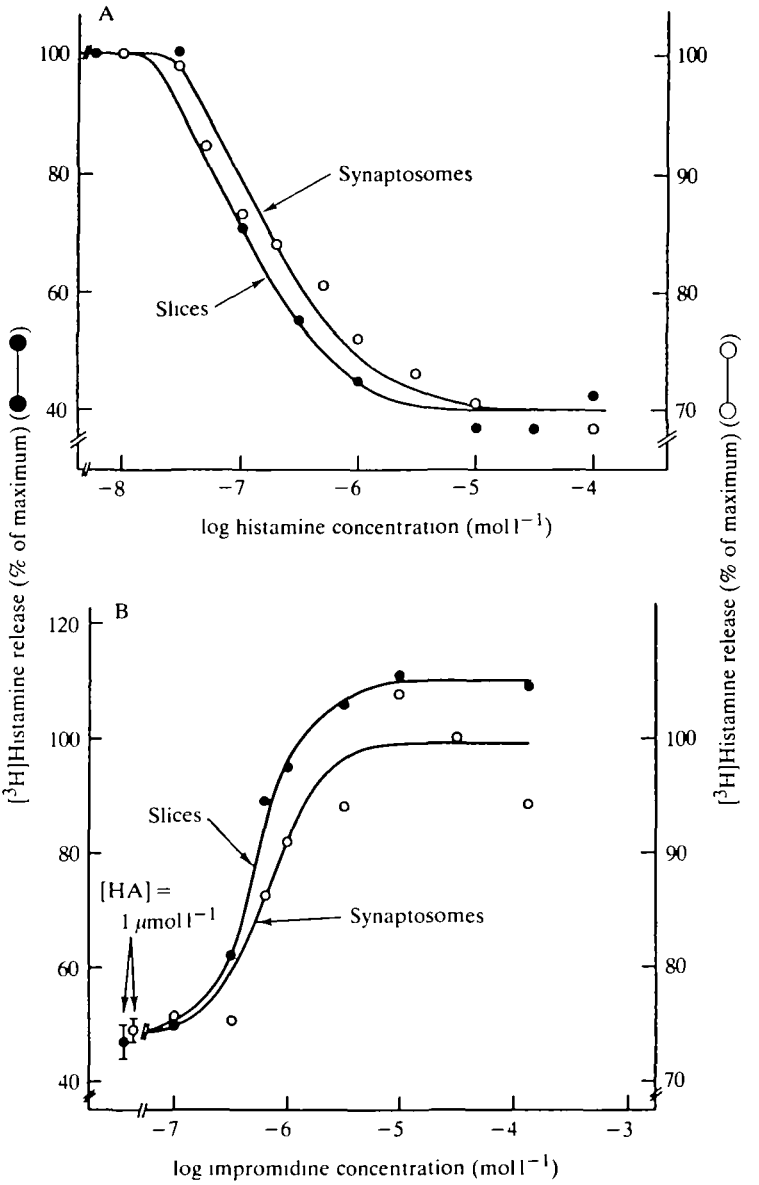


Fig. 5. Inhibition by exogenous histamine of [^{3}H]histamine release from slices and synaptosomes of rat cerebral cortex and its reversal by impromidine. Preincubations were performed in the presence of 0·3 μmol l^{-1} [^{3}H]-L-histidine. In the absence of added agents, [^{3}H]HA release induced by 30 mmol l^{-1} K^+ represented 12·3 ± 1·6 % for slices and 23·2 ± 1·7 % for synaptosomes. When required, exogenous histamine (A) was added together with 30 mmol l^{-1} K^+ and impromidine (B) 5 min before the simultaneous addition of histamine (HA) and K^+. IC_{50} values of impromidine were of 0·68 ± 0·16 and 0·57 ± 0·11 μmol l^{-1} for synaptosomes and slices respectively.

The inhibitory action of HA was only mimicked by its two N^{α} and N^{α},N^{α}-methyl derivatives: two agonists, both of H_1- and H_2-receptors.

In contrast, neither specific H_1 agonists, such as 2-methyl-HA, nor specific H_2 agonists, such as dimaprit, had any inhibitory effect (Fig. 1). Autoinhibitory receptors show a strong stereoselectivity as demonstrated with two HA derivatives including a chiral centre: α,N^{α}-dimethyl HA and N^{α}-methyl-α-chloromethyl HA. Both compounds acted as weak agonists and the activity ratio of their respective enantiomers was higher than at H_1- and H_2-receptors. Moreover, at autoinhibitory receptors (+)-isomers of both structures (absolute configuration corresponding to S-configurated L-histidine) possessed greater activity, whereas at H_2-receptors (−)-isomers were more active (Arrang, Schwartz & Schunack, 1985*c*).

Impromidine, a selective and potent H_2 agonist, not only failed to mimic the inhibitory action of HA, but increased the K^+-evoked release of [^{3}H]HA. This facilitatory action of impromidine suggested that it might have been acting as an antagonist at autoinhibitory receptors towards endogenous HA released together with [^{3}H]HA by the K^+ stimulus. Indeed, the inhibitory action of exogenous HA on release was antagonized in a competitive manner by impromidine or burimamide (Arrang *et al.* 1983).

The antagonism was always surmountable by increasing the HA concentration and was compatible with a competitive nature. Again, a facilitation of [^{3}H]HA release similar to that elicited by impromidine alone was observed in the presence of both agents in high concentrations. In contrast, various H_1-antihistamines like mepyramine or chlorpheniramine were ineffective in concentrations at which they block H_1-receptors (Fig. 1).

Also the two potent H_2-antihistamines, tiotidine and ranitidine were almost ineffective at autoinhibitory receptors, whereas burimamide and its lower homologue, norburimamide, were about 100 times more potent than at H_2-receptors. Impromidine acted as a potent antagonist at autoinhibitory receptors (K_i value $0{\cdot}06 \pm 0{\cdot}01\ \mu\text{mol}\,l^{-1}$).

Thus, from the potencies of agonists and the apparent dissociation constants of antagonists, as well as from the lack of effect of antagonists of other neurotransmitters, it can be concluded that the autoinhibition of HA release in brain is mediated by a novel class of HA receptor that we recently proposed to term H_3 (Arrang *et al.* 1983).

H_3-receptors seem to be directly located on HA-synthesizing axon terminals and not on interneurones or nearby axon terminals impinging on the former (Arrang, Garbarg & Schwartz, 1985*b*).

Hence, the unchanged inhibitory effect of exogenous HA in brain slices, in which the traffic of action potentials was blocked by tetrodotoxin, tends to exclude the possibility of participation of interneurones. This is confirmed by the persistence of H_3-receptor-mediated effects in striatal slices after a kainate treatment. This treatment abolishes the HA-induced cyclic AMP response mediated by H_1- and H_2-receptors. It does not, however, modify the L-histidine decarboxylase activity, a presynaptic marker (Garbarg *et al.* 1978).

In addition, experiments with rat brain cortex synaptosomes left little doubt that pharmacologically-identified H_3-receptors were directly located on HA-synthesizing terminals. Thus, exogenous HA inhibited [^{3}H]HA release from depolarized synaptosomes, with an EC_{50} value ($0{\cdot}20 \pm 0{\cdot}05\,\mu mol\,l^{-1}$) similar to that found with slices (Fig. 5A) and, on the two preparations, the effect was inhibited by impromidine, a competitive H_3-receptor antagonist with similar apparent K_i values, e.g. $0{\cdot}11 \pm 0{\cdot}03\,\mu mol\,l^{-1}$ and $0{\cdot}06 \pm 0{\cdot}01\,\mu mol\,l^{-1}$, respectively (Fig. 5B).

In conclusion, all these data support the hypothesis that HA modulates its own release from cerebral neurones by interacting with H_3-presynaptic autoreceptors.

AUTOINHIBITION OF BRAIN HISTAMINE SYNTHESIS MEDIATED BY H_3-AUTORECEPTORS

As previously shown (Verdière *et al.* 1975) with hypothalamic slices, depolarization of slices from cerebral cortex in the presence of $30\,mmol\,l^{-1}$ K^+ for 30 min elicited a 100% increase in synthesis of [^{3}H]HA from [^{3}H]-L-His. However, when non-radioactive exogenous HA was present in the medium, an inhibition of this K^+-induced response occurred. The inhibitory effect of HA was concentration-dependent and saturable. As in the modulation of its release, HA in this response was about 100 times more potent (EC_{50} value $0{\cdot}34 \pm 0{\cdot}03\,\mu mol\,l^{-1}$) than in H_1- or H_2-receptor-mediated responses in the brain.

Impromidine and burimamide acted as antagonists of HA with apparent dissociation constants similar to those found at H_3-autoreceptors modulating HA release, whereas mepyramine and tiotidine were again ineffective (Fig. 6A).

Although of limited amplitude, a similar inhibitory effect of exogenous HA (EC_{50} value $0{\cdot}10 \pm 0{\cdot}06\,\mu mol\,l^{-1}$) was observed in cortical synaptosomes in which a facilitatory effect of impromidine occurred (Fig. 6B).

These data strongly suggest that presynaptic H_3-autoreceptors also modulate HA synthesis in brain (Arrang *et al.* 1985*a*).

It is not clear at which biochemical level this control occurs. A direct retroinhibition of the enzyme L-histidine decarboxylase by its product, HA, was previously excluded, whereas it was suggested that control of HA synthesis might occur at the step of L-His uptake into HA-synthesizing cells (Verdière *et al.* 1975). However, the latter uptake process cannot easily be studied because of the presumably small number of HA neurones in brain as compared with other cells in which active uptake of L-His occurs.

All the current data support the hypothesis that HA modulates its own release from cerebral neurones by interacting with H_3-presynaptic autoreceptors, displaying a highly specific pharmacology and stereoselectivity. The activation of these autoreceptors seems to involve mechanisms bearing many similarities with those by which other neurotransmitters self-regulate their release. H_3-autoreceptors also mediate the modulation of HA biosynthesis in cerebral neurones. The development of compounds able to stimulate or to block these autoreceptors selectively, might

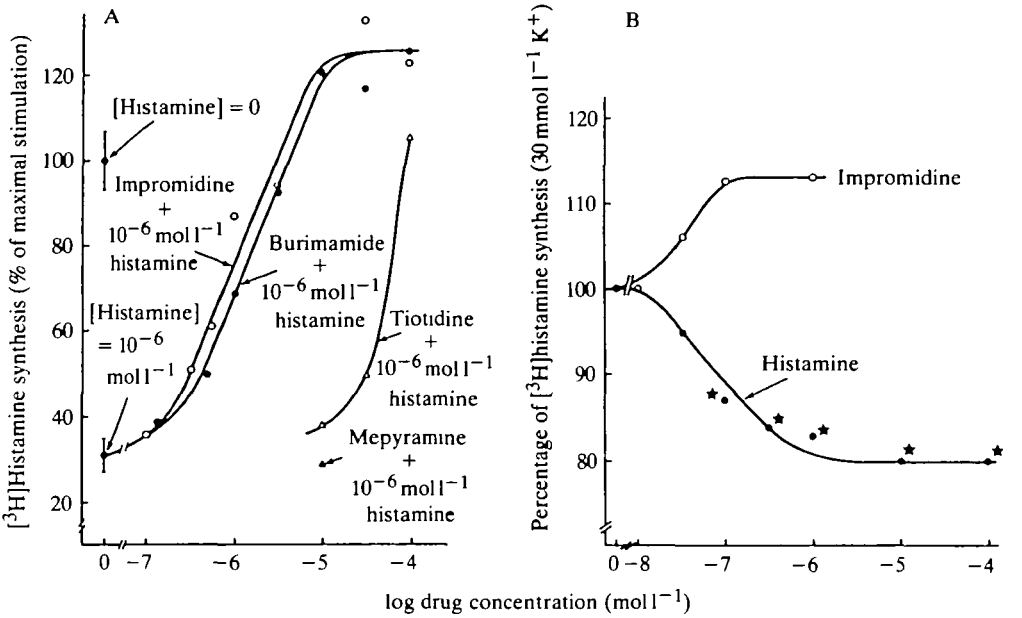


Fig. 6. Control of [^{3}H]histamine synthesis in slices (A) or synaptosomes (B) of rat cerebral cortex *via* stimulation of H_3-autoreceptors. Slices or synaptosomes were incubated for 30 min in the presence of 0·5 μmol l^{-1} [^{3}H]-L-His and either 2 mmol l^{-1} K^+ (not shown) or 30 mmol l^{-1} K^+. The depolarizing agent stimulated [^{3}H]HA synthesis by 86% (slices) or 20% (synaptosomes), in the absence of any effector. In the presence of exogenous histamine (1 μmol l^{-1}) this stimulation was largely prevented. Impromidine or burimamide reversed the response of exogenous histamine and slightly increased [^{3}H]HA synthesis.

provide a useful tool for elucidating the precise functions of histaminergic neurones in brain.

REFERENCES

ARRANG, J.-M., GARBARG, M. & SCHWARTZ, J.-C. (1983). Auto-inhibition of brain histamine release mediated by a novel class (H_3) of histamine receptor. *Nature, Lond.* **302**, 832–837.

ARRANG, J.-M., GARBARG, M. & SCHWARTZ, J.-C. (1985*a*). Histamine synthesis and release in CNS: control by autoreceptors (H_3). In *Frontiers in Histamine Research* (ed. C. R. Ganellin & J.-C. Schwartz), pp. 143–153. London: Pergamon Press Ltd.

ARRANG, J.-M., GARBARG, M. & SCHWARTZ, J.-C. (1985*b*). Autoregulation of histamine release in brain by presynaptic receptors. *Neurosci.* **15**, 553 -562.

ARRANG, J.-M., SCHWARTZ, J.-C. & SCHUNACK, W. (1985*c*). Stereoselectivity of the histamine H_3-presynaptic autoreceptor. *Eur. J. Pharmac.* **117**, 109–114.

BARBIN, G., GARBARG, M., SCHWARTZ, J.-C. & STORM-MATHISEN, J. (1976). Histamine synthesizing afferents to the hippocampal region. *J. Neurochem.* **26**, 259–263.

BARBIN, G., HIRSCH, J. C., GARBARG, M. & SCHWARTZ, J.-C. (1975). Decrease in histamine content and decarboxylase activities in an isolated area of the cerebral cortex of the cat. *Brain Res.* **92**, 170–174.

BARBIN, G., PALACIOS, J. M., RODERGAS, E., SCHWARTZ, J.-C. & GARBARG, M. (1980). Characterization of the high-affinity binding sites of [^{3}H]histamine in rat brain. *Molec. Pharmac.* **18**, 1–10.

BAUDRY, M., MARTRES, M.-P. & SCHWARTZ, J.-C. (1973). The subcellular localisation of histidine decarboxylase in various regions of rat brain. *J. Neurochem.* **21**, 1301–1309.

BAUDRY, M., MARTRES, M.-P. & SCHWARTZ, J.-C. (1975). H_1 and H_2 receptors in the histamine-induced accumulation of cyclic AMP in guinea pig brain slices. *Nature, Lond.* **253**, 362–363.

BEN ARI, Y., LE GAL LA SALLE, G., BARBIN, G., SCHWARTZ, J.-C. & GARBARG, M. (1977). Histamine synthesizing afferents within the amygdaloid complex and bed nucleus of the stria terminalis of the rat. *Brain Res.* **138**, 285–294.

BLACK, J. W., DUNCAN, W. A. M., DURANT, G. J., GANELLIN, C. R. & PARSONS, E. M. (1972). Definition and antagonism of histamine H_2-receptors. *Nature, Lond.* **236**, 385–390.

BOVET, D. & STAUB, A. M. (1937). Action protectrice des éthers phénoliques au cours de l'intoxication histaminique. *C.r. Séanc. Soc. Biol.* **124**, 547–549.

BRISTOW, D. R., HARE, J. R., HEARN, J. R. & MARTIN, L. E. (1981). Radioligand binding studies using [^{3}H]cimetidine and [^{3}H]ranitidine. *Br. J. Pharmac.* **74**, 547P.

BROWN, E., KENDALL, D. A. & NAHORSKI, S. R. (1984). Inositol phospholipid hydrolysis in rat cerebral cortical slices: I-receptor characterisation. *J. Neurochem.* **42**, 1379–1387.

CARSWELL, H., DAUM, P. R. & YOUNG, J. M. (1985). Histamine H_1-agonist stimulated breakdown of inositol phospholipids. In *Frontiers in Histamine Research* (ed. C. R. Ganellin & J.-C. Schwartz), pp. 27–38. London: Pergamon Press Ltd.

CHANG, R. S. L., TRAN, V. T. & SNYDER, S. H. (1978*a*). Histamine H_1-receptors in brain labelled with [^{3}H]mepyramine. *Eur. J. Pharmac.* **48**, 463–464.

CHANG, R. S. L., TRAN, V. T. & SNYDER, S. H. (1978*b*). Heterogeneity of histamine H_1-receptors: species variations in [^{3}H]mepyramine binding of brain membranes. *J. Neurochem.* **32**, 1653–1663.

COUSSEN, F., HAIECH, J., D'ALAYER, J. & MONNERON, A. (1985). Identification of the catalytic subunit of brain adenylate cyclase: a calmodulin binding protein of 135 kDa. *Proc. natn. Acad. Sci. U.S.A.* **82**, 6736–6740.

DAUM, P. R., DOWNES, C. P. & YOUNG, J. M. (1983). Histamine-induced inositol phospholipid breakdown mirrors H_1-receptor density in brain. *Eur. J. Pharmac.* **87**, 497–498.

DAUM, P. R., DOWNES, C. P. & YOUNG, J. M. (1984). Histamine stimulation of inositol-1-phosphate accumulation in lithium-treated slices from regions of guinea-pig brain. *J. Neurochem.* **43**, 25–31.

DAUM, P. R., HILL, S. J. & YOUNG, J. M. (1982). Histamine H_1-agonist potentiation of adenosine-stimulated cyclic AMP accumulation in slices of guinea-pig cerebral cortex: comparison of response and binding parameters. *Brit. J. Pharmac.* **77**, 347–357.

DONALDSON, J. & HILL, S. J. (1985). Histamine-induced inositol phospholipid breakdown in the longitudinal smooth muscle of guinea-pig ileum. *Brit. J. Pharmac.* **85**, 499–512.

DURANT, G. J., GANELLIN, C. R., GRIFFITHS, R., HARVEY, C. A., OWEN, D. A. A. & SACH, G. S. (1985). Some newer H_1-receptor histamine antagonists. In *Frontiers in Histamine Research* (ed. C. R. Ganellin & J.-C. Schwartz), pp. 3–12. London: Pergamon Press Ltd.

FLEISCH, J. H., KRZAN, M. C. & TITUS, E. (1973). Pharmacologic receptor activity of rabbit aorta: effect of dithiothreitol and *N*-ethylmaleimide. *Circulation Res.* **33**, 284–290.

FLEISCH, J. H., KRZAN, M. C. & TITUS, E. (1974). Alterations in pharmacologic receptor activity by dithiothreitol. *Am. J. Physiol.* **227**, 1243–1248.

FONTAINE, J., FAMAEY, J. P. & REUSE, J. (1984). Potentiation by sulphydryl agents of the responses of guinea-pig isolated ileum to various agonists. *J. Pharm. Pharmac.* **36**, 450–453.

GAJTKOWSKI, G. A., NORRIS, D. B., RISING, T. J. & WOOD, T. P. (1983). Specific binding of [^{3}H]tiotidine to histamine H_2-receptors in guinea-pig cerebral cortex. *Nature, Lond.* **304**, 65–67.

GANELLIN, C. R. (1982). Chemistry and structure activity relationships of drugs acting at histamine receptors. In *Pharmacology of Histamine Receptors* (ed. C. R. Ganellin & M. E. Parsons), pp. 10–102. London: Wright PSG.

GANELLIN, C. R. (1985). A survey of recently described H_2-receptor histamine antagonists. In *Frontiers in Histamine Research* (ed. C. R. Ganellin & J.-C. Schwartz), pp. 47–59. London: Pergamon Press Ltd.

GARBARG, M., BARBIN, G., FEGER, J. & SCHWARTZ, J.-C. (1974). Histaminergic pathway in rat brain evidenced by lesions of the MFB. *Science* **186**, 833–835.

GARBARG, M., BARBIN, G., PALACIOS, J. M. & SCHWARTZ, J.-C. (1978). Effects of kainic acid on histaminergic systems in guinea-pig hippocampus. *Brain Res.* **150**, 638–641.

GARBARG, M., YERAMIAN, E., KORNER, M. & SCHWARTZ, J.-C. (1985). Biochemical studies of cerebral H_1-receptors. In *Frontiers in Histamine Research* (ed. C. R. Ganellin & J.-C. Schwartz), pp. 19–26. London: Pergamon Press Ltd.

GERVAIS, P., GERVAIS, A., DE BEULE, R. & VAN DER BIJL, W. (1975). Essai comparé d'un nouvel antihistaminique, la mequitazine et d'un placébo. *Acta allergol.* **30**, 286–297.

GLOVER, W. E. (1979). Effect of dithiothreitol on histamine receptors in rabbit colon and guinea-pig ileum. *Clin. exp. Pharmac. Physiol.* **6**, 151–157.

GROSS, P. M. (1985). Multiple actions of histamine on cerebral blood vessels. In *Frontiers in Histamine Research* (ed. C. R. Ganellin & J.-C. Schwartz), pp. 341–349. London: Pergamon Press Ltd.

HAAS, H. L. (1985). Histamine actions in the mammalian central nervous system. In *Frontiers in Histamine Research* (ed. C. R. Ganellin & J.-C. Schwartz), pp. 215–224. London: Pergamon Press Ltd.

HAAS, H. L. & KONNERTH, A. (1983). Histamine and noradrenaline decrease calcium-activated potassium conductance in hippocampal pyramidal cells. *Nature, Lond.* **302**, 432–433.

HAAS, H. L., WOLF, P., PALACIOS, J. M., GARBARG, M., BARBIN, G. & SCHWARTZ, J.-C. (1978). Hypersensitivity to histamine in the guinea-pig: microiontophoretic and biochemical studies. *Brain Res.* **156**, 275–291.

HILL, S. J., EMSON, P. C. & YOUNG, J. M. (1978). The binding of [^{3}H]-mepyramine to histamine H_1-receptors in guinea-pig brain. *J. Neurochem.* **31**, 997–1004.

HILL, S. J. & YOUNG, J. M. (1980). Histamine H_1-receptors in the brain of the guinea-pig and the rat: differences in ligand binding properties and regional distribution. *Br. J. Pharmac.* **68**, 687–696.

HILL, S. J., YOUNG, J. M. & MARRIAN, D. H. (1977). Specific binding of [^{3}H]mepyramine to histamine H_1-receptors in intestinal smooth muscle. *Nature, Lond.* **270**, 361–363.

HOLLINGSWORTH, E. B., SEARS, E. B. & DALY, J. W. (1985). An activator of protein kinase C (phorbol 12-myristate-13-acetate) augments 2-chloroadenosine elicited accumulation of cyclic AMP in guinea-pig cerebral cortical particulate preparations. *FEBS Letts* **184**, 339–342.

HOUGH, L. B. & GREEN, J. P. (1984). Histamine and its receptors in the nervous system. In *Handbook of Neurochemistry*, vol. 6 (ed. A. Lajtha), pp. 145–211. New York: Plenum Press.

JOHNSON, C. L. (1982). Histamine receptors and cyclic nucleotides. In *Pharmacology of Histamine Receptors* (ed. C. R. Ganellin & M. E. Parsons), pp. 146–216. London: Wright PSG.

KANOF, P. D., HEGSTRAND, L. R. & GREENGARD, P. (1977). Biochemical characterization of histamine-sensitive adenylate cyclase in mammalian brain. *Archs Biochem. Biophys.* **182**, 321–334.

KARNUSHINA, I. L., PALACIOS, J. M., BARBIN, G., DUX, E., JOO, F. & SCHWARTZ, J.-C. (1980). Studies on a capillary-rich fraction isolated from brain: histaminic components and characterization of the histamine receptors linked to adenylate cyclase. *J. Neurochem.* **34**, 1201–1208.

KORNER, M., BOUTHENET, M. L., GANELLIN, C. R., GARBARG, M., GROS, C., IFE, R. J., SALES, N. & SCHWARTZ, J.-C. (1986). [^{125}I]-Iodobolpyramine, a highly sensitive probe for histamine H_1-receptors in guinea-pig brain. *Eur. J. Pharmac.* **120**, 151–160.

LANGER, S. Z. (1977). Presynaptic receptors and their role in the regulation of transmitter release. *Br. J. Pharmac.* **60**, 481–498.

NORRIS, D. B., GAJTKOWSKI, G. A. & RISING, T. J. (1984). Histamine H_2 binding studies in the guinea-pig brain. *Agents Actions* **14**, 543–545.

PALACIOS, J. M., GARBARG, M., BARBIN, G. & SCHWARTZ, J.-C. (1978*a*). Pharmacological characterization of histamine receptors mediating the stimulation of cAMP accumulation in slices from guinea-pig hippocampus. *Molec. Pharmac.* **14**, 971–982.

PALACIOS, J. M., SCHWARTZ, J.-C. & GARBARG, M. (1978*b*). High affinity binding of [^{3}H]histamine in rat brain. *Eur. J. Pharmac.* **50**, 443–444.

PALACIOS, J. M., WAMSLEY, J. K. & KUHAR, M. J. (1981*a*). GABA, benzodiazepine and histamine H_1-receptors in the guinea-pig cerebellum: effects of kainic acid injections studied by autoradiographic methods. *Brain Res.* **214**, 155–162.

PALACIOS, J. M., WAMSLEY, J. K. & KUHAR, M. J. (1981*b*). The distribution of histamine H_1-receptors in the rat brain: an autoradiographic study. *Neurosci.* **6**, 15–38.

PALACIOS, J. M., YOUNG, W. S. & KUHAR, M. J. (1979). Autoradiographic localization of H_1-histamine receptors in brain using [^{3}H]mepyramine: preliminary studies. *Eur. J. Pharmac.* **58**, 295–304.

PANULA, P., YANG, H. Y. T. & COSTA, E. (1984). Histamine containing neurons in the rat hypothalamus. *Proc. natn. Acad. Sci. U.S.A.* **81**, 2572–2576.

POLLARD, H., LLORENS-CORTES, C., BARBIN, G., GARBARG, M. & SCHWARTZ, J.-C. (1978). Histamine and histidine decarboxylase in brainstem nuclei: distribution and decrease after lesions. *Brain Res.* **157**, 178–181.

POLLARD, H., PACHOT, I., LEGRAIN, P., BUTTIN, G. & SCHWARTZ, J.-C. (1985*a*). Development of a monoclonal antibody against L-histidine decarboxylase as a selective tool for the localisation of histamine-synthesising cells. In *Frontiers in Histamine Research* (ed. C. R. Ganellin & J.-C. Schwartz), pp. 103–117. London: Pergamon Press Ltd.

POLLARD, H., PACHOT, I. & SCHWARTZ, J.-C. (1985*b*). Monoclonal antibody against L-histidine decarboxylase for localization of histaminergic cells. *Neurosci. Letts* **54**, 53–58.

QUACH, T. T., DUCHEMIN, A. M., ROSE, C. & SCHWARTZ, J.-C. (1979). In vivo occupation of cerebral histamine H_1-receptors evaluated with [^{3}H]mepyramine may predict sedative properties of psychotropic drugs. *Eur. J. Pharmac.* **60**, 391–392.

QUACH, T. T., DUCHEMIN, A. M., ROSE, C. & SCHWARTZ, J.-C. (1980*a*). [^{3}H]Glycogen hydrolysis elicited by histamine in brain slices: selective involvement of H_1-receptors. *Molec. Pharmac.* **17**, 301–308.

QUACH, T. T., DUCHEMIN, A. M., ROSE, C. & SCHWARTZ, J.-C. (1980*b*). Labeling of histamine H_1-receptors in the brain of the living mouse. *Neurosci. Letts* **17**, 49–54.

RICHELSON, E. (1978). Tricyclic antidepressants block histamine H_1 receptors of mouse neuroblastoma cells. *Nature, Lond.* **274**, 176–177.

RICHELSON, E. (1985). Histamine receptors, cyclic nucleotides and psychotropic drugs. In *Frontiers in Histamine Research* (ed. C. R. Ganellin & J.-C. Schwartz), pp. 237–250. London: Pergamon Press Ltd.

RISING, T. J. & NORRIS, D. B. (1985). Histamine H_2-receptor radioligand binding studies. In *Frontiers in Histamine Research* (ed. C. R. Ganellin & J.-C. Schwartz), pp. 61–68. London: Pergamon Press Ltd.

SCHWARTZ, J.-C. (1975). Histamine as a neurotransmitter in brain. *Life Sci.* **17**, 503–518.

SCHWARTZ, J.-C., BARBIN, G., DUCHEMIN, A. M., GARBARG, M., LLORENS, C., POLLARD, H., QUACH, T. T. & ROSE, C. (1982). Histamine receptors in the brain and their possible functions. In *Pharmacology of Histamine Receptors* (ed. C. R. Ganellin & M. E. Parsons), pp. 351–391. London: Wright PSG.

SCHWARTZ, J.-C., BARBIN, G., DUCHEMIN, A. M., GARBARG, M., PALACIOS, J. M., QUACH, T. T. & ROSE, C. (1980*a*). Histamine receptors in the brain: characterization by binding studies and biochemical effects. In *Receptors for Neurotransmitters and Peptide Hormones* (ed. G. Pepeu, M. J. Kuhar & S. J. Enna), pp. 169–182. New York: Raven Press.

SCHWARTZ, J.-C., GARBARG, M. & POLLARD, H. (1986). Histaminergic transmission in brain. In *Handbook of Physiology. The Nervous System* IV (ed. F. E. Bloom). (in press).

SCHWARTZ, J.-C., LAMPART, C. & ROSE, C. (1970). Properties and regional distribution of histidine decarboxylase in rat brain. *J. Neurochem.* **17**, 1527–1534.

SCHWARTZ, J.-C., POLLARD, H. & QUACH, T. T. (1980*b*). Histamine as a neurotransmitter in mammalian brain. *J. Neurochem.* **35**, 26–33.

STARKE, K. (1981). Presynaptic receptors. *A. Rev. Pharmac. Toxicol.* **21**, 7–30.

STEINBUSCH, H. W. M. & MULDER, A. H. (1984). Immunohistochemical localization of histamine in neurons and mast cells in the rat brain. In *Handbook of Chemical Neuroanatomy*, vol. 3, *Classical Transmitter and Transmitter Receptors in the CNS*, part II (ed. A. Bjorklund, T. Hökfelt & M. J. Kuhar), pp. 126–140. New York: Elsevier.

STEINBUSCH, H. W. M. & MULDER, A. H. (1985). Localization and projections of histamine immunoreactive neurons in the central nervous system of the rat. In *Frontiers in Histamine Research* (ed. C. R. Ganellin & J.-C. Schwartz), pp. 119–130. London: Pergamon Press Ltd.

STRAUSS, W. L. (1984). Sulfhydryl groups and disulfite bonds: modification of amino acid residues in studies of receptor structure and function. In *Membranes, Detergents and Receptor Solubilization* (ed. J. C. Venter & L. C. Harrison), pp. 85–97. New York: Alan P. Liss, Inc.

TAYLOR, K. M. & SNYDER, S. H. (1973). The release of histamine from tissue slices of rat hypothalamus. *J. Neurochem.* **21**, 1215–1223.

TOLL, L. & SNYDER, S. H. (1982). Solubilization and characterization of histamine H_1-receptors in brain. *J. biol. Chem.* **257**, 13 593–13 601.

VERDIÈRE, M., ROSE, C. & SCHWARTZ, J.-C. (1975). Synthesis and release of histamine studied on slices from rat hypothalamus. *Eur. J. Pharmac.* **34**, 157–168.

WARRANDER, S. E., NORRIS, D. B., RISING, T. J. & WOOD, T. P. (1983). [^{3}H]cimetidine and the H_2-receptor. *Life Sci.* **33**, 1119–1126.

WATANABE, T., TAGUCHI, Y., SHIOSAKA, S., TANAKA, J., KUBOTA, H., TERANO, Y., TOHYAMA, M. & WADA, H. (1984). Distribution of the histaminergic neuron systems in the central nervous system of rats: a fluorescent immunohistochemical analysis with histidine decarboxylase as a marker. *Brain Res.* **295**, 13–25.

WATANABE, T., TAGUCHI, Y., TAKEDA, N., SHIOSAKA, S., TOHYAMA, M. & WADA, H. (1985). Purification of and antibodies against L-histidine decarboxylase. In *Frontiers in Histamine Research* (ed. C. R. Ganellin & J.-C. Schwartz), pp. 91–102. London: Pergamon Press Ltd.

WELLS, J. W., CYBULSKY, D. L., KANDEL, M., KANDEL, S. I. & STEINBERG, G. H. (1985). Cognitive properties of H_2 receptors revealed by [^{3}H]histamine. In *Frontiers in Histamine Research* (ed. C. R. Ganellin & J.-C. Schwartz), pp. 69–78. London: Pergamon Press Ltd.

YERAMIAN, E., GARBARG, M. & SCHWARTZ, J.-C. (1985). *N*-ethylmaleimide-induced changes in agonist affinity for histamine H_1-receptors in the guinea-pig brain. *Molec. Pharmac.* **28**, 155–162.