Histamine H₃ Receptor: A Potential Drug Target for the Treatment of Central Nervous System Disorders

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Abstract: Histamine H₃ receptors were first described in the eighties but finally cloned four years ago. They are G-protein coupled, mostly presynaptic, and are involved in the control of the synthesis and/or release of different neurotransmitters both in the central nervous system and the periphery. The availabiliy of specific ligands has permitted the study of potential therapeutic applications of either stimulating or blocking the function of these receptors. There is experimental evidence that drugs targeted at histamine H₃ receptors could be beneficial for neurodegenerative diseases such as Alzheimer and Parkinson's disease, epilepsy, drug abuse and several affective, appetite and sleeping disorders, among others. This review presents recent advances in this field.

HISTAMINE IN THE CENTRAL NERVOUS SYSTEM.

The role of histamine as a cellular mediator was known from the first decades of the twentieth century [1]. However the data demonstrating its role as a neurotransmitter did not arrive until the 1970s [2-4]. Since then numerous studies have contributed to the present knowledge of the anatomy, physiology and pharmacology of the central histaminergic system, most of which can be consulted in the excellent reviews of Schwartz *et al.* [5], Onodera *et al.* [6], Hill *et al.* [7] and Brown *et al.* [8].

In the brain histamine is mainly located in neurons and mast cells [9] although the later are scarce and their function is at present not well established [10]. Cell bodies of the histaminergic neurons are located exclusively in the tuberomammillary nucleus of the posterior hypothalamus but their projections are distributed in almost all regions of the brain, especially in ventral areas (hypothalamus, basal forebrain, amygdala). Histaminergic neurons receive afferent input mainly from prefrontal cortex, septal regions and preoptic areas of the hypothalamus [6, 8].

Histamine is synthesised in mammalian brain from L-histidine by L-histidine decarboxylase, an enzyme that can be inhibited by S-α-fluoromethylhistidine [5]. After its release from neurons and binding to its specific receptors on the postsynaptic membrane, histamine is degraded by histamine N-methyl transferase to tele-methylhistamine, which is further oxidised by monoamine oxidase B to tele-methylimidazole acetic acid [5]. It also appears that glial cells are endowed with a carrier that could uptake histamine from the synaptic cleft [11].

Four histaminergic receptors have been identified so far [7, 12, 13] all of which are coupled to G proteins. The H_1

receptor is coupled positively to phospholipase C [14], the H_2 receptor mediates stimulation of adenylate cyclase [15] and both the H_3 and H_4 receptors couple to $G_{i/o}$ proteins [16, 12] The four types of histamine receptors have been detected in brain, although it seems that H_4 receptors are scarce and little is known about their function *in vivo* [7, 17]. Contrary to H_1 and H_2 receptors, which are postsynaptic, brain histamine H_3 receptors are located presynaptically on histaminergic neurons, where they regulate the synthesis and release of histamine, but also in non-histaminergic neurons (cholinergic, noradrenergic, dopaminergic and serotonergic, among others), where they control the release of the respective neurotransmitter [7], Fig. (**1**). Taking into account the multiple roles of these neurotransmitters and the widespread distribution of H_3 receptors in brain [18, 19], the use of histamine H_3 receptor ligands could be expected to have therapeutic potential in the treatment of a range of central nervous system disorders. This review is focused on the recent advances in this field.

THE HISTAMINE H₃ RECEPTOR

Auto and Heteroreceptors

Histamine H_3 receptors were first described by Arrang and co-workers [20] as presynaptic receptors regulating histamine release. Thus, they showed that histamine inhibited the K^+-evoked [³H]-histamine release from rat brain cortex slices preloaded with [³H]-L-histidine. However, the pharmacological properties of this effect did not conform to those expected from H_1 or H_2 receptors. In the following years, the first selective histamine H_3 receptor ligands were developed and it was demonstrated that H_3 receptors modulated not only the release but also the synthesis of histamine both *in vitro* and *in vivo* [21-23]; thus, the existence of the new receptor and its role as autoreceptor was finally confirmed.

The ability of central histamine H_3 receptors to function as heteroreceptors was also soon described *in vitro*.

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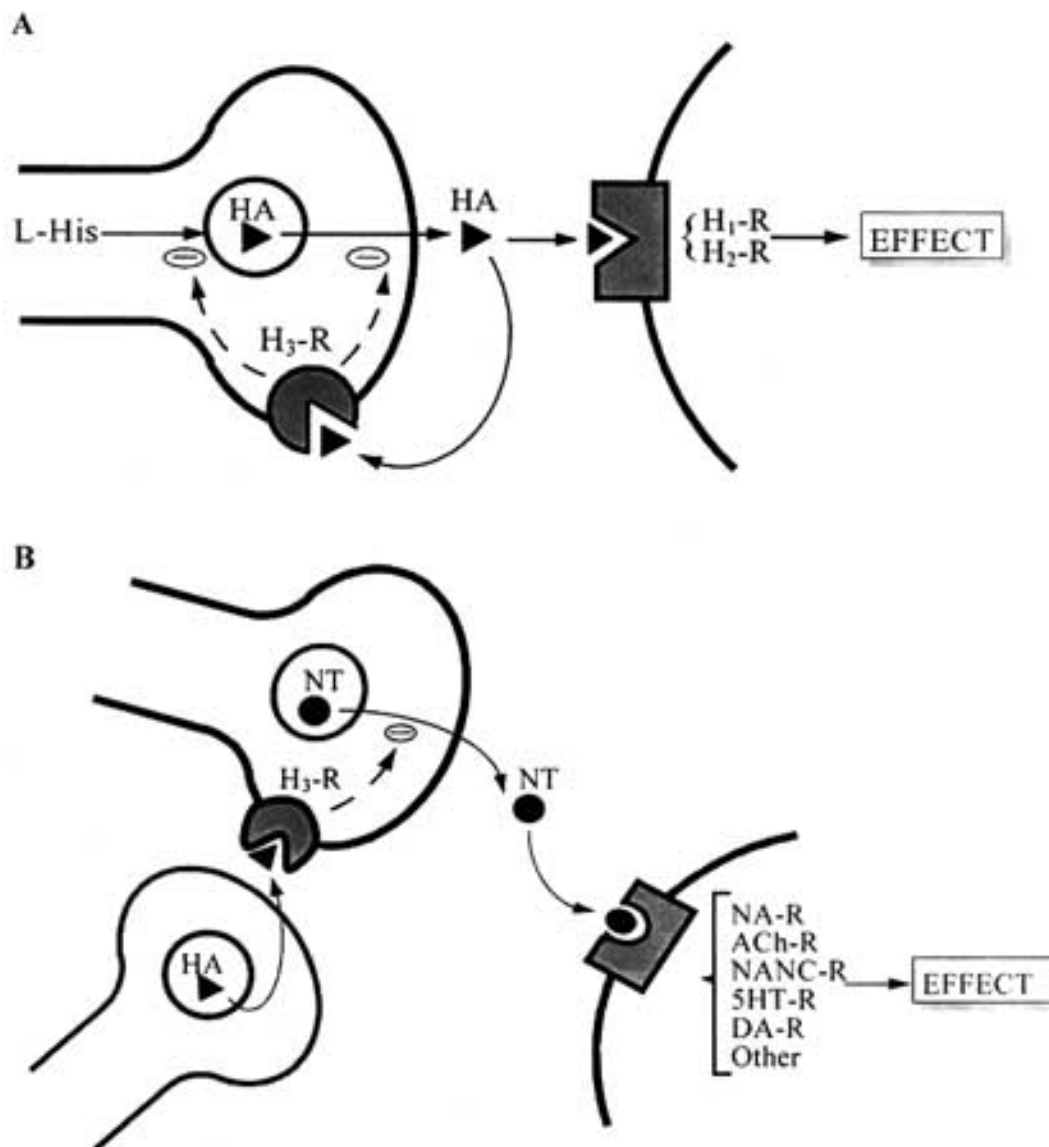
Fig. (1). Histamine H_3 receptor can act either as an autoreceptor or as a heteroreceptor.
A: histamine H_3 autoreceptors are located presynaptically in histaminergic neurons where they control both the synthesis and release of histamine from L-histidine. **B**: histamine H_3 heteroreceptors are located presynaptically in non-histaminergic neurons where they control the release of the respective neurotransmitter. L-his: L-histidine; HA: histamine; NT: neurotransmitter; NA: noradrenaline; ACh: Acetylcholine; NANC: non-adrenergic-non cholinergic; 5HT: serotonin; DA: dopamine; R: receptor.

Activation of H_3 receptors inhibits the release of noradrenaline in rat hypothalamus and cortex [24, 25]. The release of serotonin is also modulated by H_3 receptors, as shown in rat cortex, striatum and hypothalamus [26, 27, 24]. Dopaminergic neurons of the rat striatum are also endowed with histamine H_3 receptors whose activation inhibits both the release and the synthesis of dopamine [28, 29]. Moreover, histamine H_3 receptor agonists inhibit the spontaneous and dopamine D_1 receptor-dependent release of gamma-aminobutyric acid (GABA) from rat hypothalamus and striatum, respectively [30, 31]. Glutamate release is also modulated by presynaptic histamine H_3 receptors in hippocampus and striatum [32, 33]. With respect to central cholinergic transmission, a histamine H_3 receptor-mediated

inhibition has been described not only *in vitro* [34] but also *in vivo*, as detected in rat hippocampus [35, 36], cortex [37], ventral striatum [38] and basolateral amygdala [39].

Histamine H_3 heteroreceptors have also been described in the periphery. Thus, activation of H_3 receptors inhibits noradrenergic neurotransmission in kidney [40] and heart, especially under ischaemic conditions [41]. Cholinergic neurotransmission is also inhibited in airways [42] and in the gastrointestinal tract [43]. Release of neuropeptides (substance P, calcitonin gene-related peptide) from sensory C fibres is reduced by H_3 receptor activation in airways [44], heart [45], dura mater [46] and skin [47] among other areas. In addition, there is evidence for the presence of H_3 receptors

regulating secretory mechanisms in nonneuronal cells, mainly in the gastrointestinal tract [48-50].

Manipulation of peripheral H_3 receptors provides opportunities for therapeutic intervention in a range of disorders [51-54] including the treatment of neurogenic airway inflammation, migraine, gastric ulcer and myocardial ischaemia and infarction. The possible therapeutic applications for ligands acting on central H_3 receptors will be discussed below.

Structure

Although the cloning of the H_1 and H_2 receptor genes was reported in the early 90s [55, 56], it was 1999 before the gene of the human H_3 receptor was finally cloned by Lovenberg *et al.* [16] after the identification of a partial sequence of an orphan G-protein-coupled receptor in a private expressed sequence tags database. The delay in cloning could be due to the special features of the H_3 receptor gene and protein: phylogenetic analysis of the nucleotide sequence revealed a low homology between the gene encoding the H_3 receptor and those encoding other G-protein coupled receptors, including the H_1 and H_2 receptors; moreover, comparison of the full amino acid sequence indicated that the H_3 receptor resembles the α_2–adrenoceptor more than the other two histamine receptors [57]. However, the structure of the H_3 receptor protein possesses several typical features of the G-protein coupled receptors family, as described by Leurs and co-workers [57].

The cloning of the H_3 receptor gene contributed to confirm the H_3 receptor heterogeneity, which had long been suspected based on radioligand binding [58, 59] and functional studies [60, 61]. Thus, several molecular studies showed that a single form of the H_3 gene can give rise to three receptor isoforms in the rat brain [62], and to two receptor isoforms in the guinea pig brain [63]. According to Liu *et al.* [64] it was not possible to detect splice variants of the central human histamine H_3 receptor. However, more recent studies indicate that the human histamine H_3 receptor can also be alternatively spliced [65, 66, 67]. These isoforms vary in the structure of the third cytoplasmic loop and have distinct pharmacological properties and different tissue distribution [62, 68].

Ligands

Since the discovery of the histamine H_3 receptor by Arrang *et al.* [20] numerous efforts have been made to develop selective histamine H_3 receptor ligands and several reviews on this topic have been published [7, 51, 69, 70]. We will focus on the ligands that are more commonly used as tools for current pharmacological studies.

Potent H_3 agonists have been obtained by simple modifications of the histamine molecule (Fig. (**2**)). Methylation of the α-carbon atom of the ethylamine side-chain of histamine lead to the selective and potent agonist *R*-α-methylhistamine [22]. More potent agonists were produced later; replacing the amine group by an isothiourea moiety (imetit) or elongating and cyclizing the side chain (immepip) [71, 72]. The early availability of *R*-α-methylhistamine has made it the accepted reference H_3

agonist and it has been used extensively *in vitro* and *in vivo*. However, due to its basicity and polarity it hardly penetrates biological membranes and furthermore it is rapidly inactivated *in vivo*. To overcome these pharmacokinetic problems, lipophilic, non-basic azomethine prodrugs of *R*-α-methylhistamine have been developed [73, 74]. Currently the parent compound of the prodrugs, BP2-94, is under clinical development phase II for the treatment of asthma [75].
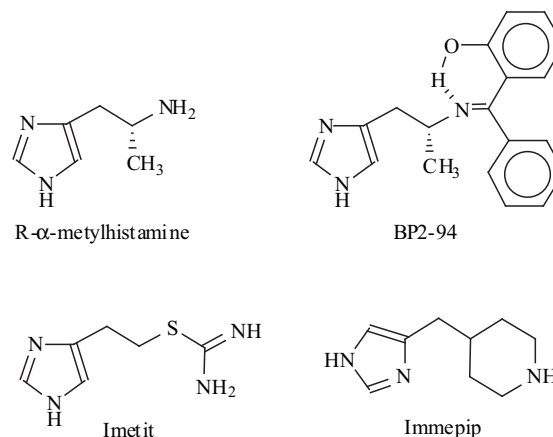
Fig. (2). Histamine H_3 receptor agonists.

Thioperamide was the first potent and selective H_3 receptor antagonist described [22] (Fig. (**3**)). Higher potency *in vitro* was soon reported for clobenpropit [76] as well as for its iodinated analogue, iodophenpropit, which was radiolabelled with [^{125}I] and extensively used for binding assays as well for autoradiographic studies [77]. More recently, several other potent H_3 antagonists have been described, including impentamine [78], iodoproxyfan –which is also used as radioligand labelled with [^{125}I] [79]- ciproxifan [80] and imoproxifan [81], the two later displaying a high oral *in vivo* potency. Finally, GT-2331 (Perceptin ®) is a highly selective H_3 antagonist with a favourable central nervous system penetration profile and a long duration of action in rats [82] and is being evaluated in phase II of clinical trials for the treatment of Attention Deficit Hyperactivity Disorder.

Very recent studies have revealed that the histamine H_3 receptor displays a high level of "constitutive activity", that is, spontaneous activity in the absence of agonist [83, 84]. Therefore, some of the H_3 ligands previously identified as H_3 antagonists can act either as inverse agonists, reducing the constitutive activity of the receptor, or as neutral antagonists, which do not affect the basal activity of the receptor but prevent the action of both agonists and inverse agonists. Thus, in SK-NMC cell lines stably expressing either the human or rat H_3 receptors at physiological receptor densities, thioperamide, clobenpropit and iodophepropit acted as inverse agonists; surprisingly impentamine acted as agonist while its analog VUF4904 acted as neutral antagonist [80]. In CHO cells expressing either the human or the rat H_3 receptor, ciproxifan and proxifan have been identified as inverse agonist and neutral antagonist, respectively [81]. Moreover, constitutive activity can be also found in vivo, as showed in rat brain by Morriset *et al.* [83]. It remains to be established whether the inverse agonists or
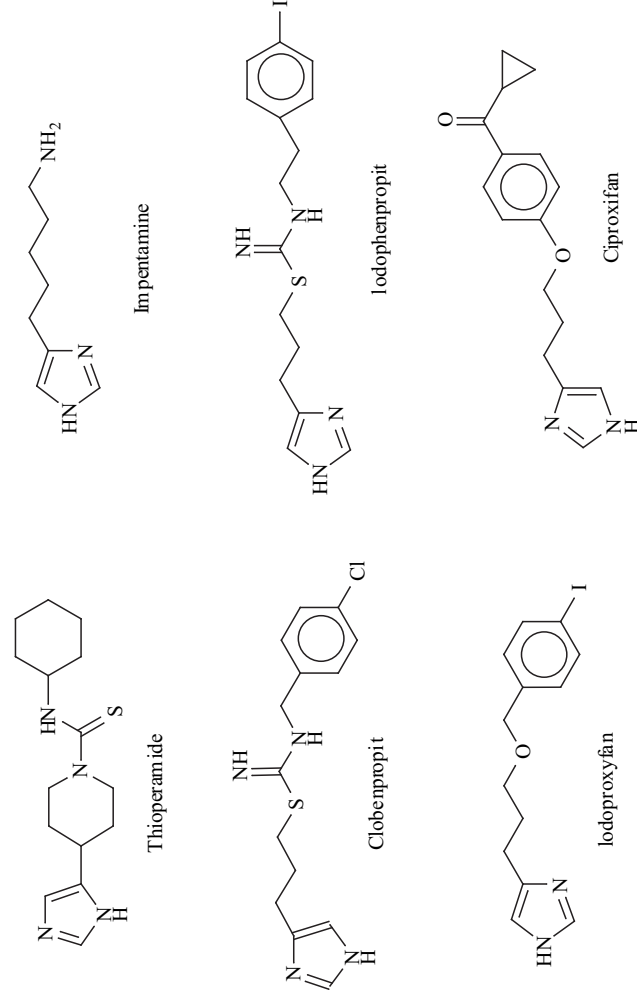
Fig. (3). Histamine H₃ receptor antagonists.

neutral antagonists will be favoured for therapeutic application.

Distribution in Central Nervous System

The detailed distribution of H₃ receptors in the rat brain was first reported by Pollard et al. [18] using [³H]R-α-methylhistamine; they observed a widespread distribution with high densities in cerebral cortex, nucleus accumbens, striatum, olfactory tubercles, substantia nigra, globus pallidus and the tuberomammillary nucleus of the posterior hypothalamus. In a recent study Pillot et al. [19] have mapped the H₃ receptor and its mRNA in rat brain by binding with [¹²⁵I]iodoproxyfan and *in situ* hybridazation, respectively. The mRNA localization did not exactly match the [¹²⁵I]iodoproxyfan binding, as was expected based on the fact that the H₃ receptor functions mainly as an inhibitory presynaptic receptor. Thus, although many of the high density H₃ receptor areas described by Pollard et al. [18] showed a marked mRNA expression, others like substantia nigra or globus pallidus had low mRNA density, suggesting that the H₃ receptors of those areas belong to afferents from other brain regions. Conversely, hippocampal, thalamic, cerebellar and vestibular nuclei exhibited low [¹²⁵I]iodoproxyfan binding but high mRNA expression, which might indicate that H₃ receptors are mainly located in projections of those areas.

Signal Transduction

Early studies on the signal transduction pathways used by the histamine H₃ receptor suggested that this receptor was linked to G_i/G_o proteins [7] similar to many other presynaptic inhibitory receptors. For example, experiments with rat cerebral cortical membranes showed that H₃ receptor agonists stimulated the binding of [³⁵S]GTP-γ-S while the effect of these drugs was abolished by pre-treatment of membranes with pertussis toxin [86]. The cloning and

functional characterisation of human and rat H₃ receptor cDNA [16, 87] confirmed that the receptor belongs to the family of G-protein-coupled receptors. These studies demonstrated that histamine H₃ receptors coupled negatively to adenylate cyclase, since H₃ receptor agonists decreased cAMP accumulation elicited by forskolin stimulation of adenylyl cyclase in receptor-transfected cells. Moreover, very recent experiments have shown that the adenylate cyclase-protein kinase A pathway is involved in the modulation of histamine synthesis by H₃ autoreceptors in rat brain cortical preparations, since protein kinase A blockers impair the synthesis of histamine induced by several histamine H₃ receptor antagonists [88]. However it can not be excluded that additional signal transduction mechanisms, other than the adenylate cyclase pathway, are involved in H₃ receptor-mediated effects. In fact it seems that a direct G protein-mediated inhibition of Ca^{2+} channels is involved in the inhibitory effect of H₃ receptor agonists on the release of several neurotransmitters, as reported for acetylcholine in ileum [89, 90], noradrenaline in heart [91], glutamate in striatum [33] and GABA in hypothalamus [30]. Finally, other signal transduction mechanisms have been also identified: the activation of the mitogen activated kinase pathway [62], and the stimulation of Na^+/H^+ exchange [91, 92].

A characteristic feature of G-protein-coupled receptors is that in the face of continuing stimulation, signalling becomes attenuated or desensitized. We studied the possible desensitization of H₃ agonist-induced effects in the longitudinal muscle-myenteric plexus of guinea pig ileum, a bioassay widely used for the evaluation of H₃ receptor ligands, where H₃ agonists inhibit electrically evoked twitches by reducing acetylcholine release from postganglionic cholinergic neurons [43]. A cumulative concentration-response curve for the H₃ receptor agonist R-α-methylhistamine was made; when a second curve was made 30 min afterwards, a marked decrease of pD₂ and a more modest decrease of E_{max} were observed [93]. This

Table I. **Histamine H$_3$ Receptor Desensitization in Longitudinal Muscle-Myenteric Plexus Strips of Guinea Pig Ileum. *R*-α-Methylhistamine (*R*αMH)-Induced Twitch Inhibition was Quantified in some of the Strips (*R*αMH-Pretreated Strips) by Means of a Cumulative Concentration-Response Curve, Whilst the others (Naive Strips) Continued Receiving Electrical Stimulation in Parallel. Once the Emax was Obtained in the Pretreated Strips, the Strips were Washed and Electrical Stimulation was Switched Off. After 30 min, the Strips were Stimulated Again and Concentration-Response Curves were Constructed with one or the Other of the Following Agonists: *R*αMH, Morphine (μ-Opioid), Clonidine (α$_2$ Adrenergic) and N^6-Cyclopentyladenosine (N^6-CPA, Adenosine A$_1$ Receptor Agonist) [94]**

Drug	Naive strips			*R*αMH-pretreated strips		
	n	pD$_2$	E$_{max}$ twitch inhibition,%	n	pD$_2$	E$_{max}$ twitch inhibition,%
*R*αMH	11	7.74±0.10	72.97±5.16	19	6.92*±0.18	59.46*±3.24
Morphine	7	7.49±0.20	86.91±2.95	5	7.38 ±0.20	92.79 ±2.97
Clonidine	8	8.11±0.18	73.68±4.77	10	8.09 ±0.18	70.16 ±5.38
N$_6$-CPA	12	8.18±0.09	90.87±1.68	13	8.06 ±0.14	90.27 ±1.72

Values are means ± SEM. * $p < 0.05$ vs naive strips.

desensitization seemed to be reversible since recovery of the agonist effect was noted at 120 min and appeared to be homologous since the presynaptic twitch inhibition provided by agonists of other G-protein-coupled receptors (i.e. μ opioid, α$_2$-adrenergic and adenosine A$_1$ receptors) remained unchanged (Table I) [94].

CENTRAL HISTAMINE H3 RECEPTORS AS DRUG TARGETS

Feeding

Many studies have shown that brain histamine levels seem to be inversely correlated to feeding behavior [95, 96, 97]. This anorectic effect of histamine may result from the stimulation of hypothalamic H$_1$ and H$_3$ receptors, whilst H$_2$ binding sites do not appear to be involved [18, 95, 98]. Histamine also promotes drinking behavior through stimulation of H$_1$, H$_2$ and H$_3$ receptors [99]. In the rat, blockade of H$_3$ receptors reduces feeding, suppresses food intake induced by peptide YY and potentiates anorexia induced by blockade of histamine catabolism or by injection of CCK-8 [95, 100-102]. Histamine H$_3$ receptor blockers also have anti-dipsogenic properties [103, 104]. In contrast, H$_3$ agonists attenuate satiety induced by bombesin or amilin [105, 106] and increase water consumption [103, 104]. From a therapeutic point of view, these findings suggest that H$_3$ blockers could be useful as anorexigens for the treatment of obesity, whilst H$_3$ agonists could enhance feeding behavior when abnormally reduced. It must be pointed out that new anorexigens will be welcomed to replace former drugs such as fenfluramine and dexfenfluramine, which were withdrawn from the market several years ago due to unacceptable cardiovascular risk. The availability of H$_3$ (-,-) mice will certainly add valuable information on the role of these receptors on body weight homeosthasis in a near future; by now, however, some controversy exists since one knockout mouse had a mild obese phenotype [107] while another did not [108].

Sleep/wake cycles

Histaminergic projections from the tuberomamillary nucleus of the posterior hypothalamus are important for wakefulness, and therefore lesions of this area promote sleep in rats, cats and monkeys [109]. Histamine release shows a circadian rhythm, the levels of the amine being higher in the periods of activity in different species [110, 111]. Recent studies with histidine decarboxylase (-,-) mice lacking histamine-immunoreactive neurons have confirmed the importance of wakefulness promotion by histamine since these animals showed deficits of waking at moments when high vigilance is required [112]. The effect of histamine seems to be related to H$_1$ receptor stimulation [113], and this partially explains the widely observed sedative effect of centrally-acting H$_1$ antagonists.

According to these observations, ligands of the H$_3$ receptors could be expected to affect sleep and wakefulness by changing central histaminergic tone. In fact, enhanced wakefulness results from H$_3$ receptor blockade with thioperamide, carboperamide or ciproxifan, which tend to increase histamine release in the central nervous system [112, 114, 115]. The wake promoting effect of thioperamide is absent in H$_3$ (-,-) mice [108]. On the other hand, H$_3$ agonists increase slow-wave sleep and, interestingly, they affect REM sleep to a lesser extent than H$_1$ receptor blockers do [114, 116]. Therefore H$_3$ blockade could be of interest in the management of hypersomnia or narcolepsia whilst H$_3$ receptor agonists could be proposed as a new class of hypnotics.

Epilepsy

Many experimental and clinical obsrvations point to the involvement of histamine in epileptic seizures. From the early fifties, central antihistaminics (H$_1$ antagonists) are known to be proconvulsant in humans [117]. Moreover, PET studies have shown that H$_1$ receptor density increases around the epileptic focus in parietal epilepsy [118].

Taken together, animal experiments suggest that central histaminergic activation reduces seizures whilst histamine blockade promotes them, at least when electrically-evoked convulsions are studied. Clonic convulsions evoked by maximal electroshock are inhibited by promoting histamine synthesis or reducing catabolism in mice; by contrast, the histamine synthesis inhibitor S-α-fluoromethylhistidine

enhances convulsions [119]. When studying the involvement of different histamine receptors a good correlation with clinical reports was obtained since H_1 blockade inhibits the anticonvulsive effect of drugs like L-histidine or morphine [119, 120]. The H_3 histamine receptor antagonists thioperamide, clobenpropit and AQ-0145 also have a protective effect against maximal electroshock probably due to their histamine releasing action; H_3 receptor agonists by themselves are devoid of effect in this model, but they prevent the protective effect of H_3 blockers [121, 122, 123].

The role of histamine and H_3 receptor ligands in other experimental models of epilepsy are much more confusing. When chemically-induced seizures are studied in mice, H_3 receptor agonists showed a biphasic effect being protective at low doses but proconvulsant at high doses; moreover, both thioperamide and the H_2/H_3 blocker burimamide dose-dependently enhance picrotoxin-induced seizures [124]. Vohora *et al.* [125] have reported that thioperamide protects against pentylenetetrazol-induced seizures in mice. However we failed to reproduce these results in our laboratory and a tendency to increase seizures was noted after injection of a relatively high dose of this drug (Fig. (**4**)). Histamine plays a complex role in the rat kindling model, where the acute injection of the synthesis precursor L-histidine decreases kindling whilst chronic administration of the drug has the opposite effect; the experiments performed so far with H_3 receptor antagonists show that these drugs reduce amygdaloid kindling in rats [126]. Therefore histamine H_3

receptor antagonists could be beneficial for the treatment of at least some epileptic diseases, either by themselves or in combination with other drugs since they were shown to potentiate the effects of phenytoin and gabapentin in several experimental models at subeffective doses [127]. Some authors highlight the fact that histamine H_3 receptor antagonists are cognition enhancers and promote arousal instead of being sedative as other antiepileptic drugs are, a key difference which could be of interest for the pharmacological management of epilepsy.

Cognition

Numerous studies have shown a major role of the central histaminergic system in learning and memory. A review of these data (see for instance [128]) tend to show that histamine acts as a regulatory center for whole brain activity. Thus, i.c.v. histamine enhances rat performance in the passive avoidance test and the synthesis precursor L-histidine has procognitive actions in the elevated plus-maze; these actions are prevented by H_1 blockers or by S-α-fluoromethylhistidine [129, 130, 131]. In these models, thioperamide was not active by itself but enhanced animal performance if combined with the H_2 antagonist zolantadine [132, 133]. Interestingly, the stimulation of H_2 histamine receptors with 4-methylhistamine has been shown to negatively modulate a passive avoidance task in mice [134], so it seems that H_1 and H_2 receptor stimulation have
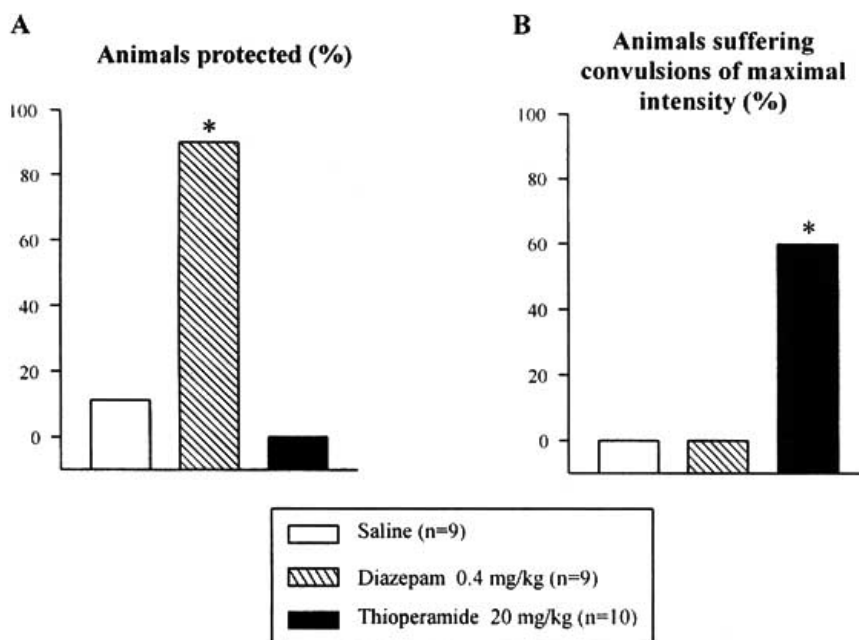

Fig. (4). Effect of the histamine H_3 receptor antagonist thioperamide on the pentylenetetrazole-induced convulsions in mice. Animals were treated i.p. either with saline, thioperamide, or diazepam and 30 min afterwards they were injected subcutaneously with pentylenetetrazole (85 mg/kg). **A**: percentage of animals that did not suffer convulsions. **B**: percentage of animals that suffered convulsions of maximal intensity (tonic extension preceded or followed by tonic and clonic convulsions). (* $p < 0.05$ vs. saline).

opposite effects on learning and memory. The effect of H_3 blockers could possibly depend on the balanced H_1/H_2 receptor stimulation of the histamine released by presynaptic H_3 receptor blockade. Nonetheless, this balance appears to be generally shifted to cognition enhancement since further studies on the effects of H_3 receptor antagonists consistently revealed a positive action on learning by using different experimental approaches, i.e. step-through passive avoidance in senescent mice [135], social memory in rats [136], scopolamine-induced memory defects in rats [137] and others. As expected, H_3 receptor agonists provoked learning and memory impairments in many studies. There are however some exceptions to these common findings: ciproxifan, clobenproprit and thioperamide impaired memory consolidation of contextual fear conditioning in one study [39], and R-α-methylhistamine improved spatial learning in another water maze experiment [138].

Recently, it has been shown that H_3 (-,-) mice are insensitive to the amnesic effect of scopolamine, which supports a major role of H_3 receptors in the control of learning impairments induced by cholinergic dysfunction [108]. This is in agreement with the idea that the effects of H_3 blockers are mainly related to the procholinergic action of central histamine, even though other neurotransmitters could also be involved. Blockade of H_3 receptors increases histamine release which in turn controls acetylcholine release in the cortex and amygdala, and also activates cholinergic neurons in the nucleus basalis magnocellularis and the medial septal-diagonal band projecting to the cortex and hippocampus, respectively [36, 139]. A procholinergic effect is then obtained in all these target areas with the apparent exception of the basolateral amygdala, where H_3 receptor blockade seems to decrease acetylcholine release [39]. Recently, the effect of thioperamide injection into the nucleus basalis magnocellularis has been studied in detail in a two-trial, delayed, place recognition task in rats [140]; the antagonist was administered 2 min before the first trial (pre-acquisition treatment), within 30 s from the end of this trial (post-acquisition) or 2 min prior to the second trial (pre-retrieval treatment), and it was found that intermediate doses of the drug (but not low and high doses) were effective only when administered post-acquisition, an effect that disappeared if the delay was extended to 90 min after the end of the first trial. Memory consolidation could then be positively influenced by H_3 receptor blockade.

All the evidence accumulated so far suggests that H_3 receptor blockade could have therapeutic implications for the treatment of disorders with cognitive deficits such as Alzheimer's disease. Interestingly, brain levels of histamine have been found to be decreased in Alzheimer's patients [141], and the acetylcholinesterase inhibitor tacrine may exert at least part of its therapeutic effect by restoring histamine deficits since this drug is able to inhibit histamine-N-methyltransferase activity [142]. Taking into account that patients with Alzheimer's disease and Down's syndrome exhibit similar changes affecting the central histaminergic system [143], the therapeutic potential of histamine H_3 receptor blockers has been extended to the latter disease. Finally, histamine H_3 receptor antagonists may have the potential to improve the cognitive deficits and motor disturbances of attention-deficit hyperactivity disorder, a condition in which histamine together with dopamine and noradrenaline transmission seem to be impaired [51, 128]. In fact, the histamine H_3 receptor antagonists GT-2331 and ciproxifan have positive effects in a validated animal model of attention-deficit hyperactivity disorder based on an inhibitory avoidance task [144], and the former drug is currently under clinical investigation for the treatment of this disease.

Parkinson's Disease

Prominent changes of histamine levels have been recently described in post-mortem brain samples taken from patients that suffered Parkinson's disease. An increase of the amine reaching 159-234% was observed in brain areas closely related to motor function such as putamen, substantia nigra and globus pallidus [145]. Changes affecting histamine H_3 receptors were previously observed in experimental models of Parkinson's disease and in clinical conditions: thus, rats treated with the dopaminergic neurotoxin 6-hydroxydopamine exhibited upregulated H_3 receptor binding in the substantia nigra and ventral striatum and a concomitant functional hypersensitivity as determined by GTP-γ-S binding upon receptor stimulation [146]. Some human studies are in agreement with these last observations since patients with Parkinson's disease showed increased H_3 receptor binding in the substantia nigra and higher H_3 receptor mRNA expression in the striatum [147]. However, Goodchild *et al.* [148] have not found significant changes of histamine H_3 binding sites in caudate, putamen, nucleus accumbens, globus pallidus, substantia nigra and insular cortex from patients of Parkinson's disease; by contrast, these authors observed histamine H_3 downregulation in cases of Huntington's disease, another degenerative disorder affecting the basal ganglia.

Although some of the above observations could suggest a significant role for histamine H_3 receptors in Parkinson's disease, the possible effects of H_3 receptor ligands have been poorly established up to date. Hemiparkinsonian rats show a turning behavior upon levodopa injection that is reduced by R-α-methylhistamine but not clearly affected by thioperamide; moreover, H_3 ligands are devoid of activity on amphetamine-induced ipsilateral turning behavior [149]. Further evidence is therefore necessary to clarify the possible interest of H_3 receptor pharmacological modulation in Parkinson's disease.

Anxiety and Depression

Both acute and chronic stress increase histamine turnover in the rat nucleus accumbens and striatum, an effect which is reversed by anxiolytics [150]. These results are in agreement with the antistress effect obtained in rats after bilateral lesions of hypothalamic histaminergic cells, a finding that suggests a link between histamine and stress-induced release of pituitary hormones; consistently, R-α-methylhistamine, imetit and BP2-94 inhibit ACTH and prolactin responses to restraint stress and lipopolysaccharide endotoxin [151]. An antianxiety effect of H_3 agonists and proanxiety effects of H_3 blockers could then be expected. Yuzurihara *et al.* [152] have found that thioperamide by itself is devoid of any activity in the light/dark test in mice, although it can potentiate the anxiogenic effect of other drugs. We also failed to obtain

significant effects with H_3 ligands in the elevated plus-maze test during our psychopharmacological screenings [153]. However, these last studies and those of Lamberti et al [154] have revealed that H_3 antagonists are positive in the forced swimming test, a model of depression based on stress. We have speculated that H_3 antagonists could have antidepressant properties mainly by affecting the release of neurotransmitters other than histamine, i.e. noradrenaline, but this hypothesis must be further explored.

Schizophrenia

Histamine H_1 receptor density was found to be elevated postmortem in the frontal cortex of schizophrenics [155]. This finding suggested that changes of the histaminergic tone could be present in this disease, which was confirmed by Prell *et al.* [156] when they observed an increased level of histamine metabolites in cerebrospinal fluid taken from treatment-resistant schizophrenics. Moreover, this increase correlated with the severity of the symptoms. There are some clinical reports showing that the histamine H_2 receptor antagonist famotidine could help to control some symptoms of shizophrenia, and therefore it has been suggested as an adjunct to antipsychotic therapy [157, 158]. Several authors have studied the binding profile of different antipsychotics and found that clozapine blocked the receptor at relevant concentrations, thus raising the possibility that this effect could be involved in the "atypical" antipsychotic properties of the drug [159, 160, 161, 162]. However, the affinity of clozapine was lower than that exhibited for D_4 or $5\text{-}HT_2$ binding sites and, whilst this drug very effectively increases tele-methylhistamine brain levels in mice, the effect seems to be mainly elicited by $5\text{-}HT_{2A}$ blockade [163]. Recently, Pillot *et al.* [164] have observed that the H_3 antagonist

ciproxifan potentiates the striatal effects of haloperidol, leading to enhanced locomotor hypoactivity and catalepsy and increased proenkephalin expression. This synergistic effect could result from H_3/D_2 interactions and led the authors to suggest that H_3 antagonists (and inverse agonists) could improve the symptoms of schizophrenia. However, in another study, blockade of H_3 receptors suppressed haloperidol-induced c-fos expression in the dorsolateral striatum, which is involved in the extrapyramidal motor symptoms of this neuroleptic, but not in the nucleus accumbens, which is involved in the therapeutic activity of the drug; the authors concluded that H_3 receptor modulation may reduce the motor side effects of antipsychotics while not changing the therapeutic action [165]. Clearly, the possible effect of H_3 receptor ligands on schizophrenia must be studied further.

Drug Abuse

Several studies can be found in the literature showing that histamine H_3 receptor modulation could have significant effects on the abuse potential of different kind of drugs. Abuse of some opiate/ H_1 receptor antagonist combinations has been reported in several countries, and thus the concomitant use of pentazocine and tripelennamine ("T's and blues") has been explained by an enhancement of the morphine-like discriminative stimulus effect of pentazocine when it was consumed with the antihistamine [166]. Japanese adolescents abused cough syrups containing dihydrocodeine and chlorpheniramine [167]. The experimental studies performed so far on this subject suggest that activation of histaminergic neurons may attenuate the rewarding effect of morphine, while inactivation does the opposite [168]. Since histamine H_3 blockers are expected to
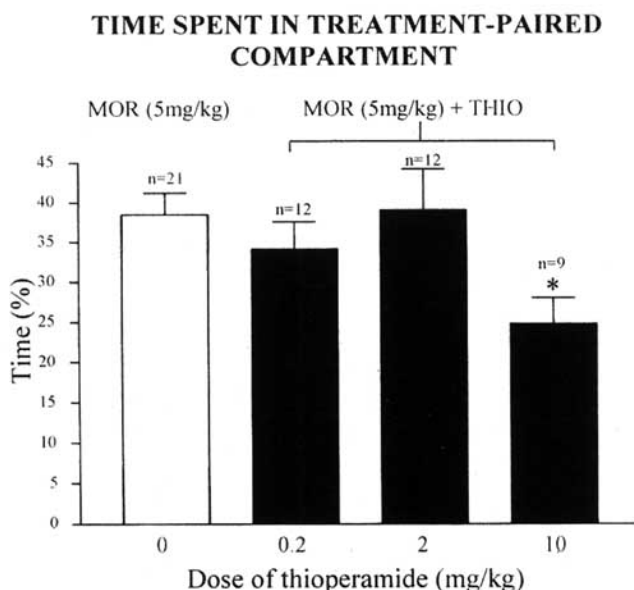

Fig. (5). Effect of thioperamide (THIO) on conditioned place preference induced by morphine (MOR). Rats were treated either with morphine (5 mg/kg) or with a mixture of morphine (5 mg/kg) and thioperamide (0.2, 2, 10 mg/kg). Bars represent means ± S.E.M. (*) p < 0.05 with respect to the treatment with morphine alone [169].

increase brain histamine levels, we have studied the effect of thioperamide on morphine-induced place preference in the rat and found that the drug was able to decrease opiate reinforcement when coadministered during conditioning (Fig. (**5**), [169]). Since many addictive drugs produce similar changes in mesolimbic pathways involved in motivation and reward, histamine H₃ receptor modulation could be expected to influence their effects. In the case of psychostimulants, preliminary data showing that brain histamine inhibits stereotyped behavior and behavioral sensitization [170] has been extended very recently with the finding that histamine H₃ receptor activation is closely involved in the changes of proenkephalin and prodynorphin gene expression induced by metamphetamine in the nucleus accumbens, a key area in drug reward [171]. Moreover, H₃ (-,-) mice show a decreased stereotypic response to metamphetamine [108]. The functional significance of these findings remains to be established but they strongly suggest that histamine H₃ receptor ligands could also influence psychostimulant reinforcement as they do with opioid drugs. There is also some preliminary evidence suggesting that histamine H₃ ligands could influence the effects of alcohol, since ethanol-sensitive selected rats exhibit lower levels of brain histamine, lower density of histamine-immunoreactive fibers and higher H₃ receptor ligand binding [172]. Finally, hyperkinesia, hyperaggression and audiogenic seizures induced by lorazepam withdrawal in the rat are potentiated by histamine H₃ antagonists and inhibited by agonists [173].

Pain

Preliminary experiments showed that neither histamine H₃ receptor agonists nor antagonists were able to modify rodent nociception in thermal tests [174-176]. More recent studies, however, have found that H₃ blockade with thioperamide produces antinociception in the mouse hot plate test whereas the agonist imetit was hyperalgesic [177]. In contrast to these results, *R*-α-methylhistamine potentiates morphine analgesia whilst thioperamide blocks it [174] and, even more confusingly, there is one report showing that both drugs exhibit spinal antinociceptive action in the rat [178]. Suh *et al.* [176] have found that intrathecal thioperamide does not affect the tail-flick response in the mouse but inhibits the analgesic effect produced by supraspinal administration of the opioid agonists morphine, DPDPE and U50,488H. Taken together, these results and others suggest that H₃ ligands are not potent central analgesics and their utility for the management of pain, if any, could be limited to enhance opioid analgesia in the case of agonists. Although this review is focused on the central nervous system, it is interesting to note that H₃ agonists have consistent peripheral antiinflammatory and antinociceptive activity and inhibit protein extravasation [46, 179]. These actions have led to the suggestion that these compounds could be useful for treating migraine, an effect that could be partially related to the inhibition of histamine-induced NO production and dilation of meningeal vessels [180].

ACKNOWLEDGEMENTS

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