Perspective

Histamine H_3 Ligands: Just Pharmacological Tools or Potential Therapeutic Agents?

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Histamine was already known as a chemical compound before its presence in mammals was established early this century. It has lasted until the 1920s before a physiological role for histamine was found, showing that histamine was more than a product of putrefaction.

Since then histamine has been combined with allergic reactions especially. Several antihistamines became available. Antihistamines like the diphenhydramines proved to be potent antagonists of several effects caused by histamine, such as contractions of the smooth muscle of the respiratory tract and skin reactions.

Some effects of histamine, however, were refractory to histamine; these effects include the increased production of gastric secretion and cardiac stimulation. In the 1960s the presence of two types of histamine receptors was proposed, and subsequently selective ligands for both types of receptor, coined H_1 and H_2, both agonists and antagonists became available.

Intensive research within the field of histamine and histamine antagonists has resulted in many interesting agents, several of which have found therapeutical applications. The larger part of these compounds are antagonists. The H_1 antagonists are being applied in allergic conditions mainly; the newer compounds such as terfenadine, astemizole, and loratadine have the advantage that they do not cause sedation, which made the older compounds of limited use. The H_2 antagonists (e.g. cimetidine, ranitidine, famotidine) have become important and safe gastric-healing ulcer agents. Recently, H_2 agonists have been suggested as cardiac stimulants (e.g. impromidine), though the gastric acid production could constitute a problem; H_1 agonists—of which no potent and selective example is available, however—seem to be important as research tools only.

H_1 antagonists—the classical antihistamines—have been found to induce rather strong central nervous system (CNS) effects, of which sedatory effects have even found a use, as some antihistamines are being applied as sleeping aids. Moreover, some classes of CNS agents, e.g. the tricyclic antidepressants and the neuroceptions, have originally been found as antihistamines. It is therefore rather remarkable that it has lasted until the 1980s before the role of histamine as a neurotransmitter in the CNS was accepted. In our decade, modern techniques, especially in the field of immunocytochemistry, made it possible to show that histamine is a neurotransmitter in the CNS indeed. The general organization patterns of histaminergic pathways in mammalian brains seem to be rather analogous to those of e.g. noradrenaline. These findings suggest that histamine and histaminergic neurons may constitute a system of the CNS which up to now has been difficult to understand. It has been suggested that histamine plays a role in e.g. states of wakefulness, energy metabolism, and cerebral circulation.

Histamine as a Neurotransmitter

Together with hormones neurotransmitters are the most important endogenous compounds which transfer information. Hormones are produced locally and transported by body fluids to the place which should receive information. Neurotransmitters are present in nerve endings and released upon the arrival of an action potential, bringing the information to another neuron or to the "innervated" organ.

For release of a neurotransmitter an action potential is required. However, the neuronal organization is very complex.[1] The release of a transmitter may be controlled by several factors; the transmitter itself may influence its own release by special receptors, but also other mediators could regulate the release. Moreover, the effect of the released transmitter is in many cases to be seen as only one component of a complex system in which several different neurons are transferring information, and in which several components together are responsible for the ultimate effect.

Upon the arrival of an action potential (AP), a certain amount of a neurotransmitter is released from the nerve ending into the synaptic cleft; subsequently, the neurotransmitter might react with different receptors (Figure 1).

In the case of histamine so-called postsynaptic receptors might belong either to the H_1 or the H_2 class. H_1 receptors are linked to a phosphatidylinositol pathway and mobilization of intracellular Ca^{2+}, whereas H_2 receptors mediate, via adenylcyclase activation, the production of cAMP. Stimulation of either the H_1 or H_2 system will lead to an ultimate physiological or pharmacological effect. Important differences between these two receptor types also include localization, function, and affinity for endogenous and exogenous ligands. For both the H_1 and H_2 receptor, histamine is the endogenous agonist; the antagonists for both receptors belong to rather different chemical classes of compounds.

Presynaptic receptors have a rather different function. These receptors modify the amount of agonist released (and in this way also the transfer of a signal). The presynaptic membrane might also be equipped with different receptors. A presynaptic receptor, stimulation of which influences the release of an agonist, is called an autoreceptor; heteroreceptors are known as well (Figure 1).

The system of the presynaptic autoreceptors are considered more and more as important for the fine regulation of neurotransmission. It seems that at high concentrations of a neurotransmitter the release is diminished by a negative feedback (e.g. the α_2 autoreceptor for noradrenaline). For noradrenaline we see a positive feedback at low con-

(1) Bloom, F. E. *FASEB J.* **1988**, *2*, 32-41.

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Table I. Activities at H_1, H_2, and H_3 Receptor of Some Selected Histamine Analogues and H_2 Agonists

compound	activity at H_1 (ref 3)	activity at H_2 (ref 3)	inhibition of [^{3}H]histamine release	
			ref 3	ref 4
histamine	100	100	100	$pD_2 = 7.4$
N^τ-methylhistamine	0.42	<0.1	<4	
N^π-methylhistamine	<0.01	<0.1	<4	
N^α-methylhistamine	72	74	270	$pD_2 = 8.7$
N^α,N^α-dimethylhistamine	44	51	170	$pD_2 = 7.3$
N^α-isopropylhistamine				$pD_2 < 5.5$
2-methylhistamine	16.5	4.4	<0.008	
4-methylhistamine	0.23	43	<0.008	
dimaprit	<0.0001	71	<0.03	
impromidine	<0.001	4800	*a*	

[a] Impromidine is an H_3 antagonist; see Table II.

Table II. Activities of Some Selected H_1 and H_2 Ligands at the H_3 Receptor (Taken from Ref 3)[a]

compound	K_B antagonist H_1	K_B antagonist H_2	K_B antagonist H_3
mepyramine	4.4×10^{-10}		$>5.8 \times 10^{-8}$
D-chlorophenyramine	5×10^{-10}		$>5.8 \times 10^{-8}$
L-chlorophenyramine	1.5×10^{-8}		$>5.8 \times 10^{-8}$
burimamide		7.8×10^{-6}	7×10^{-8}
metiamide	$>10^{-3}$	9.2×10^{-7}	2.5×10^{-6}
cimetidine		7.9×10^{-7}	3.3×10^{-5}
ranitidine		6.3×10^{-8}	1.2×10^{-6}
tiotidine		1.5×10^{-8}	1.2×10^{-5}
impromidine		4.8×10^{-7}	6.5×10^{-8}

[a] NB impromidine is an agonist!

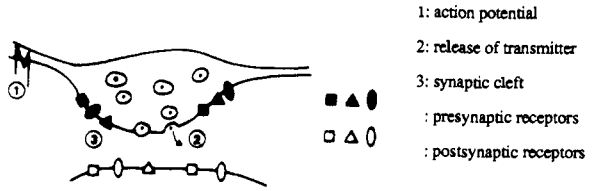


Figure 1. Neurotransmitter release and receptors at a varicosity.

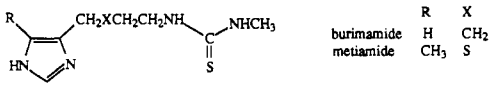


Figure 2. Metiamide and burimamide.

centrations (the presynaptic β-receptor?). In case both the positive and negative feedback are present, it should be clear that the receptor systems responsible for these effects have to show different characteristics.

Histamine is intraneuronally synthesized from histidine by means of a specific decarboxylase. In different ways we might interfere in the histaminergic neuronal system. Blockade of the L-histidine decarboxylase by a suicidal inhibitor (α-fluoromethylhistidine), however, opened the possibility to reduce the neuronal histamine levels drastically, and this approach is now an important tool in histamine research.[2] So far no selective and useful blockers of the main pathway of degradation of histamine (the formation of N^τ-methylhistamine) have become available. A third way is being constituted by the presynaptic autoreceptor of histamine.

The Presynaptic Histamine Receptor

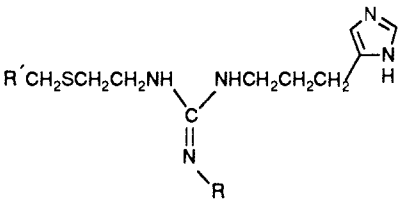
In 1983 a paper from the research group of Schwartz[3] showed that histamine inhibits its own release from depolarized slices of rat cerebral cortex. In the methods applied K^+ was used to depolarize the slices. Histamine release was followed by measuring [^{3}H]histamine release from preloaded slices. We subsequently studied the release by applying electrical stimulation in superfusion experiments and confirmed the findings of Schwartz.[4]

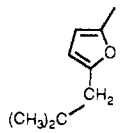
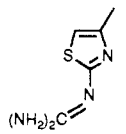
The inhibitory effects of histamine on its own release are very comparable to those seen with other neutrotransmitters. The process is Ca^{2+}-dependent, and the level of the release is higher after stronger electrical stimulation. As seen with other biogenic amines, the rate of formation of histamine from histidine is also controlled by extra neuronal histamine, probably by interfering with the same machinery which influences the histamine release from nerve endings.[5]

The presence of a histamine-dependent mechanism that reduces the histamine release points to a presynaptic inhibitory autoreceptor. In such a case it is very important to establish the characteristics of the receptor, whether it differs from already defined classes or not. From the first paper from the group of Schwartz,[3] it became already clear that we are dealing with a new receptor, for which the indication H_3 was introduced. [It is confusing that the term H_3 has also been used for a subclass of H_1 receptors[6] as well as for a subclass of H_2 receptors.[7]] Schwartz and our group investigated a number of compounds known for their affinities for H_1 or H_2, including both agonists and antagonists. In Tables I–III some striking examples are presented. We see that the structure–activity relationship (SAR) of histamine analogues is different for the three histamine mediated activities. If a receptor system exists, there should be a possibility to block the activity. In the

(2) For a review: Wada, H.; et al. Physiological function of histamine in the brain. In *Frontiers in Histamine Research*; Ganellin, C. R., Schwartz, J.-C., Eds.; Pergamon Press: Oxford, 1985; pp 225–234.
(3) Arrang, J. M.; Garbarg, M.; Schwartz, J. C. *Nature* **1983**, *302*, 832–837.
(4) Van der Werf, J. F.; Bast, A.; Bijloo, G. J.; van der Vliet, A.; Timmerman, H. *Eur. J. Pharmacol.* **1987**, *138*, 199–206.
(5) Arrang, J. M.; Garbarg, M.; Schwartz, J. C. *Neuroscience (Oxford)* **1987**, *23*, 149–157.
(6) Barbe, J.; Andrews, P. R.; Lloyd, E. J.; Brouant, P.; Soyfer, J. C.; Galy, J. P. *Eur. J. Med. Chem.* **1983**, *18*, 531–534.
(7) Fleisch, J. H.; Calkins, P. J. *J. Appl. Physiol.* **1976**, *41*, 511–519.

Table III. H_2 and H_3 Activities of Some Impromidine Analogues



compound	R′	R	H_3 activity: concn, M	H_3 activity: recovery histamine release,[a] %	H_2 activity (guinea pig): atrium α	atrium pD_2	atrium pA_2	gastric secretion α	gastric secretion pD_2	gastric secretion pA_2
impromidine		H	5×10^{-6}	100	1.0	7.8		1.0	8.5	
VUF8413		H	5×10^{-6}	100	0.9	7.8		0.9	8.5	
VUF8407		H	2×10^{-6}	100	1.0	7.9		1.0	8.2	
VUF8406		C≡N	5×10^{-6}	100	0		7.8	*b*	*b*	*b*

[a] Agonist 5×10^{-7} M histamine, causing almost 100% blockade of release. [b] Not tested.

Schwartz paper the activity at H_3 was presented for several H_1 antagonists and H_2 agonists and antagonists. From the data shown in Table II it is obvious that some, but not all, H_2 ligands, both agonists and antagonists, are H_3-receptor antagonists. It becomes clear that we are dealing with an independent receptor type indeed.

It is very striking that the weak H_2 antagonist burimamide is a potent H_3 antagonist, whereas some more active H_2 antagonists, including cimetidine, ranitidine, and tiotidine, are much weaker H_3 antagonists; also metiamide is not very active at the H_3 system. The most striking finding might be the activity of impromidine, which shows a very potent antagonistic effect at H_3. However, the difference in potency at H_3 between metiamide and burimamide is also very remarkable (see Figure 2). From the data available it is not possible to conclude which feature of these compounds is important at H_3: the methyl substituent at the imidazole (in histamine analogues the presence of this group reduces especially H_1 and H_3 agonistic potency) or the change from CH_2 to S in the side chain (the thio compound is more potent at H_2).

The thiourea-containing burimamide and the guanidino moiety containing impromidine are equipotent H_3 antagonists. This finding, too, is remarkable, as in the H_2 antagonistic series it became clear that at this receptor these two groups play a crucial role. Within the impromidine series compounds with an unsubstituted guanidino moiety, therefore having a high pK_a value, are agonists; the analogous derivatives with a cyano group bearing guanidino moiety, showing a low pK_a, are antagonists. The two H_2 antagonists burimamide and metiamide, being isothioureas, are not protonated at physiological pH either and are both H_3 antagonists.

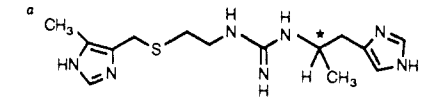
We therefore investigated a series of impromidine analogues on the H_3 receptor. Within this series we find both H_2 agonists and antagonists.[8] The activity depends on the pK_a of the guanidino moiety, as a protonated guanidino group at physiological pH leads to agonistic activity; substituents at this group which lower the pK_a turn the compound into an antagonist. Some of our results are summarized in Table III. From these data it is clear that impromidine analogues are all active as antagonists at H_3; clearly the guanidino moiety has different roles at H_2 and H_3.

Another interesting finding concerns the activity of the enantiomers of a chiral impromidine analogue as summarized in Table IV. Here again we notice the different affinities of ligands for and their qualitative properties at the three histamine receptors.

We may conclude that the H_3 receptor differs from both H_1 and H_2 receptors.

Table IV. Activities of Enantiomers of a Chiral Analogue of Impromidine[9,10]

compound[a]	agonistic act. at H_1; guinea pig ileum: ia[e]	agonistic act. at H_1; guinea pig ileum: potency	agonistic act. at H_2; guinea pig atrium: ia[e]	agonistic act. at H_2; guinea pig atrium: pD_2	antagonistic act. at H_3; rat cerebral cortex: K_i, μM
histamine	1	100	1	5.95	*d*
R enantiomer[b]	0.2	1.7	1	6.92	0.06
S enantiomer	0	0	0	0[c]	0.04
racemate	*d*	*d*	0.8	6.48	*d*

[a] 

[b] Sopromidine. [c] Antagonist; ca. 0.25 times that of metiamide. [d] Not available. [e] Intrinsic activity.

H_3 Agonists

Histamine seems to be the natural agonist of the H_3 receptor; however, the possibility that N^{α}-methyl or N^{α},N^{α}-dimethyl has a physiological role cannot be excluded. Several investigators have reported the presence of these methylated histamine analogues in body fluids.[11]

(8) Van der Werf, J. F.; Bijloo, G. J.; van der Vliet, A.; Bast, A.; Timmerman, H. *Agents Actions* 1987, *20*, 239–243.

Table V. Activities of Some Histamine Analogues at H_1, H_2, and H_3 Receptors

compound	H_1: agonist; guinea pig ileum	H_2: agonist; guinea pig atrium	H_3: inhibition of histamine release rat cerebral cortex
histamine	100	100	100
(*R*)-α-methylhistamine	0.49	1.02	1550
(*S*)-α-methylhistamine	0.49	1.74	13

As recently also a synthesizing enzyme (indolethylamine *N*-methyltransferase) has been identified and purified,[12] a study on the distribution of this enzyme as well as on that of the methylated products could in an important way contribute to our understanding of the H_3 receptor and more generally of the role of histamine.

In Table I the H_3-agonistic effects of some close analogues of histamine are being presented; the high activity of the N^{α}-methyl analogues is remarkable, as is the quick drop in activity seen with N^{α}-isopropylhistamine.

So far the most active H_3 agonist is the (*R*)-α-methylhistamine (Table V). Apparently, stereoselectivity is much more pronounced at the H_3 receptor than at either the H_1 or the H_2 receptor. Taking into account that histamine is active at much lower concentration (about 100-fold) at H_3 than at H_1 and H_2, (*R*)-α-methylhistamine is a very potent agonist indeed.

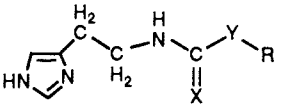
The very high activity of α-methylhistamine is somewhat surprising. Some years ago Arrang et al. reported on the stereoselectivity of N^{α}-methyl-α-(chloromethyl)histamine and α,N^{α}-dimethylhistamine at H_3 receptors; the stereoselectivity was reverse for H_2 and H_3 receptors, both compounds being weak agonists in both cases (about 1–4% of histamine).[13] We cannot but conclude that both α-methyl and N^{α}-methylhistamine are potent H_3 agonists, whereas the combination of both substitutions leads to a weak compound; an intriguing problem! From the high activity of α-methylhistamine and the high degree of stereoselectivity speculations on the structural requirement would seem to be possible. So far, no analogous compounds with a comparable activity have been found, reason why even speculations are not possible. The compound (*R*)-α-methylhistamine is currently being clinically investigated.

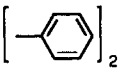
H_3 Antagonists

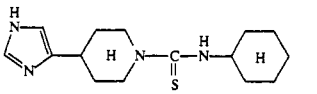
The first potent antagonist found was impromidine. This strong H_2 agonist (about 48 times histamine) is a potent H_3 antagonist (see Table II). It is striking that some other, but not all H_2 ligands, also have H_3-antagonizing properties (see Table II); no clear picture of the SAR of these compounds has emerged. The large difference in activity of the two antagonists burimamide and metiamide could be explained by several features of these two molecules.

Recently, a class of new H_3 antagonists has been published.[14] It concerns a series of N^{α}-substituted histamine analogues. In Table VI data on the available compounds have been summarized. The high selectivity (H_3 versus H_1 and H_2) is very striking; the compounds are either inactive or weak agonists at the H_1 and H_2 receptor.

Table VI. Some H_3 Antagonists

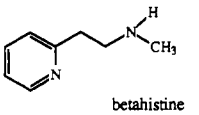


substance	X	Y	R	H_3(antag) $-\log K_i$
1	O	CH_2		6.0
2	O	$(CH_2)_2$		6.2
3	O	$(CH_2)_3$		7.1
4	O	$(CH_2)_4$		6.7
5	2H	$(CH_2)_3$		6.2
6	O	$(CH_2)_3$		6.0
7	O	$(CH_2)_3$		6.0
8	O	$(CH_2)_3$		7.3
9	O	$(CH_2)_2CH$		6.0



thioperamide

Figure 3. Thioperamide.



betahistine

Figure 4. Betahistine.

In a qualitative way the SAR show that the chain length between the amino nitrogen and the lipophilic R group is crucial. The R substituent should apparently be not too bulky (compound **9**, versus compound **3**, Table VI). Obviously, the amides are more potent than the corresponding amines (compounds **7** and **3** of Table VI). So far one can only speculate on the possible cause of this phenomenon.

A related compound with H_3-antagonistic properties is thioperamide (Figure 3). Thioperamide has been selected from a series of histamine analogues with reduced side-chain flexibility;[15] no data on this series have been pub-

(9) Schunack, W.; Schwartz, S.; Gerhard, G.; Buyuhtimkin, S.; Elz, S. Chiral agonist of histamine. In *Frontiers in Histamine Research*; Ganellin, C. R., Schwartz, J.-C., Eds.; Pergamon Press: Oxford, 1985; pp 38–46.

(10) Arrang, J. M.; Garbarg, M.; Schwartz, J. C. Histamine synthesis and release in CNS: control by autoreceptors (H_3). In *Frontiers in Histamine Research*; Ganellin, C. R., Schwartz, J.-C., Eds.; Pergamon Press: Oxford, 1985; pp 143–154.

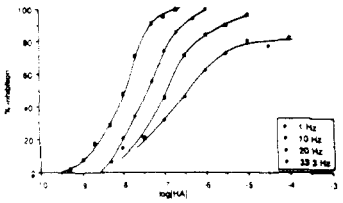
(11) See 30 for references.

(12) Herman, K. S.; Bowsher, R. R.; Henry, D. P. *J. Biol. Chem.* **1986**, *260*, 12336–12340.

(13) Arrang, J. M.; Schwartz, J. C.; Schunack, W. *Eur. J. Pharmacol.* **1985**, *117*, 109–114.

(14) Lipp, R.; Schunack, W.; Arrang, J. M.; Garbarg, M.; Schwartz, J. C. Poster at 10th International Symposium on Medicinal Chemistry, Budapest, August 15–18, 1988.

(15) Arrang, J. M.; Garbarg, M.; Lancelot, J. C.; Lecomte, J. M.; Pollar, H.; Robba, M.; Schunack, W.; Schwartz, J. C. *Nature* **1987**, *327*, 117–123.



pD_2 · 1Hz	8.1 ± 0.1
pD_2 · 10Hz	7.4 ± 0.1
pD_2 · 20Hz	7.0 ± 0.1
pD_2 · 33.3Hz	6.6 ± 0.1[a]

a) max. blockade about 80%.

Figure 5. Concentration-dependent inhibition of electrically evoked [^{3}H]HA release from superfused rat brain cortex slices by exogenously added HA at different stimulation frequencies. Superfused rat brain cortex slices (previously loaded with [^{3}H]HA) were electrically stimulated during 2 min, 90 min after onset of superfusion with various concentrations of HA present in the superfusion medium. Inhibition data were derived from HA release studies. Mean values of three to six determinations from one or two experiments are given (SD less than 10% of calculated values).

lished up to now, however. Thioperamide is a competitive antagonist of histamine at the H_3 receptor; the apparent K_i is 4 nM approximately (the effects of endogenous histamine are ignored). The compound is active very weakly at both the H_1 and H_2 receptors, showing affinities higher than 10 μM. In vivo thioperamide antagonized the effects of (*R*)-α-methylhistamine at the level of cerebral histamine levels.

Another example of an H_3 antagonist is the weak H_1 agonist betahistine (Figure 4). Betahistine has been reported as a moderately active antagonist at H_3 receptors.[16]

So far no clear picture of the requirements for H_3-receptor affinity has emerged. Many more derivatives have to be investigated. A number of rather obvious questions have to find an answer in future research.

Some Characteristics of the H_3 Receptor

Although the researchers of the group of Schwartz reported that the release of histamine (HA) was blocked to a maximal degree of 60% approximately when K^+ depolarization was applied in order to induce release,[3] we were able to show that a 100% blockade is possible when using electrical stimuli as induction of release.[4] In subsequent studies we showed a strong effect of the frequency of the stimulation on the inhbitory effect of histamine (Figure 5).[17]

At high frequencies, histamine becomes less effective; a similar effect has been found by Langer for noradrenaline.[18] At higher frequencies higher concentrations of released histamine will be reached. At such higher frequencies one would expect increased effects of an antagonist, as, for instance, was reported for dopamine release by Dubocovich.[19] We find, however, no increased effects of impromidine at higher frequencies (Table VII).

Our results might be explained by the presence of spare receptors at low frequencies (<20 Hz); at a higher frequency all receptors are needed for complete blockade, complete blockade of release being impossible at sufficient

Table VII. Effect of Impromidine on [^{3}H]HA Release at Various Stimulation Frequencies[a]

frequency, Hz	control	10^{-7} M imp[f]	10^{-6} M imp	10^{-5} M imp
2[b]	8.8 ± 1.1	NM	14.3 ± 1.9[c] (+63%)	NM
10	21.3 ± 1.5	25.3 ± 2.4[c] (+19%)	25.9 ± 2.3[c] (+22%)	25.5 ± 1.4[c] (+20%)
20	28.7 ± 3.0	31.2 ± 1.7[ns d]	35.5 ± 2.3[c] (+24%)	35.1 ± 2.0[c] (+22%)
33.3	37.8 ± 1.7	NM[e]	39.5 ± 4.7[ns]	38.4 ± 3.1[ns]

[a] Release % (±SD) are given, after stimulation with different frequencies for 2 min; values represent the means of three to twelve determinations from one to three experiments. [b] In this case the duration of stimulation was 2.5 min. [c] $p < 0.05$ as compared with corresponding control values. [d] Not significantly different from the corresponding control value. [e] Not measured. [f] Impromidine.

high frequency. The presence of a receptor reserve is also likely, because no effect of the frequency on the pA_2 of the antagonist impromidine was observed.[17]

Recently, we have found indications for the presence of spare receptors; at high frequencies histamine, which is a full agonist at a low frequency, becomes a partial agonist. The pD_2 of agonists proved to be independent of the frequency of stimulation, over the range of frequencies studied.[17]

As yet, not much has been elucidated regarding the mechanisms involved in the signal transfer at the H_3 receptor. It is known, though, that the process is Ca^{2+} dependent;[5] the role of Ca^{2+} might explain the reported differences between the results obtained with K^+ depolarization and electrical stimulation; the Ca^{2+} gating mechanisms might be different for both depolarization models.[20]

Localization of H_3 Receptors

The selective H_3 agonist (*R*)-α-methylhistamine has been used in a tritiated form to visualize and assay the H_3 receptor.[15] However, a word of caution is in place against the use of a radiotagged agonist for the purposes of assaying the receptor. As it is not known whether the H_3 receptor is present in one or two activity states, a potent radiolabeled antagonist would constitute a "safer" probe. Indeed, the first studies indicate that in binding assays with [^{3}H]-(*R*)-α-methylhistamine K_i values have been found which differ from those from functional studies.[15]

Applying the same [^{3}H]-(*R*)-α-methylhistamine it was found that the label is found mainly in the cerebral cortex (rats).[15] These findings are in line with data obtained from immunocytochemical studies showing the distribution of histaminergic neurons. The distribution of the label, however, is not exactly the same as previously found, which might mean that the presence of H_3 receptors is not restricted to histaminergic neurons. The receptor density is low (30 ± 3 fmol/mg of protein) and much lower than for H_1 and H_2.

In some peripheral tissues H_3 receptors have also been identified, though the receptor densities are even lower than in brain protein.[15] In lung tissue the H_3 receptor is found in a concentration of ca. 5 fmol/mg of protein. In Table VIII the effects of thioperamide and (*R*)-α-methylhistamine on the [^{3}H]histamine level—after a dosage of [^{3}H]-L-histidine—are presented.[15] In the studies from which these data have been taken rats received [^{3}H]-L-histidine intravenously; (*R*)-α-methylhistamine (10 mg/kg iv) was given together with [^{3}H]-L-histidine and

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Table VIII. The Influence of an H_3 Agonist and Antagonist on Tissue Histamine Levels

	histamine level after treatment[a]				
tissue	controls	relative to cerebral cortex	(R)-α-methylhistamine	thioperamide	both agents
cerebral cortex	100	-	68	234	196
hypothalamus	100	321	85	245	205
lung	100	159	72	188	143
abdominal skin	100	450	65	75	73
spleen	100	81	69	123	96
colon	100	130	81	80	75

[a] Calculated from data in ref 15.

thioperamide (10 mg/kg ip) 1 h before. The figures are relative to the level of [^{3}H]histamine in the cerebral cortex. In all tissues studied the effects of the agonist are about the same, but the reversal of the reduction of the synthesis of [^{3}H]histamine by thioperamide is less pronounced in skin and colon especially. Schwartz and colleagues speculate that the synthesis and release of histamine in and from mast cells are partly controlled by H_3 receptors.

Obviously, more extended studies are needed for completing the precise distribution of histamine H_3 receptors.

Physiological Roles of H_3 Receptors

Selective ligands have only very recently become available and as yet almost no detailed pharmacological studies have been published on their effects. Most of the available data stem from investigations in which less selective ligands have been applied, which is why suggestions on the physiological roles of H_3 receptors are still speculative in general.

As regards the functions of histamine in the central nervous system, control of hormonal secretions, production of energy, and state of sleep and waking have been mentioned, as well as control of cerebral circulation.[21] Besides this, one has also speculated on a role of histamine in controlling muscle activity. The newly available compounds will most probably become very important tools.

It has been found that H_3 receptors are present in brain regions in which histaminergic neurons are hardly present. Indeed, H_3 receptors are also heteroreceptors for other neurotransmission systems. Recently, Schlicker et al.[22] showed an inhibitor effect of histamine on the release of serotonine from slices of the cortex of the rat brain, the effect of which was antagonized by H_3 antagonists.

Another approach for elucidating the role of histamine constitutes the study of agents with a well-established pattern of activity for their effect on the histaminergic pathways. So far, not many studies on this subject have become available. From the early paper of Schwartz et al.[3] it became evident that the central effects of classical H_1 antihistaminics are unlikely to be due to an effect on H_3 receptors. From other investigations we might conclude that the sedative properties of these agents correspond to their blocking effects at the H_1 receptors.

One of the very few clinically used agents with histamine-receptor-stimulating properties is betahistine. Betahistine, N^α-methyl-2-pyridylethylamine, has a relatively weak H_1 agonistic activity and virtually no H_2 activity. It is reported to be active in certain CNS affections and has shown to be a cerebral and peripheral vasodilator. Its weak H_1-agonistic effects can hardly explain its pharmacological profile and therefore it seemed probable that the compound might derive its pharmacological profile from its interaction with the H_3 receptor. In a study, again by Schwartz et al.,[16] betahistine proved to be an H_3 antagonist (rat cerebral cortex) with an activity in the same concentration range as its K_d on H_1 receptors. These findings could mean that betahistine not only stimulates the H_1 receptor directly, but also enhances release of histamine and other transmitters from nerve endings. The profile of betahistine might therefore be a combination of H_1-agonistic (directly and indirectly via H_3), H_2-agonistic (indirectly via H_3) effects; it cannot be excluded that still other systems are involved as well. As rather strong effects of an H_3 agonist are seen when given separately (Table VIII) and because of the in vivo effects of H_3 agonists when given separately (see next paragraph), the H_3-antagonistic effects of betahistine might be important indeed.

In a recent report Bristow and Bennett[23] show that after intraaccumbens administration in mice of selective H_1, H_2, or H_3 agonists, H_1 agonists induce a weak hyperactivity, whereas an H_3 agonist causes hypoactivity (reduced by H_3 antagonists); H_2 agonists do not induce any effects. The data support both the role of H_1 (direct) and H_3 (indirect) receptors in activity patterns.

Traditionally, histamine has been investigated intensively in peripheral systems. Major stores of histamine are available in mast cells, but also in immunoactive cells. Most of the established activities of histamine can be explained by interference with either H_1 or H_2 receptors, though not all effects seen can be explained satisfactorily. The H_3 receptor seems to constitute a pathway by which histamine in peripheral systems could reach certain of its physiological activities.

As we have seen above,[15] H_3 receptors have proved to be present in certain tissues such as in lung tissue or they are likely to be found in, for example, spleen, skin, and GI tract.

In vascular smooth muscle (VSM) cells of the guinea pig mesenteric artery, Ishikawa and Sperelakis[24] showed a facilitation of the excitatory junction potentials (ejp) by repetition of the stimulation which reaches a steady state; the ejp are of a neurogenic, sympathetic origin. Histamine is depressing the ejp in a dose-dependent fashion. Though at a high concentration certain H_2-mediated effects are being observed, the ejp depression occurs at low concentrations of histamine (10^{-8}–10^{-6} M). As N^α-methylhistamine is more potent than histamine and as compounds like impromidine antagonize the effects, it is likely that H_3 receptors are involved. Ishikawa and Sperelakis conclude that H_3 receptors may, upon stimulation, produce vasodilation by inhibition of sympathetic tone.

In studies with isolated rabbit cerebral arteries Ea-kim and Oudart very recently showed a role for H_3 receptors in the vasoactive effects of histamine, which are mediated

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by both H_1 and H_2 receptors; in their study histamine reduces the contraction induced by either K^+ or uridine triphosphate.[25] The presence of H_1, H_2, and H_3 receptors makes pharmacological studies a bit suspicious, unless selective ligands are applied. The findings of Ottoson et al., who in a recent study[26] conclude that both H_1 and H_2 receptors are present in human cerebral arteries, might be, at least partly, explained by assuming the presence of H_3 receptors as well.

Ea-kim and Oudart[25] propose that H_3 receptors could also be present in tissue other than nerve terminals. This proposal is made, because the simultaneous application of both an H_1 and H_2 antagonist could not block the effects of H_3 stimulation. It might, however, well be that this absence of blockade by H_1 and H_2 antagonists results from the release of a transmitter other than histamine itself.

In other investigations on the role of histamine H_1 and H_2 receptor in cardiovascular systems it has often been concluded that both receptors are involved in the histamine-induced effects. It has also been reported that histamine effects are accompanied by changes in plasma noradrenaline concentrations.[27] For final conclusions on the role of the different receptors, the application of selective ligands is conditional, however. In the study of Knigge et al.[27] on the effects of histamine on cutaneous blood flow in man it is said that H_1 and H_2 receptors are both involved in vasodilation; the choice of histaminergic agents makes a definitive conclusion quite difficult, as the H_3 receptor could have been affected as well.

About 20 years ago Ambache and Aboo Zar[28] established an inhibitory effect of histamine on the guinea pig ileum. The effect was neither of the H_1- nor of the H_2-mediated type. In subsequent studies by the same investigators[29] burimamide was shown to prevent the inhibitory effects observed after histamine. Recently, two research groups reinvestigated these inhibitory properties of histamine on the GI tract.

Tamura et al.[30] studied the effects of histamine and some analogues at nicotinic synapses in enteric ganglia of the guinea pig. Excitatory postsynaptic potentials were intracellularly recorded. Histamine reduced the postsynaptic potentials induced by a single electrical stimulus. Histamine is effective in relatively low concentrations (10^{-9}–10^{-7} M). As N^{α}-methylhistamine is about 25 times as active as histamine, H_1 or H_2 agonists do not mimic these effects and H_1 antagonists are inactive; compounds like impromidine and burimamide behave as antagonists, and H_3 receptors seem to be involved again. These results might be explained by a reduction of acetylcholine release by histamine suppressing the transmission at nicotinergic synapses. The effects could also mean that histamine is functional in the enteric synaptic circuits and might affect GI activity in more than one way. Results of detailed studies by Snow and Weinreich[31] on presynaptic and postsynaptic effects of histamine and analogues on the superior cervical ganglion of the rat are explained by the authors by the presence of pre- and postsynaptic histamine receptor of both the H_1 and H_2 type. However, the choice of the ligands and the concentrations used make any conclusion in this respect suspicious. At least some of the findings of Snow and Weinreich might be explained by an involvement of H_3 receptors as well.

Trzeciakowski[32] studied the inhibitory effects of histamine on contractions induced by electrical stimulation of the isolated guinea pig ileum. The inhibitory effects of histamine which are recorded in the presence of blockade of H_1 receptors occur at relatively low concentrations ($-\log EC_{50}$ is 7.2) and are also seen with N^{α}-methylhistamine ($-\log EC_{50}$ is 8.8); moreover, impromidine is an antagonist of the effect ($-\log K_B$ is 7.6). These findings point again to the presence of H_3 receptors. This conclusion is supported by the blockade of the effects by tetrodoxin. The effects, however, were not affected by hexamethonium, α- and β-antagonists, naloxone, or the adenosine antagonist 8-PT, all when applied in their usual pharmacological concentrations. The spasmogenic effects of acetylcholine are not changed by N^{α}-methylhistamine.

Also on the rat vas deferens recent findings seem to indicate that H_3 receptors are involved in histamine mediated effects. A study by Todorov and Zamfirova[33] show the influence of histamine and several histaminergic ligands on contractions of the vas deferens induced by electrical field stimulation. Although the applied concentrations in this study are extremely high, making any definite conclusion almost impossible, the results are compatible with the presence of inhibitory presynaptic histamine receptors. Those H_3 receptors are most likely connected to the sympathetic system.[34]

On the urinary bladder prejuctional effects of histamine have been found by Poli et al.[35] The results of this study are preliminary; it is not clearly established to which class the presynaptic histamine receptors belong and which transmitters are involved, although it seems likely that acetylcholine plays a role here.

Taking the findings from the several peripheral systems together, it becomes attractive, but at the same time more speculative, to suggest a role for N^{α}-methyl or even N^{α},N^{α}-dimethylhistamine at such receptors. Several investigators have reported the presence of these methylated histamine analogues in body fluids.[11] As recently also a synthesizing enzyme, indolethylamine-*N*-methyltransferase, has been identified and purified,[11] a study on the distribution of this enzyme as well as on that of the mono- and dimethylated histamine analogues could considerably contribute to our understanding of the meaing of the H_3 receptor and in more general terms of the physiological roles of histamine.

For pharmacological investigations the availability of selective agonists and antagonists is very important. The newly published agonist (*R*)-(α)-methylhistamine and the antagonist thioperamide seem to be first examples of selective H_3 ligands. More compounds, also of the nonbrain-penetrating type, are highly wanted. Also detailed pharmacological investigations with such compounds are of great importance.

So far, only a limited number of compounds have been screened for their effect on H_3 receptors. Probably, one

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of the reasons is the rather tedious procedure of establishing the effect on the induced histamine release from cortical slices. Therefore more simple test systems are highly wanted.

For therapeutical applications several possibilities seem to exist.

In the central nervous system the profile of selective H_3 agonists and antagonists is still largely unknown. The paper of Bristow and Bennett prove, however, that the H_3 receptor may constitute a new way to influence the activity pattern. Detailed studies are highly wanted here, too; it also seems attractive to investigate whether the effects of betahistine could be explained by the antagonistic effects of this compound on H_3 receptors indeed.

In peripheral systems too, suggestions have to remain rather speculative. However, the relaxation effects of histamine on the gut by interfering with the release of e.g. acetylcholine indicate that selective, nonabsorbed H_3 agonists could probably constitute an alternative way (to e.g. morphine) in order to reduce GI activity and to treat diarrhea. The prospects of H_3 antagonists are less clear in this perspective, although selective, nonabsorbed compounds could become of interest as well by means of their stimulatory properties.

The effects of H_3 agonists and antagonists on circulatory events have to be established. So far, the results point to attractive possibilities. It has been known for a long time that histamine itself is very active, on many systems; the effects of histamine on H_1 and H_2 will be antagonized by the effects mediated by H_3. A selective H_3 agonist could therefore antagonize histamine induced effects, without inducing histaminergic effects itself directly (e.g. smooth muscle contractions of gastric acid production); in a reverse way the same is true for H_3 antagonists. Investigations into the effects of both selective agonists and antagonists are highly wanted; one might speculate that the compounds constitute alternatives for existing agents which are useful for controlling blood pressure and circulation.

Note Added in Proof. After the manuscript of this paper had been finished, several new studies have been published. A very interesting finding has come from the group of Barnes. In two papers (Ichinose, M.; Stretton, C. D.; Schwartz, J.-C.; Barnes, P. J. Histamine H_3-receptors inhibit cholinergic neurotransmission in guinea-pig airways. *Br. J. Pharmacol.* **1989**, *57*, 13-15. Ichinose, M.; Barnes, P. J. Inhibitory histamine H_3-receptors on cholinergic nerves in human airways. *Eur. J. Pharmacol.* **1989**, *163*, 383-386.), it is shown that H_3-receptor stimulation reduces the cholinergic contractile responses to electrical stimulation without affecting acetylcholine-induced effects. The facts seem to indicate that H_3 receptors present on the vagus nerves modulate cholinergic neurotransmission in the airways and would open other appealing therapeutical possibilities of selective H_3 agonists.

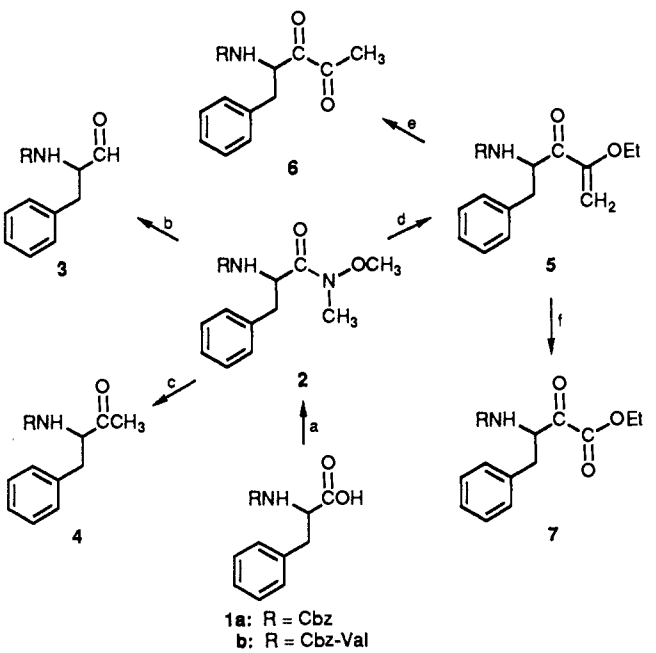
Communications to the Editor

α-Diketone and α-Keto Ester Derivatives of N-Protected Amino Acids and Peptides as Novel Inhibitors of Cysteine and Serine Proteinases

Sir:

A successful approach to specific inhibition of proteinases has been to design small peptide substrate analogues in which the scissile amide unit has been replaced by a functionality incorporating an electron-deficient carbonyl group, such as an α-fluorinated ketone[1-4] or an α-keto ester.[4,5] In this communication, we describe the synthesis and the evaluation of α-diketone derivatives of amino acids and dipeptides as a novel class of electron deficient carbonyl containing inhibitors for the serine and cysteine proteinases, α-chymotrypsin and calpain, respectively. We also demonstrate that peptidyl α-keto esters, which have been previously shown to inhibit serine proteinases,[4,5] are also potent inhibitors of calpain.

Scheme I[a]



1a: R = Cbz
b: R = Cbz-Val

[a] (a) *i*-BuOCOCl, *N*-methylmorpholine, *N,O*-dimethylhydroxylamine hydrochloride; (b) $LiAlH_4$, THF; (c) MeLi, THF; (d) *t*-BuLi, ethyl vinyl ether, $MgBr_2$, THF; (e) HCl, dioxane, H_2O; (f) O_3, CH_2Cl_2, pyridine.

Chemistry. The chemistry used to construct the α-diketone and α-keto ester inhibitors is shown in Scheme I. *N*-Carbobenzyloxy- and *N*-(*N*-carbobenzyloxyvalyl)-

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