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Review

Selective ligands as tools to study histamine receptors

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Abstract – In this review the histaminergic ligands for the histamine H_1, H_2 and H_3 receptors, which are currently used as tools in pharmacological studies, are described. To study interactions with the histamine H_1 receptor, the H_1 agonist 2-aminoethylthiazole has long since been used. However, during the last decade, 2-phenylhistamine derivatives emerged with interesting binding features. So far no radiolabelled selective H_1 agonist has been commonly used. As H_1 antagonists mepyramine, triprolidine and chlorpheniramine are described together with radiolabelled H_1 antagonists [^{3}H]mepyramine and [^{3}H]doxepin. Special attention has been paid to the PET ligands [^{11}C]doxepin and [^{11}C]mepyramine and the [^{125}I] labelled antagonists [^{125}I]iodobolpyramine and [^{125}I]iodoazidophenpyramine. Concerning H_2 agonists, especially dimaprit, amthamine and impromidine are discussed. There are several H_2 antagonists; amongst them cimetidine, tiotidine and ranitidine are used most frequently. Many of these antagonists behave as inverse agonists. As radiolabelled H_2 antagonists, [^{3}H]cimetidine, [^{3}H]tiotidine, [^{125}I]iodoaminopotentidine and [^{125}I]iodoazidopotentidine are included. Commonly used histamine H_3 agonists are N^{α}-methylhistamine, (R)α-methylhistamine, imetit and immepip. Both methylhistamines are also available as [^{3}H] labelled ligands. As reference compounds, often used H_3 antagonists are thioperamide, clobenpropit, iodophenpropit and impentamine. Most important radiolabelled H_3 antagonists are S-[^{3}H]methylthioperamide, [^{3}H]thioperamide, [^{125}I]iodophenpropit and [^{125}I]iodoproxyfan. The use of all these compounds as a tool in pharmacology is also discussed. © 2000 Éditions scientifiques et médicales Elsevier SAS

tools / histamine / H_1 agonists / H_1 antagonists / radiolabelled H_1 antagonists / H_2 agonists / H_2 antagonists / radiolabelled H_2 antagonists / H_3 agonists / radiolabelled H_3 agonists / H_3 antagonists / radiolabelled H_3 antagonists

1. Introduction

Nowadays it is well established that histamine exerts its effects by activating histamine receptors, of which three subtypes (H_1, H_2, and H_3) are recognised. Specific activation or blockade of these receptor subtypes has led to a tremendous increase in the knowledge of the roles of histamine in physiology and pathology and the mechanisms involved.

The histamine H_1 receptor, which is found throughout the whole body, is in fact the classical histamine receptor. Via stimulation of this receptor histamine causes contraction of smooth muscles in, e.g. airways and intestines. Moreover, histamine plays a role in allergic conditions which have often been treated successfully with antihistamines, more precisely, histamine H_1 antagonists.

The observation that gastric acid secretion caused by histamine could not be blocked with classical antihistamines led to the conclusion that another histamine receptor type was involved, which was subsequently called the histamine H_2 receptor and the classical one since then the H_1 receptor. During the last decades, research for histamine H_2 ligands has been very fruitful as a few hundred histamine H_2 agonists have been described and more than one thousand H_2 antagonists. Among them are compounds that are very potent and selective. Several of them are succesfully used for the treatment of gastric ulcers by inhibiting gastric acid secretion.

In 1983 Arrang et al. [1] described a functional assay in which it was found that histamine inhibits its own synthesis and release in rat brain (cortical) slices. Based on the absence of effects of H_1 and H_2 agonists and antagonists in this assay, it was concluded that a third histamine receptor (H_3) had to be involved. Later it was found that this receptor is not only localised on presynaptic membranes in the CNS, but also peripherally regulates the release of histamine (via its autoreceptor)

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and other transmitters (via heteroreceptors). After Arrang et al. [1] described in 1983 the selective H_3 agonist (R)α-methylhistamine and the H_3 antagonist thioperamide, huge efforts have been made to develop other selective histamine H_3 ligands. More recently, H_3 receptors have been found to occur postsynaptically as well.

In this review we describe the histaminergic ligands for the H_1, H_2 and H_3 receptors which are currently used as a tool in pharmacological studies.

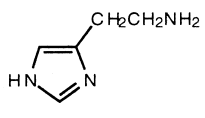
2. Histaminergic H_1 ligands

2.1. H_1 agonists

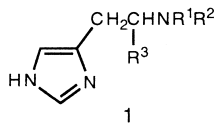
Although, for therapeutic application, potent and specific H_1 agonists do not seem to have any role, such compounds are of importance for fundamental research on the function of H_1 receptors in several physiological and pathophysiological conditions. Several attempts to obtain active H_1 agonists by modification of the histamine molecule led to a strong decrease or complete loss of activity. Recently a few reviews on this subject have been published [2, 3].

In the histamine molecule, two major structural elements can be distinguished: the imidazole ring and the aminoethyl side chain. Modifications in both parts have been reported.

Introduction of substituents in the ethylene sidechain of histamine has not revealed interesting H_1 agonists. Methylation of the alpha or beta position leads to reduction of H_1 activity [4]. Remarkably, the activities of the enantiomers of the alpha methylated histamine (**1**, R^1 = R^2 = H, R^3 = CH_3) are equal, indicating that the spatial orientation of the methyl group is rather unimportant [5]. Methylation of the amino group of histamine (**1**, R^1 = CH_3, R^2 = R^3 = H) yields an active H_1 agonist as well. However, there is no selectivity because this compound is a relatively potent H_2 agonist [3] and also an H_3 agonist [1]. The same is true for the N,N-dimethylated histamine (**1**, R^1 = R^2 = CH_3, R^3 = H). Alkylation with higher alkyl groups leads to a strong decrease in H_1 agonistic activity [6]. Replacement of the aminoethyl sidechain by more rigid moieties also causes a strong decrease in activity [7].



histamine



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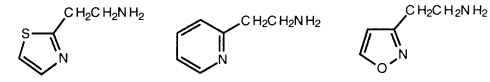
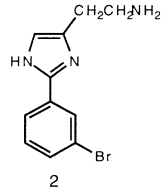
Modification of the imidazole ring of histamine has shown to be the most promising approach to develop potent and selective H_1-agonists. Thus, replacement of the imidazole ring of histamine by the 2-thiazolyl, 2-pyridyl or 3-oxazolyl group yields H_1 agonists, although their activity (only activities relative to histamine instead of pD_2 values are available) is reduced considerably compared to histamine [8, 9]. The 2-thiazole derivative has often been used as a selective H_1 agonist, the H_2 and H_3 activity being rather low.



rel. activity (histamine = 100)	29.5	8.3	0.5

Replacement of the imidazole system by the 3- and 4-pyridyl group led to inactivity suggesting that the presence of an N atom in the ring adjacent to the aminoethyl sidechain is necessary.

Substitution at the imidazole ring revealed that H_1 agonism can be retained with lower alkyl substitution at the 2- position [3]. Higher alkyl substitution in this position is not tolerated. Thus, the 2-cyclohexylhistamine is completely devoid of H_1 activity [10]. Surprisingly, several corresponding 2-arylhistamines (e.g. aryl = phenyl, 3-pyridyl, 4-pyridyl, 2-thienyl, 3-thienyl) show moderate H_1 agonistic activity. These observations introduced the development of the most potent and selective H_1 agonists known thus far. It was found that compounds of a series of 2-(substituted phenyl)histamines showed, depending on their substitution, selective H_1 agonism, whereas in some cases antagonism was also observed [11, 12]. Particularly the *meta* substituted analogues appear to exert interesting agonistic activity. Thus, 2-(3-bromophenyl)histamine (**2**) and 2-(3-trifluoromethylphenyl)-histamine (**3**) are relatively potent H_1 agonists in the guinea-pig ileum. In fact, these compounds are very useful tools to study the pharmacology of the H_1 receptor. Their agonistic activities measured on guinea-pig ileum are somewhat higher than that of histamine [13].



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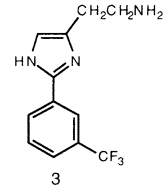

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Table I. Functional receptor activation (pD_2) by compounds **2** and **3**.

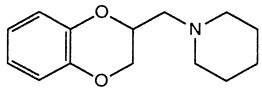
Compound	Receptor type						
	H_1	H_2	H_3	M_3	α_1	β_1	$5HT_{1A}$
2	6.75	3.37	< 5.0	3.97	5.25	4.63	5.01
3	6.81	3.48	< 5.0	3.81	4.79	3.96	4.96

The affinity of these compounds has been confirmed by in vitro binding studies. Histamine inhibited [^{3}H]mepyramine binding to H_1 receptors from guinea-pig cerebellar membranes with a K_i value of 39 μM. Compounds **2** and **3** are approximately 40 times more potent than histamine in this test, with K_i = 0.8 and 1.2 μM, respectively. Furthermore, it appeared that they are rather selective for the histamine H_1 receptor, which may be illustrated by the receptor activation profile that has been composed, including, besides the H_1 receptor, the H_2, H_3, and also the muscarinic M_3, the adrenergic α_1 and β_1 and serotonergic $5HT_{1A}$ receptors (*table I*).

From these results it is clear that these 3-Br and 3-CF_3 derivatives show substantial functional receptor selectivity with ratios in the range of 32–2 400. Considerably higher ratios are found between the H_1 and H_2 activity [13]. However, it is interesting to note that Leurs et al. [14] provided evidence that histamine and 2-phenylhistamine analogues bind in different ways to the H_1 receptor. Besides, it has been reported that a number of 2-substituted histamines, including compound **2**, cause a direct activation of the family of the G_i-proteins [15].

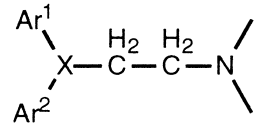
2.2. H_1 antagonists

It was about 60 years ago that the first histamine H_1 antagonists were discovered. Since that time many compounds belonging to different structural classes have become known with high antagonist activity at this receptor. Several reviews on this topic have been published [2–4, 16–18].



piperoxan

The first H_1 antagonist was described in 1937. Piperoxan and related ethers were reported to protect guinea-pigs against lethal doses of histamine. From these early studies until now a large number of H_1 antagonists have been identified. Most of these so-called classical H_1 antagonists can be depicted by the general structure **4** [19].



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Ar^1 = (subst.) phenyl or heteroaryl (preferably 2-pyridyl).

Ar^2 = phenyl or (subst.) phenylmethyl.

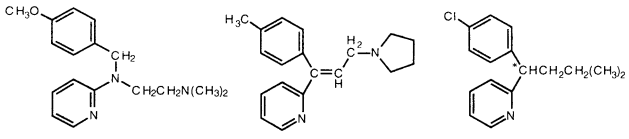
X = CH, N, CH–O or X–CH_2 replaced by CH=CH.

N = tert. amino, (preferably dimethylamino or pyrrolidino).

Therapeutic application of classical H_1 antagonists has been the symptomatic treatment of several allergic conditions, as histamine release from mast cells is induced in those cases. These compounds are usually inactive in bronchial asthma, but can be successfully used in allergic rhinitis, conjunctivitis and dermatitis. A major disadvantage of the classical H_1 antagonists is that they often produce sedation at therapeutic doses. This effect is caused by H_1-receptor blockade in the CNS, as it became clear that H_1 receptors are involved in the regulation of sleep and wakefulness [20] and because H_1 antagonists that do not reach the CNS are devoid of these side effects (see further). Other side effects are especially caused by the anticholinergic activity of some compounds [16].

During the last decades modern H_1 antagonists have been developed with negligible or only moderate affinity for other receptors, like the cholinergic and serotonergic receptors. A major breakthrough achieved with the development of non-sedating H_1-antagonists, which appeared to have a strongly reduced capacity in entering the CNS, e.g. astemizole, cetirizine, terfenadine and loratadine [2] (and refs cited there).

Although many potent and selective H_1 antagonists are available to study the H_1 receptor, one should in all cases be aware of possible muscarinic and serotonergic antagonistic properties and local anaesthetic effects of many classical H_1 antagonists. Currently mepyramine, triprolidine and, to a lesser extent, chlorpheniramine are mainly used as H_1 antagonists in pharmacological studies.



2.2.1. Triprolidine

Triprolidine is a particularly useful tool for the classification of H_1 receptors because there is also a geometric isomer. The E-isomer, triprolidine, is 1 170 times more potent than the corresponding Z-isomer, with pK_d values of 9.9 and 6.9, respectively, measured at histamine receptors in the guinea-pig ileum [21].

2.2.2. Mepyramine

The commonly used reference compound for studying the affinity of ligands to the H_1 receptor is undoubtedly mepyramine with its high affinity (pK_d = 9.4) [21] and good receptor selectivity. Its competitive nature has been confirmed by Schild analysis (slope = 1.0) in both guinea-pig ileum and cerebellar brain slices [22]. Fortunately, mepyramine is rather selective if used at concentrations below 100 nM. Above this level interactions with receptors other than H_1 cannot be excluded. Thus, responses on muscarinic receptors (> 10 μM), on H_2 receptors (> 5 μM) and on monoamine reuptake (> 1 μM) may occur [22].

2.2.3. (S)-Chlorpheniramine

Some chiral antihistamine drugs show pronounced stereoselective activity. Thus the (S)-chlorpheniramine (pA_2 = 9.30 in guinea-pig ileum) is much more potent than its (R)-enantiomer (pA_2 = 7.84 in guinea-pig ileum). (S)-Chlorpheniramine has been reported to be 200 times more effective than its enantiomer in protecting guinea-pigs against histamine in vivo. Likewise, ratios of 100 have been observed in competitive binding studies in brain homogenates [3].

2.3. Radiolabelled H_1 antagonists

2.3.1. [^{3}H]-Mepyramine

The knowledge about the localisation, distribution and biochemical properties of the H_1 receptor have been enhanced tremendously by the development of radioactive probes for the receptor site. The first selective radioligand for the H_1 receptor, [^{3}H]mepyramine, was introduced in 1977 [23]. The [^{3}H]mepyramine binding in homogenates of the longitudinal smooth muscle showed a saturable specific binding and the dissociation constants determined for several H_1 antagonists, including both stereoisomers of chlorpheniramine, agreed with those obtained with functional experiments [24]. Since then H_1 receptor binding sites have been demonstrated using [^{3}H]mepyramine in many tissues, amongst them mammalian brain [25], airways [26], intestines [24], genitourinary tract [27] and vascular system [28, 29]. However, it has also been shown that significant species differences can occur. The dissociation constant for mepyramine binding in rat brain was found to be 9.1 nM, which was higher than 0.83 nM found in an equivalent experiment with guinea-pig brain. Also, the regional distribution of [^{3}H]mepyramine binding in rat brain was not the same as that in guinea-pig brain, suggesting a different distribution of H_1 receptors [25]. Also, for studying the cerebellar H_1 receptor distribution in mice, [^{3}H]mepyramine has been shown to be a valuable tool [30].

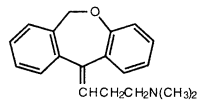
The binding of [^{3}H]mepyramine to H_1 receptors expressed in HeLa cells has been studied as well. The dissociation constants of mepyramine and (S)-chlorpheniramine were similar for binding to H_1 receptors in other mammalian tissues [31].

The nature of the binding of [^{3}H]mepyramine to homogenates of guinea-pig cerebral cortex has been studied in detail and, as a result, a two site binding model was proposed. The dissociation constants of the high affinity site appear in good agreement with those obtained from inhibition of histamine induced responses [32].

It should be noted, however, that in some tissues, e.g. rat liver, [^{3}H]mepyramine additionally binds to non-H_1 receptor sites. This was based on the fact that a relatively low affinity for the H_1 antagonists *trans*-triprolidine, d- and l-pheniramine and a reversed stereoselectivity of the binding site to (S)- and (R)-chlorpheniramine was observed [33]. It is suggested that this binding site is actually the enzyme debrisoquine 4-hydroxylase [34].

2.3.2. [^{3}H]-Doxepin

Besides [^{3}H]mepyramine, several other radioactive ligands have been used to label H_1 receptors. However, only a few can be applied without complications [35]. In most cases these ligands are not selective for the H_1 receptor and additional precautions have to be taken in order to avoid undesired labelling of other receptors. Thus, the use of the tricyclic antidepressant [^{3}H]doxepin (a 15:85 mixture of the Z and E isomers, with the Z isomer being 3.5 times more potent than the E isomer on the guinea-pig ileum [19]) for labelling the H_1 receptor is not widely accepted, although it is one of the most potent H_1 antagonists with a pK_d = 10.4 (guinea-pig instestinal smooth muscle) and 10.1 (guinea-pig cerebellar homogenate) and Hill coefficients did not differ from unity [36].



doxepin

[^{3}H]doxepin binds to brain homogenates from rats and guinea-pigs involving two saturable sites. The high affinity site with a pK_d of 10.4 is associated with histamine H_1 receptors. This high affinity binding shows stereospecificity in that (S)-chlorpheniramine is 100 times more potent than its (R)-enantiomer. Also, its regional variation in binding closely parallels that of [^{3}H]mepyramine. The drug specificity of the low affinity site is distinct from that of histamine H_1 receptors with no stereospecificity of the chlorpheniramine enantiomers. Furthermore several H_1 antagonists display nanomolar potency at the high affinity site and weak affinity at the low affinity site [37]. In rat brain it was found that various tricyclic antidepressants were potent inhibitors at the high affinity [^{3}H]doxepin site. Their potencies, however, did not correlate with their potencies previously reported for the H_1 receptor [38].

Comparable results were obtained with [^{3}H]doxepin in human brain [39].

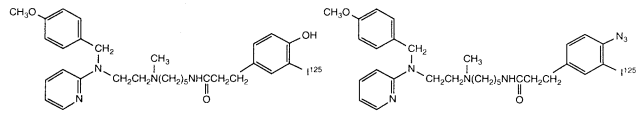
2.3.3. Ligands for PET studies

As might be expected from its antidepressant activity, [^{3}H]doxepin is not selective for the H_1 receptor, it also has affinity for the histamine H_2 and muscarinic receptors [40]. Still, [^{11}C]doxepin has been used to visualize H_1 receptors in human brain in vivo by positron emission tomography (PET). It was found that in healthy young men H_1 receptor occupancy could be measured, since (S)-chlorpheniramine (i.v.) almost completely (98%) abolished the binding of [^{11}C]doxepin. The nonsedative H_1 antagonist terfenadine on the other hand, occupied only 17% of the available H_1 receptor in the frontal human cortex after a single oral dose [41].

The other H_1 antagonist used in PET studies is [^{11}C]mepyramine. Its application for mapping the functional H_1 receptors in the brain has been reported for guinea-pigs [42] and dogs [43]. Moreover, Yanai et al. [44] demonstrated the specific [^{11}C]mepyramine binding in the brain of human volunteers. It was found that the regional distribution of [^{11}C]mepyramine coincided well with that determined, in vitro, in autopsied material and that its binding was blocked by pretreatment with (S)-chlorpheniramine. Such PET studies are useful to measure H_1 occupancy with the classical and new antihistamines to predict their sedative side effect. Furthermore, it is possible to determine alterations in the distribution of H_1 receptors in the human brain in diseases in which histaminergic transmission is changed [44].

2.3.4. [^{125}I]Iodobolpyramine

Thus far, besides [^{11}C] ligands for PET studies, only [^{3}H] labelled ligands have been discussed and in general they are useful tools to study H_1 receptors. However, high affinity [^{125}I] labelled ligands have some technical advantages, since they can be obtained with a 50–100 higher specific radioactivity and thereby provide increased sensitivity for receptor assays. For that reason Korner et al. [45] developed [^{125}I]iodobolpyramine as a radiolabelled ligand for the H_1 receptor. In fact iodobolpyramine is an analogue of mepyramine in which the aliphatic dimethylaminoethyl chain has been extended with the iodine containing moiety.



[^{125}I]-iodobolpyramine [^{125}I]-iodoazidophenpyramine

It was found that in guinea-pigs, [^{125}I]iodobolpyramine uniformly labels a single population of sites in cerebellar membranes. These sites correspond to H_1 receptors, as was shown by additional pharmacological experiments. Binding of [^{125}I]iodobolpyramine to cerebellar membranes of guinea-pigs occurred slowly, the equilibrium was reached only after 3 h at 25 °C and after 5 h at 20 °C, with an observed pKd of 9.8 [45]. [^{125}I]Iodobolpyramine has been used for mapping of the H_1 receptors in the guinea-pig brain [46] and in the human brain [47]. Besides that, [^{125}I]iodobolpyramine shows remarkable species differences. Whereas H_1 receptors in guinea-pig brain are labelled with high affinity, in rat brain tissue no specific labelling could be established, despite the moderate differences in affinity of [^{3}H]mepyramine for guinea-pig and rat brain H_1 receptors [25]. Moreover, on human lymphocytes [^{125}I]iodobolpyramine binds to a site which does not show the expected affinity for (S)-chlorpheniramine [48]. In view of these data, [^{125}I]iodobolpyramine may be used as a general radioligand for the H_1 receptor, however, one should always be aware of the occurrence of artifacts.

2.3.5. [^{125}I]Iodoazidophenpyramine

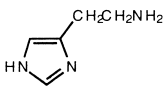
For irreversible binding to receptors, the technique of photoaffinity labelling with an azide upon UV irradiation, resulting in blockade of the involved receptor, is well

known. Major progress was made by the development of a radioactive photoaffinity label for the H_1 receptor. The compound is in fact an azido derivative of [^{125}I]iodobolpyramine and is called [^{125}I]iodoazidophenpyramine ([^{125}I]APP) [49]. Using guinea-pig brain in the dark, reversible binding of [^{125}I]APP to cerebellar membranes occurred with a pK_d of 10.9 and was inhibited by various H_1 antagonists with the expected potencies. Upon UV irradiation, 5% of the reversibly bound radioactivity became bound covalently to cerebellar membrane polypeptides, which served as a marker for further research on the structure elucidation of the H_1 receptor [49].

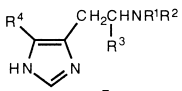
2.4. H_2 agonists

2.4.1. Histamine analogues

Although several attempts have been made, simple modifications of the histamine molecule did not achieve potent and selective agonists for the H_2 receptor [50]. The most favourable results are obtained with introduction of a methyl or ethyl group in position 5 of the imidazole ring (**5**, $R^1 = R^2 = R^3 = H$, $R^4 = CH_3$ or C_2H_5); the relative H_2 agonistic activity (histamine = 100) decreased to 71 and 53, respectively, whereas their H_1 agonistic activity is substantially lower, being less than 1% of histamine [2]. As no other selective H_2 agonists were available for many years, 5-methylhistamine has been used intensively to study the interactions with H_2 receptors (pD_2 = 5.8 at guinea-pig right atria; histamine pD_2 = 6.0); its pD_2 at the H_1 receptor is 4.8 (guinea-pig ileum) [51].



histamine



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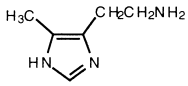
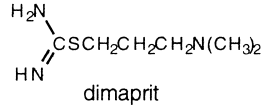


5-methylhistamine

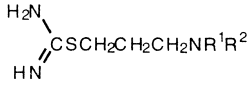
Mono- or dimethylation of the amino group in histamine (**5**, $R^1 = CH_3$, $R^2 = R^3 = R^4 = H$ or $R^1 = R^2 = CH_3$, $R^3 = R^4 = H$) is tolerated, but due to the affinity to the H_1 and H_3 receptor of these compounds these modifications do not lead to selective agents. Introduction of higher alkyl groups for R^1 and/or R^2 causes a substantial reduction of the H_2 activity. Alkylation in other positions of the imidazole ring of the histamine molecule has shown to have a strong negative effect on H_2 agonism [50]. Methylation of the ethylene side chain also causes a strong decrease in H_2 activity. Remarkably, after chloromethylation (**5**, $R^3 = CH_2Cl$, $R^1 = R^2 = R^4 = H$) only a moderate decrease in activity was observed with interesting stereoselectivity [52].

2.4.2. Dimaprit

The first selective H_2 agonist was found in a search for H_2 antagonists in a series of isothiourea derivatives. It appeared that the S-(dimethylaminopropyl)isothiourea (dimaprit) was a highly selective H_2 agonist with virtually no affinity for the H_1 receptor [53]; later it was found that the compound had a considerable H_3 antagonistic effect (see section on H_3).



dimaprit



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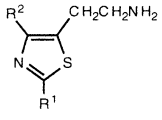
Several modifications of the dimaprit structure have been made in order to evaluate the structure-activity relationships in this type of compound. It appeared that variation of the length of the propylene chain is deleterious to activity. Hence it has to be concluded that the chain length of dimaprit is optimal for H_2 activity. N-demethylation (**6**, $R^1 = R^2 = H$) causes a negative effect on activity as well, which might rather be ascribed to its tendency to cyclise followed by conversion into a guanidine than to lack of affinity, since, notwithstanding this instability, H_2 agonistic activity could be demonstrated [54]. Active compounds have also been found with higher alkyl groups (**6**, R^1 and/or R^2 = higher alkyl), but potencies are low [55]. Replacement of the dimethylamino group by cyclic amines (e.g. NR^1R^2 = pyrrolidino, piperidino, morpholino) renders the compound inactive in stimulating cat gastric secretion [56]. Substitution of the isothiourea part is extremely unfavourable for activity. Even introduction of a small methyl group causes a complete loss of activity [57].

Dimaprit itself has been shown, in vitro, to have 17.5% of the potency of histamine on the rat uterus and 71% on the guinea-pig right atrium. Furthermore it was found to stimulate gastric acid secretion in the rat, dog and cat with approximately 19%, 58% and 400–500%, respectively, of the potency of histamine [4]. Later, the following activites for dimaprit were reported: guinea-pig right atrium, pD_2 = 5.8 (α = 1.0), guinea-pig gastric secretion, pD_2 = 5.3 (α = 1.0) and guinea-pig cortex, pK_d = 4.3 (displacement of [^{3}H]tiotidine) [58]. As already mentioned, dimaprit has a very low activity at the guinea-pig ileum, i.e.

only 0.001% of the activity of histamine. The separation of activity between H_2 and H_1 receptor stimulation is thus greater than 10^5 [4]. However, studies with dimaprit concerning H_3 activity demonstrated its affinity, although no stimulation was observed. In fact, dimaprit is a moderately potent H_3 antagonist with pA_2 = 5.8, measured on rat cortex [59]. In the case of receptor stimulation, dimaprit is a selective agent for the H_2 receptor, but if affinity is concerned binding to the H_3 receptor should also be taken into account.

2.4.3. Amthamine

Over the past decades much attention has been paid to the activation mechanism of the H_2 receptor. The first model, postulated by Weinstein et al. [60], was based on a tautomeric shift of a proton of the amidine system in the imidazole ring of histamine, which was thought to trigger the H_2 receptor. A comparable 1,3-prototropic shift is also possible in the isothiourea part of dimaprit. However, for sterical reasons, such a receptor interaction has to be considered as unlikely. Therefore, we proposed a modified mechanism for interaction [61] in which, instead of two N-atoms, one N-atom and the S-atom of the isothiourea group were involved. In this model the pKa of the ring system governs the affinity to the H_2 receptor. Recently, theoretical calculations strongly supported this mechanism [62]. Considering the structural requirements in view of the new mechanism led to the development of a series of 5-aminoethyl-1,3-thiazoles (**7**).



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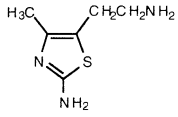
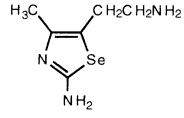


amthamine



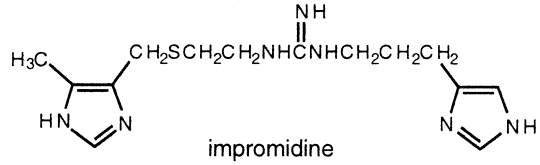
amselamine

The resemblance of **7** (R^1 = R^2 = H) with histamine is obvious, whereas **7** (R^1 = NH_2, R^2 = H) may be regarded as a cyclised dimaprit analogue. Structure–activity studies showed that affinity to the H_2 receptor is mainly governed by the basicity of the heterocyclic ring, i.e. the thiazole ring; consequently electron donating groups for R^1 and R^2 are favourable for activity. In the series described (**7**, R^1 = H, CH_3, NH_2 and R^2 = H, CH_3) amthamine is the most potent, with a pD_2 = 6.21 (guinea-pig right atrium). Based on calculations performed with the model of Eriks, the S-atom in amthamine was replaced by the Se-atom giving amselamine. Indeed amselamine proved to be a more potent H_2 agonist, with a pD_2 = 6.41 (guinea-pig right atrium), than amthamine. This compound is a potent and selective H_2 agonist, since activities on the H_1 and H_3 receptor are low with a pA_2 = 3.85 and a pD_2 = 4.44, respectively [63].

During the last decade the pharmacology of amthamine has been evaluated in more detail. In displacement studies with [^{3}H]tiotidine a pK_d of 5.30 was found for the binding to guinea-pig cerebral cortex membranes [64]. Coruzzi et al. [65] confirmed the stimulatory action of amthamine on gastric acid secretion in conscious cats (ED_{50} = 0.0069 μmol/kg/h) and in anaesthetized rats (ED_{50} = 11.69 μmol/kg, i.v.). In the last model the efficacy of amthamine was significantly higher than that of histamine and dimaprit. Amthamine is also an effective secretagogue in the isolated gastric fundus, behaving as a full agonist (EC_{50} = 18.9 μmol/L). The effect of amthamine on various cardiovascular preparations may be summarized as follows: pD_2 = 6.72 (guinea-pig right atrium), pD_2 = 6.17 (guinea-pig pappillary muscle), pD_2 = 5.38 (human atrium). In all these systems amthamine behaves as a full agonist equipotent to, or slightly more active than, histamine [66]. Further studies on cardiovascular effects of amthamine in the rat have established that it is a potent and selective H_2 agonist in the dose range of 0.01–3 μmol/kg, i.v. Higher doses, however, evoked effects which are caused by activation of adrenergic mechanisms [67].

Since amthamine, up to concentrations of 10^{-4} mol/L, did not show any significant functional effect on the H_1 receptor, and as a pD_2 of 4.70 for stimulation of the H_3 receptor is reported [63], it can be concluded that amthamine is a potent and selective H_2 agonist and as such a valuable tool to study the histaminergic pathways in which H_2 receptors are involved.

2.4.4. Impromidine



impromidine

Another important highly potent H_2 agonist is impromidine. It was found to be 48 times more potent than histamine on the spontaneously beating guinea-pig right atrium, having a pD_2 = 7.8 [58, 68]. On the uterus it behaves as a partial agonist (α = 0.8) with 9.3 times the potency of histamine [69]. Also, on the guinea-pig pap-

pilary muscle, impromidine is reported to be a partial agonist (α = 0.8) with a pD_2 = 7.65 [68]. Further, it is a potent stimulant of gastric acid secretion with 17–27 times the potency of histamine in the rat, cat and dog preparations when given i.v. [4], whereas in the guinea-pig a pD_2 of 8.4 was found [58]. Also, in man, impromidine has been investigated for its effect on gastric acid secretion. It was found that impromidine produced a dose-dependent increase in acid output similar to histamine, and there was a competitive antagonism by tiotidine and cimetidine. In man the ED_{50} was calculated to be approximately 1.1×10^{-8} mol/kg/h [70]. Furthermore, it was shown that impromidine has a positive inotropic effect on the human ventricle mediated via histamine H_2 receptors. In this model impromidine appeared to be a partial agonist compared to histamine. These studies provided further evidence for the potential clinical use of impromidine in severe cardiac failure [71]. Thus it was suggested that treatment of certain types of cardiac failure in combination with catecholamine-induced damage of sarcolemnal β-receptors with impromidine, together with β-adrenergic antagonists, may have a beneficial effect [72].

Large numbers of impromidine related compounds have been prepared and evaluated for histaminergic activity. These investigations revealed that the guanidinopropylimidazole part mimics histamine in triggering the H_2 receptor. Moreover, it was found that the guanidine moiety must be capable of binding a proton at physiological pH and that the imidazolylmethylthio part may be replaced by various (di)arylmethyl groups [50]. In accordance with these findings its amthamine analogue (thiapromidine) was found to be a potent H_2 agonist as well (pD_2 = 7.90, guinea-pig right atrium) [73]. From other studies it has been shown that impromidine is a moderate antagonist for the H_1 receptor with a pD_2 = 5.5 [68, 74] and a relatively potent antagonist for the H_3 receptor with a pD_2 = 7.3 [1], making it a non-selective compound for histamine receptors as far as affinity is concerned.

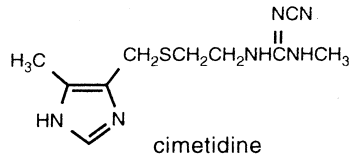
2.5. H_2 antagonists

In 1972 a new class of histamine antagonists were reported which were capable of antagonizing the histamine induced gastric acid secretion and having no effect on the, until then well-known, (H_1) histamine receptors. From that time on we are familiar with histamine H_1 receptors located in, e.g. ileum and trachea, and H_2 receptors located in e.g. right atrium, stomach and uterus. The discovery of the H_2 antagonists was initiated with the modification of the side chain of the histamine molecule. The guanidinoethylimidazole (often referred to as guanylhistamine) appeared to be a partial H_2 agonist, thereby possessing some H_2 antagonistic properties [50]. Since the seventies many research groups have put effort into the development of H_2 antagonists, eventually leading to a thousand compounds described in the literature [50].

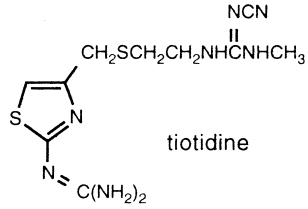
Based on their chemical structure, at least six classes of H_2 antagonists can be distinguished. Representitives of each class are depicted below.

2.5.1. Cimetidine

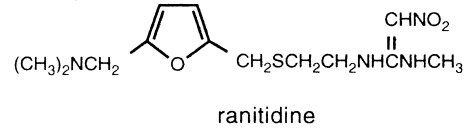
The first H_2 antagonist in clinical use for the treatment of gastric ulcers was cimetidine [75]. It has a pA_2 value of 6.1 (guinea-pig right atrium) and its ID_{50} in inhibiting the rat gastric acid secretion is 1.4 μmol/kg [75]. Cimetidine has been used for several years as a reference compound in studies on interactions with the histamine H_2 receptor. It has been shown that cimetidine has virtually no affinity for the H_1 receptor (pK_d = 3.3) [75] and has a weak affinity for H_3 receptors with pK_d =



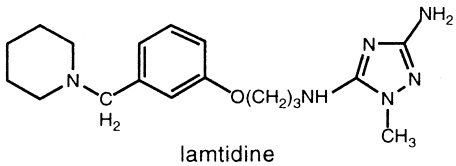
cimetidine



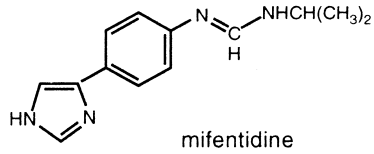
tiotidine



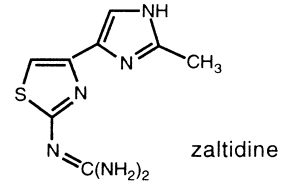
ranitidine



lamtidine



mifentidine



zaltidine

4.5 [76]. However, during the last decade it has been replaced by tiotidine and ranitidine that are more potent and selective for the H_2 receptor.

Recently it was reported that in Chinese hamster ovary (CHO) cells, wild type histamine H_2 receptors are up-regulated by long term exposure to cimetidine [77]. It was concluded that the effect is H_2 receptor mediated, since H_1 and H_3 antagonists, and an analogue closely related to cimetidine devoid of H_2 activity, did not affect H_2 receptor expression. In the same study it was shown that the H_2 receptor displays, in the absence of agonists, a basal activity measured as cAMP production. Treatment of these CHO cells with cimetidine resulted in a decrease of the basal cAMP level, indicating a negative intrinsic activity or inverse agonism. In these experiments ranitidine also acted as an inverse agonist, whereas burimamide did not alter the basal cAMP level, thereby classifying itself as a neutral antagonist. The observed inverse agonism is suggested to be the mechanistic basis for the H_2 receptor upregulation by cimetidine. In line with this hypothesis is the observation that the neutral antagonist burimamide does not cause upregulation of the H_2 receptor [77].

2.5.2. Tiotidine

Tiotidine may be regarded as an analogue of cimetidine in which the imidazole ring has been replaced by a guanidinothiazole moiety. Physicochemical studies indicate, both in crystal form and in solution, a cyclic hydrogen-bonded structure with a hydrogen bond between a guanidino NH_2 group and the thiazole N-atom. Notwithstanding the fact that guanidines are generally very basic compounds, the pK_a of this substituted guanidine has been reported to be as low as 7.05, which is close to the value of cimetidine. Structure–activity relationships of tiotidine analogues were described by Gilman et al. [78]. Contrary to what has been found with cimetidine, replacement of the S-atom in the side chain does not result in a decrease in activity. Moreover, introduction of a 5-methyl group, which is favourable in the cimetidine series, causes a drop in activity in the tiotidine series. Therefore different binding sites at the H_2 receptor for cimetidine and tiotidine are likely. Also, potent tiotidine analogues are obtained on replacement of the cyanoguanidine part with thiourea or nitroethenediamine moieties; pA_2 values of 7.9 and 8.2, respectively, have been observed [78]. Tiotidine itself is a potent H_2 antagonist with reported pA_2 values of 7.3–7.8 (guinea-pig right atrium) [50]. Its low affinity for the H_1 receptor ($pA_2 <$ 4.5) and the H_3 receptor ($pA_2 = 4.8$) [22] makes it a selective tool to establish interactions with the H_2 receptor. Although the H_2 antagonistic activity of tiotidine would justify clinical use, it has not reached the market because of serious side effects, including parietal cell atrophy [79].

2.5.3. Ranitidine

The discovery of ranitidine demonstrated that a nitrogen heterocycle is not a necessary structural feature for an H_2 antagonist, but that the N-atom may be incorporated into the substituent of the aromatic ring. In ranitidine the methylimidazole ring of cimetidine has been replaced by the dimethylaminomethylfuranyl part, while the cyanoguanidine is replaced by the nitroethenediamine group. Ranitidine is more potent than cimetidine, with reported pA_2 values ranging from 6.7–7.3 [50], albeit that a pA_2 value of 7.2 is mentioned frequently [80, 81]. Ranitidine is a potent drug to inhibit gastric acid secretion. Thus ID_{50}'s of the inhibition of the histamine-stimulated gastric acid secretion in the rat of 0.13 mg/kg (i.v.) [82] and 0.6 μmol/kg (i.v.) [83] have been established. Nowadays it is a frequently prescribed drug for the treatment of gastric ulcers.

Structural modifications revealed that the dimethylamino group can be replaced by secondary amino functions without affecting the activity. However, replacement with a pyrrolidino group causes a marked decrease in activity. Since the trifluoroethylamino derivative, being less basic than ranitidine, is still a potent H_2 antagonist, it was suggested that the species interacting with the H_2 receptor is, like cimetidine, the non-protonated form [84]. Structure–activity patterns amongst ranitidine analogues, however, appear to differ from those in the cimetidine series. These observations suggest that the furan and imidazole are not behaving as simple bio-isosteres [3]. Ranitidine has, if any, a very low affinity to H_1 receptors with a $pKd < 4$ [85]. Regarding the affinity to the H_3 receptor a $pKd < 5.9$ has been mentioned [1].

2.6. Radiolabelled H_2 antagonists

2.6.1. [^{3}H]Cimetidine

The first radioligand developed as a probe for the H_2 receptor was [^{3}H]cimetidine [86]. Early studies in both guinea-pig cerebral cortical membranes and membranes from various rat brain areas [22] demonstrated a saturable binding that could be displaced by burimamide, metiamide or cimetidine, with dissociation constants that correlate well with those found on the isolated guinea-pig atrium. However, it is now clear that the binding site labelled by [^{3}H]cimetidine represents some imidazole recognition site other than the H_2 receptor. Potent non-imidazole H_2 antagonists like tiotidine and ranitidine appeared to be inactive in displacing [^{3}H]cimetidine, whereas imidazole containing compounds lacking any H_2

affinity were found to be effective in displacing [^{3}H]cimetidine [87, 88]. Thus, it is now widely accepted that [^{3}H]cimetidine binds to a non-H_2 receptor imidazole recognition site [35]. Also, tritiated forms of metiamide, impromidine and ranitidine have been used in an attempt to label H_2 receptors, but appeared to be unsuitable [35].

2.6.2. [^{3}H]Tiotidine

Tritiated tiotidine was the first radiolabel that was used successfully for labelling H_2 receptors in various tissues. In guinea-pig cerebral cortical membranes the saturable binding of [^{3}H]tiotidine (pK_i = 7.5) can be displaced by a wide range of H_2 receptor antagonists of diverse chemical structure, with potencies that correlate well with their ability to inhibit H_2 effects in the guinea-pig right atrium and gastric mucosa [89, 90]. It should be noted that earlier studies in rat and guinea-pig hippocampus were not successful [91]. Furthermore, it has been noticed that for labelling brain H_2 receptors with [^{3}H]tiotidine, the composition of the incubation medium is very important [58]. Although several examples described that H_2 receptor binding can be measured in brain tissue, the very high level of nonspecific binding may be regarded as a drawback in its use. In several peripheral tissues it has even been impossible to label the H_2 receptor with [^{3}H]tiotidine [90], although in guinea-pig lung parenchymal tissue [92] and guinea-pig and rabbit left atria [93, 94], labelling with [^{3}H]tiotidine was successful. Moreover, binding of [^{3}H]tiotidine to gastric mucosal cells revealed the presence of several binding sites which may not be related to the histamine H_2 receptor [95]. Also, in kidney membranes, [^{3}H]tiotidine seems to label non-H_2 receptor sites [90]. Therefore, it may be concluded that the use of [^{3}H]tiotidine for labelling peripheral H_2 receptors is rather limited.

2.6.3. [^{125}I]Iodoamino/azidopotentidine

A high affinity iodoligand, [^{125}I]iodoaminopotentidine, has subsequently been devised and used successfully to reveal H_2 receptor distribution in brain tissue. This ligand binds with high affinity (pK_d = 9.5) to H_2 receptors in guinea-pig, primate and human brain [47, 96, 97]. Compared with [^{3}H]tiotidine, [^{125}I]iodoaminopotentidine is more sensitive and shows a considerably lower amount of nonspecific binding in brain tissue. Additionally, this ligand showed promising results in autoradiographic studies in both rodent, primate and human brain [47, 96, 97]. Interestingly, an azido analogue of [^{125}I]iodoaminopotentidine, viz. [^{125}I]iodoazidopotentidine, has been developed to label the H_2 receptor irreversibly [96]. These [^{125}I] radiolabelled H_2 antagonists appear to be promising tools to study H_2 receptors, since they are available with high specific activity and show thusfar relatively low nonspecific binding.

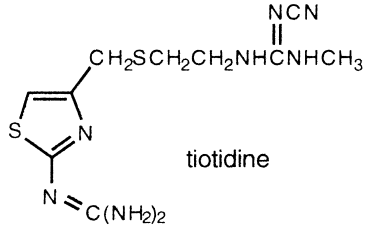
2.7. Histaminergic H_3 ligands

2.7.1. H_3 agonists

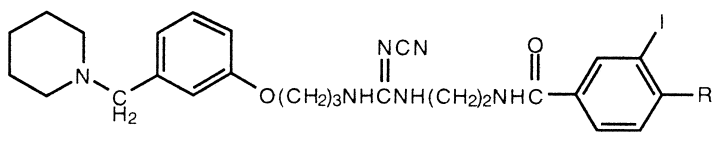
The histamine H_3 receptor was discovered as a presynaptic receptor localised on histaminergic nerve terminals in the central nervous system. Its stimulation decreases the release and synthesis of histamine [98, 99]. Recent studies revealed that H_3 receptors also modulate the release of several other neurotransmitters both in the CNS and in the gastrointestinal tract [100–103].

2.7.2. Histamine analogues

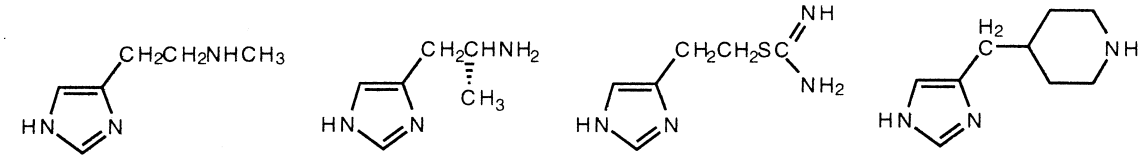
Potent H_3 agonists are obtained by simple modifications of the histamine molecule. It was found that the imidazole ring is a common structural feature in almost all H_3 agonists. Methylation of the aminoethyl sidechain of histamine can be very favourable for H_3 activity. Thus, introduction of one or two methyl groups to give N^{α}-methylhistamine and N^{α},N^{α}-dimethylhistamine affords potent H_3 agonists, but they show little selectivity between the three histamine receptors [1]. Also, introduction of a methyl group in α-position substantially increased the potency at the H_3 receptor [104]. The increased activity has to be ascribed almost completely to its R-isomer ((R)α-methylhistamine with a pD_2 = 8.4 (K^+-stimulated histamine release on rat cortex) [104] and pD_2 = 7.8 (electrically evoked cholinergic contractions of guinea pig intestine)) [105]. Moreover, it was reported that (R)α-methylhistamine has only weak affinity to the



tiotidine



iodoaminopotentidine (R = NH_2)
iodoazidopotentidine (R = N_3)



N^{α}-methylhistamine (R)α-methylhistamine imetit immepip

H_1 receptor (0.5% of histamine) and the H_2 receptor (1% of histamine) making it a selective ligand for the H_3 receptor [4]. Hence, in combination with its less active enantiomer, (S)α-methylhistamine with a pD_2 = 6.3 (K^+-stimulated histamine release on rat cortex) [104], it is a very useful tool for the pharmacological characterization of H_3 receptor mediated responses.

2.7.3. Imetit

Investigations for H_3 agonists that are structurally less related to histamine have led to the discovery of imetit. It is in fact a histamine analogue in which the amino group has been replaced by the isothiourea moiety. Imetit is a potent and selective agonist of the H_3 receptor with a pD_2 = 9.0 (K^+-stimulated histamine release on rat cortex) and a pD_2 = 8.1 (electrically evoked cholinergic contractions of guinea-pig intestine) [106, 107]. Several analogues of imetit have been prepared. It has been postulated that the S-atom in imetit is not involved in binding to the H_3 receptor, since replacement of the S-atom by the apolar CH_2 group giving the corresponding amidine does not cause a decrease in activity [108]. Replacement of the isothiourea group by the more basic guanidine group results in a drastic decrease in activity. However, since imetit has been shown to be devoid of appreciable H_1 activity (pD_2 = 5.0) and H_2 activity (pD_2 = 3.9) [59], it is a valuable tool in pharmacological studies. Moreover, imetit is also active at low doses in vivo [107], which makes it an important drug for in vivo studies as well.

2.7.4. Immepip

A third type of H_3 agonist that was developed recently is immepip. Its structure is obviously related to histamine, it may be considered as an analogue with an elongated and cyclised side chain. Immepip has been found to be a highly effective H_3 agonist in the guinea-pig intestine with a pD_2 = 8.0, whereas for (R)α-methylhistamine, 7.8 was found [109]. Since for the H_1 and H_2 receptor only weak affinities were observed with a pK_i = 4.8 and < 3.5, repectively, it is a highly selective ligand to study interactions at the H_3 receptor [110]. In in vivo experiments in the rat, immepip produced only minimal changes in basal arterial pressure, whereas (R)α-methylhistamine gave, at higher doses, a probably adrenergic induced increase in blood pressure and immetit a serotonergic (5-HT_3)-induced decrease in blood pressure [111]. These results emphasize the in vivo selectivity of immepip for the H_3 receptor. Additionally immepip may serve as an interesting compound for modelling the H_3 receptor because of its remarkable structural deviation in relation to the other H_3 agonists.

2.8. Radiolabelled H_3 agonists

2.8.1. [^{3}H]Methylhistamines

With the introduction of (R)α-methylhistamine as a selective ligand for the H_3 receptor, Arrang et al. [104] also presented the tritiated compound as a radioligand for the H_3 receptor. Binding of [^{3}H](R)α-methylhistamine to membranes of the rat cerebral cortex was reversible and saturable with a pK_d = 9.5 [112]. The affinity of several H_3 antagonists obtained from displacement studies with [^{3}H](R)α-methylhistamine correspond rather well with their potency found in the histamine release assay. Agonists, on the other hand, show a 5–10-fold higher affinity in the binding assay compared to the histamine release assay. This last observation might be explained by the selective labelling of the high affinity state of the G-protein-coupled receptor [112].

Also, N^{α}-methylhistamine has been evaluated as a radiolabelled probe for the H_3 receptor. Since this ligand has a higher specific activity it provides a higher sensitivity than [^{3}H](R)α-methylhistamine and might therefore have advantages. Competition binding studies revealed that [^{3}H]N^{α}-methylhistamine labels H_3 receptors both in guinea-pig brain [113] and rat brain [114] with a pK_d = 9.4. Kinetic analysis of displacement curves indicate that the binding characteristics of these labelled agonists are very complex. It has been found that, e.g., binding of [^{3}H](R)α-methylhistamine to the low affinity site is inhibited by guanine nucleotides instead of binding to the high affinity site [112]. Nevertheless, the use of these radiolabelled H_3 agonists has provided valuable information on the location of H_3 receptors in the

CNS [113, 115]. Also in peripheral tissue, like guinea-pig lung, pancreas and intestine, the presence of H_3 receptors was established with these radiolabelled agonists [112, 113]. Furthermore [^{3}H](R)α-methylhistamine has been used for autoradiographic studies in guinea-pig lung and the brain of various species [47, 116, 117]. Notwithstanding their successful use in receptor studies, especially due to their high selectivity and high specific binding, these ligands have, being agonists, certain disadvantages as they may induce two affinity states of the receptor, a phenomenon which is not observed with use of antagonists.

2.9. H_3 antagonists

In their first paper on H_3 ligands Arrang et al. [1] described the activity of several H_1 and H_2 ligands at the H_3 receptor. It appeared that H_1 ligands hardly affect the H_3 receptor, but that, amongst H_2 active compounds, potent H_3 ligands were found. Thus the H_2 antagonist burimamide is an effective H_3 antagonist (pA_2 = 7.2) and also some H_2 agonists like impromidine and dimaprit are rather active H_3 antagonists [1]. Major progress on the pharmacology of H_3 receptors was made with the development of the potent and selective H_3 antagonist thioperamide [104].

2.9.1. Thioperamide

Thioperamide appears to be a competitive H_3 antagonist on rat cerebral cortex slices [104] with a pA_2 = 8.4 (K^+-stimulated [^{3}H]histamine release). Also, using guinea-pig intestine, competitive antagonism was found with a pA_2 = 8.9 (electrically evoked cholinergic contractions) [105]. Only negligible affinities for H_1 and H_2 receptors were found with pK_d's of < 4 and < 5, respectively [104], and relatively high affinities for the 5-HT_3 receptor (pK_i = 6.9) and the sigma receptor (pK_i = 6.9) [118]. Thioperamide also shows H_3 antagonism in vivo [104].

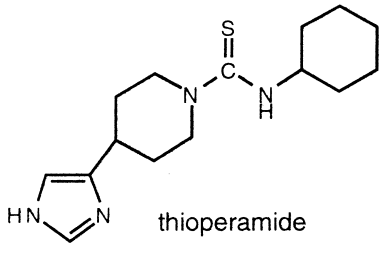
2.9.2. Imetit analogues

Some years ago a new series of H_3 antagonists were developed from the H_3 agonist imetit [106]. It was found that benzylation of the isothiourea group of imetit afforded a rather potent H_3 antagonist. The H_3 antagonistic activity could be increased further by simple modifications leading to clobenpropit, with a pA_2 = 9.9 [106]. With this result, clobenpropit has manifested itself as a very potent H_3 antagonist in vitro and is a valuable tool in pharmacological studies [119]. However, in vivo, a relatively low activity has been observed. Thus, in mice after oral administration, an ED_{50} of 26 mg/kg, measured as brain histamine turnover, was found, whereas thioperamide shows an ED_{50} of 1.0 mg/kg [120]. Probably a slow absorption is due to the low oral activity of clobenpropit.

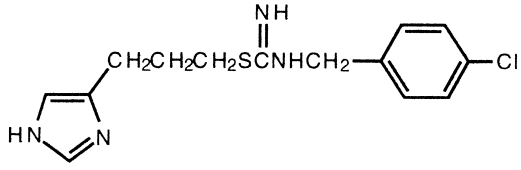
Recently, an iodonated analogue of clobenpropit, called iodophenpropit, had been prepared. This compound also appears to be a potent H_3 antagonist, with a pA_2 = 9.6 [121].

2.9.3. Impentamine

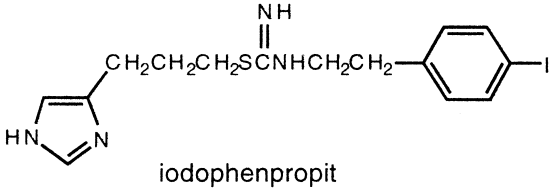
Recently it was shown that the histamine homologue impentamine behaves as a potent H_3 antagonist. It competitively antagonized the (R)α-methylhistamine induced inhibition of the electrically evoked contractions of the guinea-pig jejunum, having a pA_2 of 8.4 [122]. However, in the H_3 receptor mediated inhibition of the electrically evoked [^{3}H]noradrenaline release in mouse brain cortex slices, impentamine appeared to be a partial agonist (pD_2 = 8.2, α = 0.6). Furthermore, impentamine inhibits



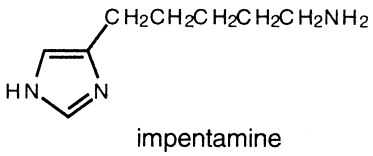
thioperamide



clobenpropit



iodophenpropit



impentamine

[^{125}I]iodophenpropit binding and [^{3}H](R)α-methylhistamine binding to rat brain cortex with pKi's of 8.5 and 9.1, respectively [122]. Although these data appear to be very heterogenous at first glance, they constitute evidence for the existence of H_3 receptor subtypes. If this is the case, impentamine could become a tool for further evaluation of the presence of such subtypes [122].

2.10. Radiolabelled H_3 antagonists

2.10.1. S-[^{3}H]Methylthioperamide

In 1993 a radiolabelled analogue of thioperamide, one of the first H_3 antagonists, was described in the literature. Yanai et al. [123] reported that S-[^{3}H]methylthioperamide labels H_3 receptors with high affinity in rat brain membranes. This radioligand has also been used to obtain autoradiographic images which were essentially the same as those obtained with [^{3}H](R)α-methylhistamine [123].

2.10.2. [^{3}H]Thioperamide

Recently, Alves-Rodrigues et al. [124] showed that [^{3}H]thioperamide binds to rat cortical membranes in a saturable and reversible manner at high and low affinity sites. The high affinity site is most likely the H_3 receptor. The low affinity binding site, with a 30-fold higher density than the H_3 receptor, is probably one of the cytochrome P450 isoenzymes.

2.10.3. [^{3}H]GR168320

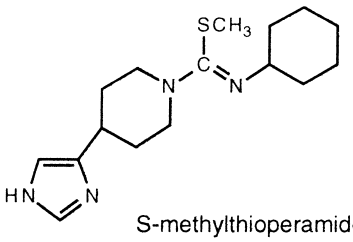
Another highly potent H_3 antagonist is GR168320, which in fact is a guanidine analogue of thioperamide, with a pA_2 = 9.7 (guinea-pig intestine) [125]. [^{3}H]GR168320 revealed, in rat cerebral cortex, a high affinity with a K_d = 0.1 nM with relatively low nonspecific binding. Furthermore, GR168320 acts as a functional antagonist with K_B = 0.2 nM. Remarkably, this compound does not seem to exhibit, at least in the rat cerebral cortex, the difference in displacement by agonists and antagonists [125].

2.10.4. [^{125}I]Iodophenpropit

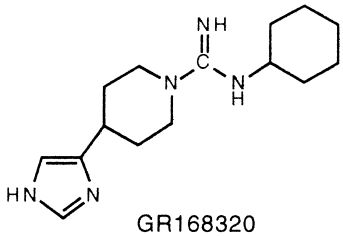
Iodophenpropit is an H_3 antagonist with only moderate affinity to the H_1 receptor (pK_i = 5.8) and the H_2 receptor (pK_i = 6.5) [118]. It has been radiolabelled with [^{125}I] and the resulting [^{125}I]iodophenpropit shows a saturable, reversible high affinity binding with a pK_i = 9.0 [118] and a pK_d = 9.2 [126] to rat cortical membranes, which can be strongly inhibited by agonists like (R)α-methylhistamine and imetit, and antagonists like thioperamide and clobenpropit. Extended testing in 39 different receptor binding assays revealed that iodophenpropit has a relatively high affinity for the 5-HT_3 receptor (pK_i = 8.0), the α_2 adrenoceptor (pK_i = 6.9) and the sigma receptor (pK_i = 6.8) [118]. Due to its high specific activity [^{125}I]iodophenpropit may be used to study the presence of H_3 receptors in peripheral tissues. Additionally iodophenpropit is a valuable tool to study the distribution of H_3 receptors by autoradiography in the rodent brain [126].

2.10.5. [^{125}I]Iodoproxyfan

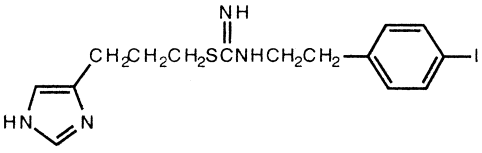
Another radioligand for the H_3 receptor is the recently described [^{125}I]iodoproxyfan [127]. It has been used successfully for binding assays as well as for autoradiographic studies [128]. Iodoproxyfan is a selective and potent H_3 antagonist with a pA_2 = 8.3, in a functional



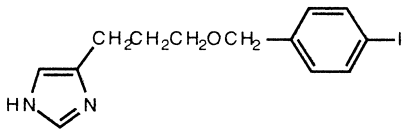
S-methylthioperamide



GR168320



iodophenpropit



iodoproxyfan

assay on synaptosomes from rat cerebral cortex, whereas a pK_i of 9.0 from a binding assay on rat cerebral cortex was reported [128]. Its selectivity is demonstrated by low H_1 and H_2 activity with pK_i's of 5.2 and 5.8, respectively. In addition, unlabelled iodoproxyfan was at least 300 times less potent at α_1, α_2 and β_1 adrenergic, 5-HT_{2A} and 5-HT_3 serotonergic, and M_3 muscarinic receptors than at H_3 receptors [128, 129]. This selectivity and the low nonspecific binding (35%) of [^{125}I]iodoproxyfan probably accounts for its successful application as a probe for autoradiographic studies [128]. However, one should be aware that with some radiolabelled antagonists, including iodoproxyfan, a difference was found between radioligand binding displaced by agonists as compared to antagonists [124, 127]. Moreover iodoproxyfan may have agonistic properties on the H_3 receptor since it inhibits the electrically induced contractions of the guinea-pig ileum, which could be antagonized by the H_3 antagonist thioperamide [130, 131].

3. Conclusion

Concerning the histamine H_1 receptor, both selective agonists and antagonists are available. Amongst the H_1 antagonists [^{3}H] and [^{125}I] radiolabelled compounds have been prepared as well as [^{11}C] radiolabelled compounds for PET studies.

Also, for the H_2 receptor, highly selective agonists and antagonists have been developed. Additionally, a number of [^{3}H] and [^{125}I] radiolabelled antagonists have been reported, including a compound suitable for photoaffinity labelling.

During the last 15 years extensive studies on ligands for the H_3 receptor have led to selective agonists and antagonists. Moreover, [^{3}H] radiolabelled agonists and [^{3}H] and [^{125}I] radiolabelled antagonists have been developed.

In fact one may conclude that for all three histamine receptors, specific tools for pharmacological studies are available.

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