The histamine H3 receptor: structure, pharmacology and function

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Non-standard abbreviations

5-HT  5-Hydroxytryptamine (serotonin)
aa   Amino acids
A1R  Adenosine A1 receptor
A2AR  Adenosine A2A receptor
ACH  Acetylcholine
AD  Alzheimer's disease
CNS  Central Nervous System
D1R  Dopamine D1 receptor
D2R  Dopamine D2 receptor
ECL  Extracellular loop
GABA  γ-Aminobutyric acid
GTS  Gilles de la Tourette's syndrome
GIRK  G protein-gated inwardly rectifying K+ channel
GPCR  G protein-coupled receptor
H1R  Histamine H1 receptor
H2R  Histamine H2 receptor
H3R  Histamine H3 receptor
H4R  Histamine H4 receptor
HDC  Histidine decarboxylase
ICL  Intracellular loop
MSN  Striatal medium-sized spiny neuron
PD  Parkinson's disease
PKA  Protein kinase A
PKC  Protein kinase C
PLC  Phospholipase C
SN  Substantia nigra
SNc  Substantia nigra pars compacta
SNr  Substantia nigra pars reticulata
TMN  Tuberomammillary nucleus
Abstract
Among the four G-protein coupled receptors (H1-H4) identified as the mediators of the biological effects of histamine, the H3 receptor (H3R) is distinguished for its almost exclusive expression in the nervous system and the large variety of isoforms generated by alternative splicing of the corresponding mRNA. Additionally, it exhibits dual functionality as auto- and hetero-receptor, and this enables H3Rs to modulate the histaminergic and other neurotransmitter systems. The cloning of the H3R cDNA in 1999 by Lovenberg et al. allowed for detailed studies of its molecular aspects. In this work we review the characteristics of the H3R, namely its structure, constitutive activity, isoforms, signal transduction pathways, regional differences in expression and localization, selective agonists, antagonists and inverse agonists, dimerization with other neurotransmitter receptors, and the main pre- and post-synaptic effects resulting from its activation. The H3R has attracted interest as a potential drug target for the treatment of several important neurological and psychiatric disorders such as Alzheimer’s and Parkinson’s diseases, Gilles de la Tourette’s syndrome and addiction.
1. Introduction

In 1910 Sir Henry Dale and his colleagues isolated histamine from ergot and later found that it had a stimulant effect on smooth muscle from the gut and the respiratory tract, caused vasodepression, stimulated cardiac contractility, and induced a shock-like syndrome when injected into animals. In 1920 Popielski demonstrated that histamine stimulated gastric acid secretion, and in 1927 the amine was isolated from the liver and the lung, evidencing that it was a natural constituent of the body (reviewed by Parsons and Ganellin, 2006).

Although histamine was detected in the brain in 1919 by John J. Abel, its role as a neuromodulator became evident only several decades later; using antibodies against the amine and its synthesizing enzyme, histidine decarboxylase (HDC), the morphological characterization of histamine-producing neurons proved the existence of a histaminergic system in the mammalian brain (Figure 1).

2. The brain histaminergic system

Histamine-synthesizing neurons are located in the hypothalamus tuberomamillary nucleus (TMN), with ~4,500 neurons found in the rat TMN and ~64,000 neurons in the human TMN. These neurons send diffuse projections to the entire Central Nervous System (CNS) through three major pathways, two ascending bundles that innervate the forebrain structures and one descending bundle reaching the spinal cord (Watanabe et al., 1984; Airaksinen and Panula, 1988; Haas et al., 2008).

Histaminergic neurons have a resting membrane potential of about -50 mV and spontaneously fire action potentials at 2.1 ± 0.6 Hz with a marked circadian rhythmicity,
that is, more active during wakefulness. A non-inactivating Na$^+$ current, active even at -70 mV, appears responsible for spontaneous firing, with low-threshold depolarizing Ca$^{2+}$ currents contributing to the repetitive firing. The action potential is broad with a significant contribution from Ca$^{2+}$ currents followed by a pronounced (15-20 mV) after-hyperpolarization, which activates a depolarizing cationic current ($I_h$) and two A-type K$^+$ currents that delay the return to the resting potential. The Ca$^{2+}$ currents mediate dendritic histamine release and are the target of the autoreceptor-mediated negative feedback on action potential firing (Haas and Reiner, 1988; Haas et al., 2008).

The behavioral state-dependent activity of the histaminergic neurons is influenced by several neuronal, humoral, and paracrine signals, and the activity is mainly regulated by excitatory glutamatergic inputs from the cerebral cortex and the hypothalamus, and by inhibitory GABAergic afferents from the hypothalamic ventrolateral preoptic nucleus (Ericson et al., 1991; Haas et al., 2008; Panula et al., 2015).

2.1 Histamine synthesis, release and catabolism

Histamine is synthesized from the amino acid (aa) L-histidine by the enzyme HDC, which is expressed both in the neuronal bodies and terminals, and the bioavailability of the precursor is the rate-limiting factor. Histamine is stored in vesicles in neuronal cell bodies and axon varicosities by the vesicular monoamine transporter 2, VMAT-2 (Merickel and Edwards, 1995), and is released by exocytosis upon the arrival of action potentials (Haas et al., 2008). The synthesis and release of histamine are regulated by H$_3$ auto-receptors (Arrang et al., 1983, 1987a; Morisset et al., 2000).
Most histaminergic fibers do not make typical synaptic contacts (Takagi et al., 1986) and the amine is released from several points along the fibers, allowing its action on a large number of cells. In the brain, histamine is also produced by mast cells, which contribute modestly to the total amine levels in the adult brain, but during early post-natal development represent the principal source of the amine (Molina-Hernández et al., 2012; Panula et al., 2015).

Inactivation of histamine in the brain is primarily due to the action of histamine-N-methyltransferase producing tele-methylhistamine that is transformed to tele-methyl-imidazolacetic acid by monoamine oxidase B (MAO-B). Diamine oxidase is the main histamine metabolizing enzyme in the peripheral tissues, but its activity in the brain is considerably low under basal conditions (Barnes and Hough, 2002; Maldonado and Maeyama, 2015). In contrast to most other aminergic neuronal cells, histaminergic neurons lack a specific re-uptake transporter, although astrocytes take up histamine with low affinity (Km 0.56 mM and 4.0 mM) through the activity of the plasma membrane monoamine transporter (PMAT) and, to a lesser extent, the organic cation transporter 3, OCT3 (Yoshikawa et al., 2013).

2.2 Histamine receptors

The differing potency of antagonists in blocking histamine action to increase contraction rate in isolated mouse atria and gastric acid secretion or to induce smooth muscle contraction in isolated guinea-pig ileum led to the first classification of histamine receptors into the H₁ and H₂ subtypes by Ash and Schild in 1966, supported by the subsequent development of selective H₂ receptor (H₂R) antagonists (Parsons and Ganellin, 2006). The
bovine H₁ receptor (H₁R) and the canine H₂R were cloned in 1991 (Yamashita et al., 1991; Gantz et al., 1991), allowing for the classification of these receptors into the class A, rhodopsin-like, of G protein-coupled receptors (GPCRs). H₁Rs and H₂Rs are expressed in the brain, and the former signals primarily through Gα₁q/11 proteins whereas the latter activates mainly Gα₂ proteins (Panula et al., 2015).

A third receptor (H₃R) was pharmacologically identified by Arrang et al. (1983) and cloned in 1999 by Lovenberg et al.; the H₃R activates Gαᵢ/o proteins and is almost exclusively expressed by neuronal cells of the CNS and the peripheral nervous system (Panula et al., 2015). Soon after, a fourth receptor was cloned by several groups (e.g. Nakamura et al., 2000 and Oda et al., 2000). The H₄R is mainly expressed by cells of the immune system and, like the structurally related H₃R, activates Gαᵢ/o proteins (Panula et al., 2015).

3. The histamine H₃ receptor (H₃R)

In 1983 Arrang et al. reported that in rat cerebro-cortical slices labeled with [³H]-histidine, the depolarization-evoked, Ca²⁺-dependent release of [³H]-histamine was reduced by exogenous histamine (IC₅₀ 41 nM, maximal inhibition 61%). The effect was insensitive to tetrodotoxin, which prevents the generation and propagation of action potentials, mimicked by Nα-methylhistamine (NAMH), and antagonized by impromidine and burimamide with potencies significantly different from those reported for H₂R blockade. More potent H₂R antagonists and selective H₁R antagonists also showed low potencies. It was therefore proposed that auto-inhibition of histamine release was mediated by a novel class of receptor (H₃R). In a later study (Arrang et al., 1985a) auto-inhibition of depolarization-evoked [³H]-histamine release was also shown for isolated nerve terminals (synaptosomes) from rat.
cerebral cortex (-30%) and slices of striatum (-49%), hippocampus (-47%) and hypothalamus (-64%). The same group reported that histamine also inhibited its own synthesis evoked by depolarization in rat cerebro-cortical slices (IC$_{50}$ 340 nM, maximal inhibition 70%; Arrang et al., 1987a). This effect was competitively antagonized by burimamide and impromidine with potencies similar to those observed for the auto-inhibition of release (Arrang et al., 1983), supporting the function of the H$_3$R as an auto-receptor.

In 1999 Lovenberg et al. identified a partial clone (GPCR97) and used it to probe a human thalamus cDNA library. This resulted in the isolation of a full-length clone encoding a 445 amino acid protein with the characteristics of the class A of GPCRs, and with 20-27% homology to biogenic amine receptors and 22% and 21.4% homology to the human H$_1$R and H$_2$R, respectively. Upon transfection into human HEK-293 cells, rat C6 glioma cells and human SK-N-MC neuroblastoma cells, GPCR97 displayed a pharmacological profile practically indistinguishable from that of the native H$_3$R. Soon after, the receptor was cloned by sequence similarity from various other species, namely rat, guinea pig, mouse and monkey, with a high level of homology ($\geq$ 93%) across these species (see Figure 2).

### 3.1 Expression in the Central Nervous System (CNS)

The H$_3$R is mainly expressed by neurons and in very low density by glial cells (Arrang et al., 1987b; Ferreira et al., 2012). The distribution of the H$_3$R in the CNS has been studied via in situ hybridization (mRNA), RT-PCR (mRNA), and autoradiography (binding sites) in rodents, human and monkey. In situ hybridization studies report very high levels of H$_3$R mRNA in the cortex (mainly in the V layer, with lower expression in the superficial layers),
hippocampus (CA1 and ventral CA3 pyramidal layers of Ammon’s horn), caudate, and putamen. Strong mRNA expression is also observed in the anterior olfactory nucleus, amygdala, bed nucleus of the stria terminalis, cerebellum, thalamus (mostly in the sensory and intralaminar nuclei), and some hypothalamic nuclei, particularly the TMN where histaminergic neurons are located. Low to moderate mRNA expression is detected in the habenula and zona incerta; the signals are very low in the globus pallidus, substantia nigra (SN) and substantia innominata, and not detected in the islands of Calleja (Tardivel-Lacombe et al., 2000; Pillot et al., 2002; Sallmen et al., 2003).

The analysis by RT-PCR of the expression of the H₃R isoforms of 445 and 365 aa in the human brain (see below for a description of the receptor isoforms) indicates high levels in cerebellum and caudate, moderate in hypothalamus and thalamus, low expression in SN, hippocampus, prefrontal cortex, corpus callosum and amygdala, and very low levels in the spinal cord (Bongers et al., 2007b).

Binding studies show a similar H₃R distribution in primate and rodent brain. The receptor is widely expressed but with heterogeneous density, and high levels are found in cerebral cortex (except in layer V), tenia tecta, nucleus accumbens, striatum, hippocampus, bed nucleus of the stria terminalis, olfactory nuclei, some hypothalamic nuclei (mainly the TMN), amygdala, and pyriform cortex. In contrast to mRNA expression, dense binding is found in the globus pallidus and the substantia nigra pars reticulata (SNr). A low density of binding sites is found in locus coeruleus and raphe nuclei, and the cerebellum and the pituitary gland are scarcely labeled (Martinez-Mir et al., 1990; Pollard et al., 1993; Anichtchik et al., 2000; Pillot et al., 2002).
The H3R location is mainly pre-synaptic, either as auto-receptor or as hetero-receptor, but there is also evidence for a post-synaptic location of the H3R in striatum, cerebral cortex, hippocampus, nucleus accumbens, lateral hypothalamus and zona incerta (Pillot et al., 2002; González-Sepúlveda et al., 2013; Parks et al., 2014).

3.2 Structure

With over 800 genes encoding GPCRs, these proteins constitute the largest family of membrane proteins contained in the human genome. These receptors share a common seven transmembrane (TM) domain structure that forms the core, an extracellular amino terminus (NT), an intracellular carboxyl terminus (CT), three extracellular (ECL), and three intracellular (ICL) loops.

The H3R possesses a DRF motif in the interface of TM3 and ICL2 (instead of the DRY sequence common to most class A GPCRs), a NPVLY motif in TM7 (corresponding to the NPXXY motif present in all GPCRs), and a palmitoylation site in the CT (Cys428) that allows for the formation of helix 8 (Figure 3). A disulfide bond is formed by Cys107 and Cys188 on ECL1 and ECL2, respectively. The H3R has a short NT (39 aa), with a glycosylation site on Asn11, and a long ICL3 (142 aa). These segments are the loci of the naturally occurring mutations D19E and A280V, respectively (Wiedemann et al., 2002), and cleavage through splicing of the ICL3 leads to several H3R isoforms as discussed below.

Since 2000, the rat, guinea pig, monkey and mouse genes encoding the H3R have been cloned (Lovenberg et al., 2000; Tardivel-Lacombe et al., 2000; Chen et al., 2003; Yao et al., 2003), and this work revealed a homology of $\geq 93\%$ in the H3R protein sequence.
among these species (Figure 2). The inter-species differences in the pharmacological profiles rely on a double change in the residues 119 and 122 located nearby Asp114 in TM3. Human and monkey receptors have Thr119 and Ala122 at these species-variant sites, whereas rodents have Ala119 and Val122, with the exception of the guinea-pig receptor (Thr119 and Val122). When compared with the human H3R (hH3R), a single-residue change (V362I) in TM6 is found in the rat, mouse and guinea pig. No differences exist between human and monkey H3Rs in the transmembrane domains (Hancock et al., 2003).

In contrast to the small changes in the helical domains, a significant number of differences among species are found in a 65-66 aa-length sequence in the ICL3 (aa 236 to 300 in the hH3R), but this does not affect the presumed Ser and Thr substrates of phosphorylation, suggesting a conserved kinase modulation profile.

### 3.3 Determinants of G-protein coupling and activation

Structurally and functionally, two faces can be identified on all GPCRs: the extracellularly accessible orthosteric binding site, and the internal region responsible for G protein activation. The crystallization of GPCRs coupled to a G protein has provided insight into the mechanisms of receptor-G protein interaction and activation of the latter. These studies show that the α-CT and the helixes 4 and 5 of the Gα subunit dock to a cavity formed mainly by TM5, TM6, and ICL2 in the GPCR, which adopts an “open” conformation upon receptor activation. The Arg of the DRY motif plays a key role in docking the α-CT of the G protein (Rasmussen et al., 2011), and residues in ICL3, helix 8 and the CT appear to contribute to stabilizing the receptor-G protein interaction because a truncation close to the NPXXY motif prevents G protein activation (van Rijn et al., 2013; Flock et al., 2015).
Computational models of the H₃R-G protein interaction have not yet been published, but functional studies suggest that ICL3 plays a role in G protein activation. Alternative splicing generates several H₃R isoforms (see below), and the hH₃R of 445 aa (hH₃R₄₄₅) inhibited forskolin-induced cAMP formation in CHO cells, but the hH₃R₃₆₅ (lacking 80 aa in ICL3) failed to do so (Cogé et al., 2001). In rat C6 glioma cells, the hH₃R₃₆₅ was less efficacious in a calcium mobilization assay (Esbenshade et al., 2006), but agonists were 3- to 20-fold more potent in the R-SAT assay, based on β-galactosidase activity (Wellendorph et al., 2002). The rat H₃R₄₁₃ and H₃R₃₉₇ isoforms that lack 32 and 48 aa in ICL3 showed similar efficacy and higher agonist potency for the inhibition of forskolin-induced cAMP formation, but for 42/44-MAPK phosphorylation the H₃R₄₄₅ coupled considerably better (Drutel et al., 2001). The naturally occurring A280V mutation on ICL3 also modifies the functionality of the hH₃R₄₄₅ to inhibit cAMP accumulation and stimulate 42/44-MAPK phosphorylation (Flores-Clemente et al., 2013).

Altogether, the previous information indicated that there is not a single structural determinant for the H₃R functionality, but that the interplay of several regions provides the appropriate physiological output.

### 3.4 Constitutive activity and determinants

The term constitutive activity refers to a ligand-independent state of the receptor that spontaneously adopts the active conformation. The H₃R possesses high constitutive activity as indicated by the reduction of basal G protein activation induced by inverse agonists (Wieland et al., 2001; Rouleau et al., 2002). Constitutive activity has mainly been shown for transfected human and rat H₃Rs, but also for native rodent receptors with inverse
agonists reducing basal $[^{35}\text{S}]$-GTP$\gamma$S binding to membranes from the cerebral cortex and hippocampus, enhancing depolarization-induced $[^{3}\text{H}]$-histamine release from cerebrocortical synaptosomes and increasing the levels of the histamine metabolite tele-methylhistamine in homogenates from the cerebral cortex (Morisset et al., 2000; Sallmen et al. 2003).

The DRY motif is responsible for the equilibrium between the inactive and active receptor conformations, and therefore plays a pivotal role in constitutive activity. A salt bridge between the Arg in the DRY motif (TM3) and an Asp or Glu in TM6 stabilizes or ‘locks’ the inactive state. Upon receptor activation, this interaction is broken and the Arg residue in TM3 rotates towards TM5 to form a hydrogen bond with a Tyr residue to establish an ‘active lock’ (Valentin-Hansen et al., 2012). Accordingly, the R112A mutation in the hH4R prevents G protein activation (Schneider et al., 2010), and the R116A mutation in the rat H2R resulted in a highly structurally instable receptor whose expression could only be detected after stabilization with either an agonist or inverse agonist; furthermore, this receptor showed increased agonist affinity and reduced efficacy (Alewijnse et al., 2000). However, there is no direct evidence for a role of the DRF motif in H3R constitutive activity.

As pointed by Morisset et al. (2000), the CT of ICL3 in the rat H3R has a stretch of eight amino acids that is similar (six identical and two conserved residues) to the corresponding sequence of a mutated human $\beta_2$-adrenoceptor with high constitutive activity (Lefkowitz et al., 1993). This region is critical for constitutive activity in other native or mutated GPCRs. Although the sequence is conserved in all H3R isoforms and species (see Figure 2),
constitutive activity varies among isoforms and it can be different between species and cell lines (Arrang et al., 2007).

3.5 H3R isoforms

The existence of H3R isoforms was initially suggested by the pharmacological heterogeneity of the receptor expressed in several brain regions in binding and functional assays (West et al., 1990). The molecular cloning of the receptor indicated that the structure of the hH3R gene comprised three exons and two introns (Tardivel-Lacombe et al., 2001; Wiedemann et al., 2002) or four exons and three introns (Cogé et al., 2001), that allows for the generation of isoforms via RNA splicing. Alternatively, for humans and rodents, the last intron is proposed to be a pseudo-intron located in the region coding for ICL3, which is retained in the hH3R445, but deleted in the hH3R413 (Tardivel-Lacombe et al., 2001; Bongers et al. 2007a). In both proposed structures the two first introns are located in the same position in the human and rodent genes, suggesting the existence of a variety of functional and non-functional isoforms with certain similarity among species (Tardivel-Lacombe et al., 2000; Drutel et al., 2001; Morisset et al., 2001; Wellendorph et al., 2002).

RT-PCR analysis identified several isoforms that differ in the length of their NT or CT, deletions in the ICL3 or shorter sequences in TMs. To date the pharmacological and functional characteristics of the H3R isoforms have only been evaluated in heterologous systems (Table 1).

Some of the identified isoforms lack regions critical for agonist binding (TM 3 and 5-7) or signaling (ICL2, ICL3 and CT), and therefore do not trigger the signaling pathways typically associated with H3R activation (Wess, 1997; Uveges et al., 2002; Oldham and
three rat isoforms (rH3R497, rH3R465 and rH3R449) that do not possess TM7, but do have an extracellular CT of 105 aa without homology to the functional isoforms reduce the cell surface expression of the rat H3R445 upon co-expression in COS-7 cells (Bakker et al., 2006). The expression and signaling of functional H3Rs could thus be regulated by isoforms incapable of triggering the signaling pathways described below. These isoforms could also directly interact with other GPCRs and affect their binding or signaling properties. Furthermore, non-canonical signaling, either agonist-independent or mediated by alternative pathways, cannot be discarded for the truncated or longer H3R isoforms, and merits further investigation.

3.6 Signaling pathways

The coupling of the H3R to Ga\_i/o proteins was first suggested by the inhibitory effect of Pertussis toxin (PTX) activity, which decreased the affinity for [3H]-NMHA of the H3R endogenously expressed by murine pituitary AtT cells (Clark et al., 1993) and prevented H3R-stimulated [35S]-GTP\_γ\_S binding in rat cerebro-cortical membranes (Clark and Hill, 1996). As for other GPCRs, both the Ga subunits and the G\_βγ complexes mediate the Ga\_i/o protein-dependent signaling of the H3R (Figure 4).

**Inhibition of adenylyl cyclases.** In the study that reported the cloning of the hH3R (Lovenberg et al., 1999), receptor activation inhibited forskolin-induced cAMP accumulation, and this effect was disrupted by PTX (Shi et al., 2012). Likewise for other Ga\_i/o protein-coupled GPCRs, a direct interaction between the Ga\_i/o subunit and sensitive
adenylyl cyclases (1, 3, 5, 6 and 8) appears to be the mechanism that mediates this effect (Cooper and Crossthwaite, 2006).

**Inhibition of the Na\(^+\)/H\(^+\) exchanger (NHE).** The activity of the Na\(^+\)/H\(^+\) exchanger represents one of the key mechanisms for restoring the intracellular pH following ischemia-induced acidosis by extruding protons concomitantly with Na\(^+\) influx (Karmazyn, 1999). A rise in intracellular Na\(^+\) may reverse the Na\(^+\)/Cl\(^-\) dependent noradrenaline transporter (NET) and induce carrier-mediated neurotransmitter release. H\(_3\)R activation reduces NHE activity in sympathetic nerve terminals leading to inhibition of noradrenaline release in myocardial ischemia (Silver et al., 2001); the mechanism is not fully understood, although a direct G\(_{\alpha_i/o}\) subunit/NHE interaction appears likely (van Willigen et al., 2000).

**Inhibition of N- and P/Q-type voltage-gated Ca\(^{2+}\) channels.** The inhibitory effect of H\(_3\)Rs on neurotransmitter release (see below) is most likely linked to the reduction in depolarization-induced Ca\(^{2+}\) entry via the binding of G\(_{\beta\gamma}\) complexes to the pore-forming \(\alpha_1\)-subunit of N- and P/Q-type voltage-gated Ca\(^{2+}\) channels (Zamponi and Currie, 2013). Accordingly, H\(_3\)R activation reduces depolarization-induced Ca\(^{2+}\) entry in dissociated hypothalamic histaminergic neurons (Takeshita et al., 1998), striatal synaptosomes (Molina-Hernández et al., 2001), transfected human neuroblastoma SH-SY-5Y cells (Silver et al., 2002), and transfected rat pheochromocytoma PC12 cells (Morrey et al., 2008). The inhibition of voltage-gated Ca\(^{2+}\) currents by H\(_3\)Rs in histaminergic neurons was abolished by PTX (Takeshita et al., 1998), and both a G\(_{\beta\gamma}\) scavenger and a phosphoducin-like anti-G\(_{\beta\gamma}\) peptide prevented the H\(_3\)R-mediated reduction in depolarization-induced Ca\(^{2+}\) entry and neurotransmitter exocytosis in pheochromocytoma PC12 cells (Morrey et al., 2008), supporting the participation of G\(_{\beta\gamma}\) dimers in these processes.
Activation of G protein-gated inwardly rectifying K⁺ channels (GIRKs). $G_\beta\gamma$ subunits bind and activate GIRK channels (Bünemann et al., 2001), and transfected $hH_3R_{445}$ and $hH_3R_{365}$ activate channels formed by the GIRK1 (Kir3.1) and GIRK4 (Kir3.4) subunits expressed in *Xenopus* oocytes (Sahlholm et al., 2012). GIRKs can inhibit synaptic transmission (Meneses et al., 2015), and activation of pre-synaptic GIRKs would thus represent an additional mechanism for $H_3$Rs to modulate neurotransmitter release.

Activation of GIRK channels by $H_3$Rs has also been observed at the postsynaptic level in neurons producing melanin-concentrating hormone (MCH), where the effect was prevented by GDP$\beta$S, an inhibitor of G protein signaling (Parks et al., 2014).

**Phospholipase C (PLC) activation.** In transfected CHO and SK-N-MC cells, activation of the $hH_3R_{445}$ induces a significant increase in the intracellular concentration of Ca$^{2+}$ ions ([Ca$^{2+}]_i$) due to PLC activation and release of Ca$^{2+}$ from intracellular stores via inositol-1,4,5-trisphosphate (IP$_3$) formation (Cogé et al., 2001; Bongers, 2008). The $H_3$R-mediated increase in [Ca$^{2+}]_i$ was prevented by PTX, which implicates the participation of $G_{\alpha_{i/o}}$ proteins (Bongers, 2008). The PLC/IP$_3$/Ca$^{2+}$ pathway is most often triggered by the $G_\alpha$ subunits of $G_{\alpha_{q/11}}$ proteins, but $G_\beta\gamma$ complexes can also activate PLC$\beta$ by binding to a region (PH and Y domains) different from the $G_{\alpha_{q/11}}$ subunit binding domain (C2 domain and carboxyl terminal region; Rebecchi and Pentyala, 2000; Rhee, 2001).

**Activation of the mitogen-activated protein kinase (MAPK) pathway.** $H_3$R activation stimulates 42/44-MAPK phosphorylation both in heterologous systems and native tissues (Drutel et al., 2001; Giovannini et al., 2003; Flores-Clemente et al. 2013). $G_\beta\gamma$ complexes appear to play a central role in this action because pharmacological inhibition and $G_{\alpha_{i/o}}$-transducin, a $G_\beta\gamma$ scavenger, prevent $H_3$R-mediated 42/44-MAPK phosphorylation (Lai et
al., 2016). However, other mechanisms, such as the binding to activated receptors of β-arrestins, that act as a signaling scaffold (Gutkind, 2000), or the transactivation of the epidermal growth factor (EGF) receptor (Lai et al., 2016), could also contribute to MAPK activation.

**Activation of the phosphatidylinositol 3-kinase (PI3K) pathway.** In transfected cells, primary cultures of rat cerebro-cortical neurons and rat striatal slices, H₃R activation stimulate the phosphorylation of Akt or protein kinase B (PKB), which subsequently phosphorylates and thereby inhibits the action of glycogen synthase kinase 3-β (GSK3β) (Bongers et al., 2007c). This action on the PI3K/Akt pathway was prevented by PTX and probably depends on G_{βγ} complexes, which are known to activate PI3K (Murga et al., 1998).

**Stimulation of phospholipase A₂.** The activation of phospholipase A₂ by the H₃R induces the release of arachidonic acid, docosahexaenoic acid, and lysophospholipids, inducing functional consequences such as the relaxation of guinea-pig bronchioles by enhanced release of the endothelium-derived relaxing factor (EDRF), a metabolite of arachidonic acid (Burgaud and Oudart, 1993).

### 3.7 Regulation of H₃R signaling

**Desensitization.** This process, which leads to changes in signaling efficacy and receptor expression at cell surfaces, represents a major mechanism to regulate GPCR functional responses. Homologous desensitization is triggered by the phosphorylation of activated receptors by GPCR kinases (GRKs), whereas in the heterologous process activation of one GPCR leads to the desensitization of one or more unrelated receptors in the same cell. The
latter process often involves GPCR phosphorylation by second messenger-activated kinases, in particular protein kinases A (PKA) and C (PKC). GPCR phosphorylation results in conformational changes that impair G protein activation and triggers receptor internalization (Gainetdinov et al., 2004; Gurevich et al., 2012).

In CHO-K1 cells stably transfected with the hH3R445, exposure to agonists results in functional desensitization as well as reduced receptor expression in the cell surface due to the action of GRKs 2/3 and clathrin-dependent endocytosis (Osorio-Espinoza et al., 2014). In the same expression system, the hH3R445 also experiences PKC-mediated heterologous desensitization upon the activation of a second GPCR coupled to the PLC/IP3/DAG pathway (Montejo-López et al., 2016).

**RGS proteins.** The regulator of G protein signaling (RGS) proteins modulate the intracellular effects of activated GPCRs. Four RGS subfamilies (RZ, R4, R7, R12) act as GTPase-activating proteins to increase the rate of GTP hydrolysis by Ga subunits (Hollinger and Hepler, 2002; Sjögren et al., 2010). Whereas there are no reports on the regulation by RGS of the H3R signaling, RGS proteins are co-expressed with H3Rs in the CNS, for example in cerebral cortex (RGS 4-8, 10), nucleus accumbens and striatum (RGS 4, 8, 9), hippocampus (RGS 7, 8, 10) and hypothalamus (RGS 4, 6, 7, 8) (Gold et al., 1997), making the RGS-mediated regulation of H3R signaling an aspect deserving attention.

**4. Pharmacology**

The H3R shows a complex pharmacology, with drugs acting as full agonists, partial agonists, neutral antagonists, inverse agonists and protean ligands. H3R affinity for ligands
has been determined by radioligand binding assays ($K_i$ or $pK_i$, Table 2) and efficacy (Emax) and potency ($pEC_{50}$, $EC_{50}$, $pK_B$, $pA_2$, $pD_2$) through the analysis of $[^{35}S]$-GTPγS binding, cAMP formation, 42/44-MAPK phosphorylation, calcium mobilization, and PI3K activation (Table 3).

In 2008 the crystal structure of the GPCR opsin was resolved (Scheerer et al., 2008), leading to a revolutionary change in the study of GPCR-drug interactions. Although the crystal structure of the H3R has yet to be elucidated, homology models and point-mutation studies allow for the assessment of ligand recognition by its binding pocket.

### 4.1 Determinants of histamine binding

Histamine (2-[4-imidazolyl]ethylamine) is an hydrophilic non-chiral molecule, consisting of an imidazole ring and an ethylamine side-chain, and its binding to the H3R depends on features shared by other biogenic amine receptors. Although the ligand binding site comprises residues in TM3 to TM7, two main residues are essential for histamine binding. One is the negatively charged Asp114 in TM3 that interacts with the protonated amine group of histamine, and the other is Glu206 in TM5 that forms a hydrogen bond with the nitrogen present in the imidazole ring.

In addition to Glu206, other residues important for histamine binding are Trp196, Thr201, Ala202, Thr204 and Phe208 in TM5. Mutations in these residues decrease binding affinity, with the E206A mutation resulting in the most marked change. Interestingly, variations in binding affinity induced by mutations are not always matched in functional assays, with the W196A, T204A, E206A and F208A mutations increasing ligand potency to inhibit cAMP formation, indicating that conformational changes in the binding pocket do not necessarily...
translate into receptor-driven G protein activation (Uveges et al., 2002). Other residues involved in histamine binding are Tyr115 and Cys118 in TM3; Trp371, Tyr374 and Leu401 in TM6; and Tyr189 in ECL2 and Trp402 in TM7 (Ishikawa et al., 2010). The high affinity of the H3R for the endogenous agonist does not rely on Asp114 (conserved in all four histamine receptors, and essential for histamine binding), but on Glu206, only present in H3Rs and H4Rs, explaining the non-selective actions of several classical H3R ligands at the H4R (Axe et al., 2006). The increased affinity of H3Rs and H4Rs for histamine may be due, at least in part, to the stronger interaction of the imidazole ring with Glu206 compared to the interaction with the Asn or Thr residue found on the same position in H1Rs or H2Rs, respectively (Uveges et al., 2002).

The previous information allows a pharmacophore structure to be drawn for the H3R (Figure 5) where Glu206 anchors the imidazole moiety of histamine and the other residues form a lipophilic bed in which histamine lays, favoring the interaction of the basic amine moiety with Asp114 in TM3 (de Esch et al., 2000).

4.2 Binding of synthetic imidazole-containing and non-imidazole ligands

Imidazole-containing agonists. So far, all agonists at the H3R are based on the structure of histamine and therefore contain the imidazole moiety, which is critical for agonist activity (Leurs et al., 1995). Modifications of the imidazole ring have been unsuccessful. By contrast, modifications of the histamine side chain that preserve the basic amino group or other proton donor moieties have allowed for the design of more selective and potent agonists. Like histamine, these molecules bind to the H3R by using Glu206 as anchor for the imidazole moiety and establishing hydrogen bonds with Asp114 (Figure 5).
The first highly selective and potent H₃R ligands were the agonist RAMH (R-α-methylhistamine) and the antagonist thioperamide, and the differences in the affinity and potency of the ligand enantiomers demonstrated the stereoselectivity of the H₃R (Arrang et al., 1987b; Kovalainen et al., 1999; De Esch et al., 1999). The replacement of the histamine amine group by an isothiourea group led to the very potent and selective H₃R agonist imetit (Garbarg et al., 1992), and other histamine analogues with a piperidine ring in the side chain, such as immepip, showed improved affinity and efficacy at the H₃R (Figure 6 and Table 2). Modifications in the piperidine nitrogen, like VUF-5681, resulted in decreased affinity and functional activity (Shih et al., 1998; Kitbunnadaj et al., 2003, 2005). The H₄R has high homology to the H₃R, and also high affinity for H₃R agonists such as imetit and immepip. The search for more selective ligands led to methimepip (N-methyl-substituted immepip), which retains high affinity and efficacy (Tables 2 and 3), and shows 2000-fold selectivity for the hH₃R over the hH₄R (Kitbunnadaj et al., 2003, 2005).

**Imidazole-containing antagonists.** In addition to the imidazole moiety, the majority of these compounds possess a central electro-negative group and a cyclic moiety (Figure 7). These groups form a conserved pharmacophore with three interactions: a) a hydrogen bond of the imidazole ring with Glu206 in TM5; b) a salt bridge of the central electro-negative group with either Tyr374 in TM6 or Asp114 in TM3, which may dictate the torsion of the antagonist; and, c) a π-stacking of the hydrophobic cyclic group with Phe198 in TM5, Tyr189 in ECL2, or Trp110 in TM3 (Levoin et al., 2010; Figure 5).

The extension of the ethylamine side chain, derived from the imidazolyl piperidine compounds by varying the piperidin-substituting groups, resulted in drugs devoid of agonist activity and led to the antagonist thioperamide (Arrang et al., 1987b). The more
potent antagonist clobenpropit was derived from imetit through the benzylolation of the isothiourea group and other modifications, and its iodinated analogue (iodophenpropit) is also a potent antagonist (van der Goot and Timmerman, 2000). Other imidazole-containing antagonists are referred to in Table 3.

The discovery of H₃R constitutive activity led to the reclassification of some antagonists as inverse agonists or protean ligands. Thioperamide and ciproxifan, classically considered antagonists, also behave as inverse agonists. Proxyfan acts as agonist, inverse agonist or neutral antagonist, depending on the level of H₃R constitutive activity, and is therefore considered a protean ligand (Morisset et al., 2000; Rouleau et al., 2002; Arrang et al., 2007).

Non-imidazol antagonists. The effects of H₃R activation on physiological and pathological conditions are the main focus of research into the therapeutic potential of selective H₃R ligands. However the imidazole ring reduces penetration of the blood-brain barrier (Young et al., 1988), and imidazole-based drugs inhibit the hepatic cytochrome P450 (Ishikawa et al., 2010). To avoid these side-effects, antagonists lacking the imidazole ring, but still capable to form a hydrogen bond with Glu206, were synthesized (Figure 7). The first two non-imidazole antagonists were UCL1972 and VUF5391 where the imidazole ring was replaced for a piperidine or pyrrolidine group, respectively. The heterocyclic nitrogen interacts with Glu206, the central oxygen forms a salt bridge with a hydroxyl-containing residue, and the heterocyclic moiety establishes π-stacking interactions (Ganellin et al., 1998, Menge et al., 1998; Levoin et al., 2010).
By exploration of the structure-activity relationship, a series of highly potent and selective non-imidazole antagonists were developed, including ABT-239, JNJ-5207852, NNC 38-1049 and GSK189254, which show anti-obesity properties, promote the wake state, and have pro-cognitive effects (Barbier et al., 2004; Esbenshade et al., 2005; Fox et al., 2005; Medhurst et al., 2007).

The imidazole-containing compounds display similar affinities for the H3R across species (see Tables 2 and 3). Nevertheless, some antagonists, such as thioperamide or ciproxifan, are less potent at the hH3R than at the rat H3R, and as mentioned previously these differences have been attributed to changes in two residues located in TM3 (T119A and A122V). In contrast, the non-imidazole compounds are more potent and selective at the hH3R (West et al., 1999; Ligneau et al., 2000; Parsons and Ganellin, 2006). Furthermore, binding studies with thioperamide and burimamide in rat, human and monkey tissues indicate the presence of high- and low-affinity sites, which could be related to either different states of the same receptor or to the expression of more than one isoform (West et al., 1990, 1999).

5. Function

5.1 Pre-synaptic effects

The best-known function of the H3R is the pre-synaptic regulation of neurotransmitter release in the central and peripheral nervous systems. This regulation has been reported for histamine itself, noradrenaline, dopamine, acetylcholine (ACh), GABA, glutamate, serotonin (5-hydroxytryptamine, 5-HT), and some neuropeptides. Whereas the evidence for
a direct action of H3Rs is consistent for most of these neurotransmitters, this is not established for acetylcholine and dopamine.

**Histamine.** The observation that histamine inhibited its own release led to the identification of the H3R by Arrang *et al.* (1983). Control by pre-synaptic H3Rs of histamine release has been shown for slices of rat cerebral cortex, striatum, hippocampus and hypothalamus, as well as for rat cerebro-cortical synaptosomes (Arrang *et al.*, 1983, 1985a,b). In vivo microdialysis experiments have shown that H3R agonists reduce histamine release in the rat hypothalamus and cerebral cortex, whereas antagonists increased the release in the hypothalamus, nucleus *basalis magnocellularis*, and cerebral cortex (Jansen *et al.*, 1998; Lamberty *et al.*, 2003; Giannoni *et al.*, 2009).

**Acetylcholine (ACh).** The modulation of cholinergic transmission by H3Rs was first reported for the peripheral nervous system; histamine and H3R agonists inhibited the electrically-evoked contraction of guinea-pig ileum strips, but had no effect on the action of exogenous ACh (Trzeciakowski, 1987). It was later shown that in the longitudinalis smooth muscle/mienteric plexus preparation the release of [3H]-ACh induced by electrical stimulation was reduced by H3R activation (Poli *et al.*, 1991).

In the CNS, H3R activation inhibited K+-induced [3H]-ACh release from rat entorhinal cortex slices, but this effect was not detected in synaptosomes from the same region or in slices from the hippocampus (Arrang *et al.*, 1995; Alves-Rodrigues *et al.*, 1998), questioning the presence of the receptor on cholinergic nerve terminals. In vivo microdialysis shows that H3R activation reduces ACh release in the rat fronto-parietal cortex, hippocampus, nucleus *accumbens* and basolateral amygdala (Blandina *et al.*, 1996;
Prast et al., 1999; Passani et al., 2001; Bacciottini et al., 2002). These studies also suggest the involvement of trans-synaptic effects. For example, H₃R-mediated inhibition of GABA or dopamine release could subsequently relieve the inhibitory control by these transmitters of ACh release (Schlicker et al., 1993; Garcia et al., 1997; Prast et al., 1999).

**Noradrenaline.** H₃R-mediated inhibition of noradrenaline release has been reported for both the CNS and the peripheral nervous system. In cardiac sympathetic terminals H₃R stimulation reduces noradrenaline release by various mechanisms, including inhibition of voltage-activated Ca²⁺ and Na⁺ channels, reduction of NHE activity, and trans-activation of prostanoid receptors (Endou et al., 1994; Imamura et al., 1995, 1996; Hatta et al., 1997; Mazenot et al., 1999; Silver et al., 2002; Seyedi et al., 2005; Levi et al., 2007).

For the CNS, in vitro experiments indicated that H₃R activation reduces depolarization-induced [³H]-noradrenaline release in rodent cerebral cortex, spinal cord, cerebellum, hippocampus, hypothalamus and olfactory bulb, and human cerebral cortex (Schlicker et al., 1989, 1992; 1994, 1999; Celuch, 1995; Timm et al., 1998; Aquino-Miranda et al., 2011). In vivo microdialysis experiments showed that H₃R activation reduces noradrenaline release in rat hippocampus and cerebral cortex (Di Carlo et al., 2000; Medhurst et al., 2007).

**5-hydroxytryptamine (5-HT).** In rat cerebral slices and synaptosomes as well as in SNr slices, H₃R activation inhibits 5-HT release evoked by electrical or chemical stimulation (Schlicker et al., 1988; Fink et al., 1990; Threlfell et al., 2004). By contrast, in rat olfactory
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bulb slices, H₃R activation had no effect on depolarization-induced [³H]-5-HT release (Aquino-Miranda et al., 2011).

**Dopamine.** In mouse striatum and rat substantia nigra pars reticulata (SNr), depolarization-evoked [³H]-dopamine release is inhibited by H₃R activation (Schlicker et al., 1993; Garcia et al., 1997), suggesting that the receptor resides on the terminals and dendrites of the dopaminergic nigro-striatal neurons. However, H₃R activation had no effect on depolarization-evoked [³H]-dopamine release from rat and rabbit striatal slices (Smits and Mulder, 1991; Schlicker et al., 1993), indicating differences between species and brain regions.

In microdialysis studies, H₃R activation increases the extracellular dopamine levels in rat prefrontal cortex, but not in the striatum (Fox et al., 2005; Medhurst et al., 2007). The systemic or local administration of H₃R antagonists augments dopamine release induced by methamphetamine in the nucleus accumbens; however, the effect of the local perfusion of the antagonists was lower than that produced by their systemic administration (Munzar et al., 2004), casting doubt on the presence of H₃Rs on the terminals of ventral tegmental area (VTA) neurons, the main source of dopaminergic innervation to nucleus accumbens (Wise, 2004).

**Glutamate.** Pre-synaptic H₃Rs inhibits glutamatergic transmission in rat hippocampus, striatum, basolateral amygdala, thalamus and globus pallidus (Doreulee et al., 2001; Jiang et al., 2005; Garduño-Torres et al., 2007; Osorio-Espinoza et al., 2011). In rat striatal and thalamic synaptosomes H₃R stimulation reduces both glutamate release and an increase in the intracellular Ca²⁺ concentration induced by depolarization (Molina-Hernández et al.,
Optogenetic experiments confirmed the H₃R-mediated inhibition of cortico-striatal and thalamo-striatal glutamatergic transmission, and the increased paired-pulse ratio supports the pre-synaptic location of H₃Rs in cortico-striatal and thalamo-striatal synapses. Of note, H₃R activation had no effect on the plasticity of cortico-striatal synapses, but the thalamo-striatal synapses became significantly facilitatory (Ellender et al., 2011).

**GABA.** In slices from rat SNr and striatum, the activation of dopamine D₁ receptors, coupled to Gαₛ proteins, enhances depolarization-evoked [³H]-GABA release and this effect is selectively counteracted by H₃R-mediated inhibition of P/Q-type voltage-activated Ca²⁺ channels (Garcia et al., 1997; Arias-Montaño et al., 2001, 2007). Likewise, in the nerve terminals of striato-pallidal neurons, H₃R activation counteracts the facilitatory action of adenosine A₂A receptors (A₂ARs), which are also coupled to Gαₛ proteins (Morales-Figueroa et al., 2014).

H₃R-mediated inhibition of GABA release has been observed *in vivo* in rat medial vestibular nucleus, and *in vitro* in primary cultures of rat cerebro-cortical neurons and dissociated neurons from the rat hypothalamus ventromedial nucleus (Jang et al., 2001; Bergquist et al., 2006; Dai et al., 2007). In contrast, H₃R activation does not modify depolarization-evoked [³H]-GABA release from rat thalamus and olfactory bulb slices (Garduño-Torres et al., 2007; Aquino-Miranda et al., 2011), indicating that not all GABAergic neurons express pre-synaptic H₃Rs.

**Neuropeptides.** Histamine inhibits non-adrenergic/non-cholinergic contraction of the bronchial and intestinal smooth muscle (Ichinose and Barnes, 1989; Taylor and Kilpatrick,
and H₃R activation reduces substance P release from sensorial nerve terminals of the rat hind paw (Ohkubo et al., 1995) and the release of the calcitonin gene-related peptide (CGRP) from the peri-arterial nerve terminals of the rat mesenteric artery (Sun et al., 2011).

### 5.2 Post-synaptic effects

There is evidence for post-synaptic H₃Rs in some areas of the brain, namely striatum, cerebral cortex, hippocampus, nucleus accumbens, lateral hypothalamus, and zona incerta (Pillot et al., 2002; Panula and Nuutinen, 2013, González-Sepúlveda et al., 2013; Parks et al., 2014).

#### 5.2.1 Activation of the MAPK and Akt pathways

The detection of 42/44-MAPK phosphorylation upon the activation of the rat H₃R₄₄₅ led to the discovery of the link between H₃Rs and the MAPK pathway (Drutel et al., 2001), which were also observed for native receptors (Mariottini et al., 2009). In rat hippocampus slices, H₃R activation stimulates the MAPK pathway in CA3 pyramidal cells, and MAPK activation seems to be required for H₃R-induced memory improvement and consolidation in rats after contextual fear conditioning (Giovannini et al., 2003).

In transfected SK-N-MC cells, hH₃R activation stimulates the activity of the Akt/GSK3β axis (Bongers et al., 2007c), and in rat cortical neurons H₃R stimulation results in the phosphorylation of Akt at Ser473 and GSK3β at Ser9, via PI3K and MAPK activation (Mariottini et al., 2009). Akt regulates the expression of apoptosis inhibitors such as Bcl-2 and Bcl-x, and thus promotes neuronal cell survival (Song et al., 2004), and in rat cortical neurons, H₃R activation increases the expression of Bcl-2 in an Akt-dependent manner. The
Akt/GSK3β pathway plays a relevant role in regulating several important cellular processes including cell plasticity and survival, proliferation and metabolism, and H₃R activation exerts a protective effect against serum deprivation-induced cell death in rat cortical neurons and NMDA-induced neurotoxicity in mixed cultures of mouse cortical cells through the activation of the PI3K-Akt pathway (Mariottini et al., 2009).

5.2.2 GIRK activation. In melanin-concentrating hormone (MCH)-producing neurons located in the rodent lateral hypothalamus, H₃R activation stimulates GIRKs and inhibits neuronal firing (Parks et al., 2014).

5.3 H₃R heterodimerization and functional consequences

GPCR dimerization is now a well-accepted phenomenon that can be defined as a transitory state in which, through protein-protein interactions, a GPCR can alter the binding, signaling or desensitization of the second GPCR that it is dimerized with. Although this phenomenon has been described as temporarily, short-lived, and the result of stochastic interactions, it has sufficient impact to cause or modify a physiological output (Milligan, 2006; Calebiro et al., 2013).

Because of its hetero-receptor nature, the H₃R is co-expressed with a large number of GPCRs in different neuronal populations. For example, in striatal medium-sized spiny neurons (MSNs), H₃Rs co-exist with dopamine D₁ (D₁R) or D₂ (D₂R) receptors (González-Sepúlveda et al., 2013), allowing for heterodimer formation. In both interactions, H₃R acts as a negative modulator of agonist binding to and signaling of dopamine receptors.

The first insight into H₃R/D₁R interactions came from functional studies where the H₃R inhibited D₁R-mediated facilitation of GABA release in SNr and striatum slices (Garcia et
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al., 1997; Arias-Montaño et al., 2001). In a heterologous expression system H₃R activation induces a shift from cooperative to a non-cooperative binding of D₁R agonists, which indicates that an intra-membrane cross-talk occurs between these receptors. In contrast, D₁R stimulation did not modify agonist binding to H₃Rs. A change in the D₁R signaling pathway from Gαₛ to Gαᵢ/o was also observed in cAMP formation assays. Furthermore, H₃R activation did not induce 42/44-MAPK phosphorylation, but did so when the D₁R was co-expressed. H₃R antagonist prevented the effect of a D₁R agonist and vice versa (Ferrada et al., 2009), and cross-antagonism in rat striatal slices of wild-type and D₁R-KO mice suggests that the interaction also occurs in vivo. The phosphorylation of 42/44-MAPKs appears to be a particular print of the H₃R/D₁R interaction, as this effect was not observed in D₂R-MSNs. In addition, behavioral analysis showed that a H₃R antagonist enhanced D₁R-induced locomotor activity (Ferrada et al., 2008; Moreno et al., 2011).

The H₃R/D₂R interaction is supported by Förster Resonance Energy Transfer (FRET) analysis in transfected cells and by binding studies in which H₃R activation reduced agonist affinity of the D₂R high- and low-affinity sites in sheep striatal membranes (Ferrada et al., 2008). However, the lack of synergism in D₂R- and H₃R-stimulated [³⁵S]-GTPγS binding in rat striatal membranes (Humbert-Claude et al., 2007) argues against a direct H₃R/D₂R interaction.

The Gαₛ-coupled A₂₅₄R is highly expressed and distinctively limited to D₂R-MSNs. A₂₅₄R activation inhibits GABA release from MSN collaterals (Kirk and Richardson, 1994), but in the striato-pallidal projections, facilitates the release of the neurotransmitter (Mayfield et al., 1993). The latter effect is functionally opposed by H₃R activation, which also decreases A₂₅₄R affinity for the agonist CGS-21680 in membranes from globus pallidus.
synaptosomes, suggesting a direct, protein-protein interaction between A2ARs and H3Rs (Morales-Figueroa et al., 2014).

6. H3Rs and neurological and psychiatric disorders

The H3R is a potential drug target for the treatment of several important neurological and psychiatric disorders and this aspect will be discussed briefly in reference to Alzheimer’s disease, attention-deficit hyperactivity disorder, Parkinson’s disease, Gilles de la Tourette’s syndrome, schizophrenia, addiction, and sleep disorders.

6.1 Parkinson’s disease (PD)

PD is a progressive neurodegenerative movement disorder that primarily results from the death of substantia nigra pars compacta (SNC) dopaminergic neurons. In addition, the noradrenergic, cholinergic and serotonergic systems are also affected and may contribute to the disorder. Parkinsonian symptoms include bradykinesia, muscular rigidity, rest tremor, and postural and gait impairment (Moore et al., 2005; Kalia and Lang, 2015).

As discussed previously, H3Rs modulate striatal GABAergic, glutamatergic and dopaminergic transmission at the pre- and post-synaptic levels in addition to controlling the release of GABA, glutamate, and 5-HT in several other nuclei of the basal ganglia. In hemiparkinsonian rats, H3R levels increase in the striatum and SNr ipsilateral to the lesioned SNC. Post-mortem analysis of PD patients shows increased histaminergic innervation to SN, augmented histamine levels in caudate/putamen, globus pallidus and SN, higher H3R density in the SNr and increased H3R mRNA in the external globus pallidus (reviewed in Panula and Nuutinen, 2013). Injection of the H3R agonist immepip into the SNr reverses apomorphine-induced contralateral turning behavior in hemiparkinsonian rats, whereas in
naive animals the compound induces ipsilateral turning (García-Ramírez et al., 2004). In hemiparkinsonian rats apomorphine-induced turning behavior is enhanced by the systemic administration of L-histidine and reduced by inhibiting histamine synthesis (Liu et al., 2008).

Dopamine-replacement therapy has dominated the treatment of PD motor symptoms since the early 1960s. However, chronic administration of the dopamine precursor L-DOPA results in severe side effects, particularly dyskinesias related to excessive D1R-mediated signaling in the basal ganglia (Santini et al., 2007). In parkinsonian marmosets the systemic administration of immepip exacerbates the symptoms in non-treated animals, but in L-DOPA-treated marmosets reduces the total dyskinesia score without affecting the anti-parkinsonian action (Gomez-Ramírez et al., 2006). Altogether, the available information indicates a role for histamine and the H3R in PD pathophysiology, and a potential use for drugs acting at the receptor in the treatment of both the disease and the complications of the pharmacological therapies.

6.2 Gilles de la Tourette’s syndrome (GTS)

GTS is a disorder characterized by motor and phonic tics (echolalia, echopraxia and coprolalia), sensory and cognitive symptoms, and co-morbidities such as obsessive-compulsive and attention-deficit hyperactivity disorders (Shan et al., 2015). GTS is related to the dysfunction of the cortico-striatal-thalamic-cortical circuitry, and involves the transmitters dopamine, noradrenaline, 5-HT, and histamine (Panula and Nuutinen, 2013; Rapanelli and Pittenger, 2015).
A mutation (W317X) in one of the HDC gene alleles is associated with the familiar occurrence of GTS (Ercan-Sencicek et al., 2010). HDC-knockout mice, with brain histamine levels almost negligible in homozygote animals, exhibit stereotypic movements and spontaneous tic-like symptoms after the intraperitoneal injection of amphetamine (Castellan Baldan et al., 2014), supporting the involvement of the histaminergic system in GTS.

6.3 Schizophrenia

Schizophrenia is a mental disorder defined by positive symptoms (hallucinations, delusions, and thought and movement disorders), negative symptoms (reduced feelings, difficulty to initiate and maintain activities, and reduced speaking), and cognitive symptoms such as poor understanding of information, trouble focusing, and problems with working memory (Flores et al., 2016).

In schizophrenic patients, H₃R receptor expression is increased in the prefrontal cortex and decreased in the hippocampus, areas closely related to memory and cognitive processes (Jin et al., 2009). An increase in the histamine metabolite tele-methylhistamine was reported for chronic patients (Prell et al., 1995), and antipsychotics drugs affect the activity of the histaminergic neurons by acting at dopamine, 5-HT, and glutamate (NMDA) receptors expressed by these neurons (Javitt et al., 1991; Morisset et al., 1999, 2002) and by antagonizing H₁, H₂, and H₃ receptors (Alves-Rodrigues et al., 1996; Green and Maayani, 1977; Richelson et al., 2000). This information suggests that the histaminergic system plays a role in the disease.
Schizophrenia is characterized by the hyperactivity of dopaminergic neurons and the hypoactivity of glutamatergic neuronal cells. H₃R antagonists/inverse agonists attenuate dopamine receptor-mediated facilitation of locomotor sensitization (Clapham and Kilpatrick, 1994) and locomotor hyperactivity resultant from blockade of NMDA receptors (Faucard et al., 2006; Mahamood et al., 2012). In DBA/2 mice, administration of a H₃R antagonist/inverse agonist reduces the natural deficits in sensorimotor gating (Fox et al., 2005). Although the evidence in animal models supports the antipsychotic properties of H₃R antagonists, two clinical trials with ABT-288 and MK-0249 failed to improve the cognitive function in schizophrenic patients (Egan et al., 2013; Haig et al., 2014).

6.4 Addiction

Addiction is a compulsive and persistent dependence on behaviors or substances in which the reward brain circuitry (the dopaminergic meso-cortico-limbic system) plays a major role. HDC-KO mice show increased cocaine tolerance and reduction in the place-preference test. Conversely, HDC-KO mice show an increase in alcohol-evoked conditioned place preference and consumption (Zimatkin and Anichtchik, 1999). Substances like alcohol, cocaine and morphine modulate histamine synthesis, release and turnover (Nishibori et al., 1985; Ito et al., 1997), suggesting the involvement of the histaminergic system in addiction processes. In H₃R-KO mice, a reduction in alcohol-induced place preference and consumption is observed, and a similar effect is induced by H₃R blockade, indicating the participation of the H₃R in alcohol addiction (Brabant et al., 2007; Nuutinen et al., 2010; Galici et al., 2011).
Hyperactivity caused by amphetamine/methamphetamine is attenuated by the H₃R antagonists thioperamide, ciproxifan, pitolisant (BF2.649), and ABT-239 (Clapham and Kilpatrick, 1994; Fox et al., 2005; Ligneau et al., 2007a,b; Motawaj and Arrang, 2011). However, other antagonists (GSK-207040, JNJ-5207852 and JNJ-10181457) failed to replicate this response (Komater, 2003; Southam et al., 2009). Moreover, thioperamide and clobenpropit increased methamphetamine self-administration (Munzar et al., 2004). On the basis of the previously described cross-talk between the dopaminergic and histaminergic systems, and the direct modulation of histaminergic transmission by addictive drugs, targeting the histaminergic neurons is a plausible therapeutic proposal.

6.5 Attention-deficit hyperactivity disorder (ADHD)

ADHD, most prevalent in children, is characterized by an ongoing pattern of inattention and/or hyperactivity-impulsivity. This disorder affects several neurotransmitter systems, mainly the dopaminergic, noradrenergic, cholinergic, and serotonergic systems (Vohora and Bhowmik, 2012). H₃R antagonists enhance the release of neurotransmitters involved in cognition including ACh and dopamine in the prefrontal cortex; ACh, dopamine and noradrenaline in the cingulate cortex; and ACh in the hippocampus (Fox et al., 2005; Medhurst et al., 2007; Ligneau et al., 2007b). In rodents, the pro-attentional and pro-cognitive actions of these drugs suggest that these compounds have a potential therapeutic use in this disorder (reviewed by Passani and Blandina, 2011, and Vohora and Bhowmik, 2012). However, the H₃R antagonist bavisant (JNJ-31001074) did not show clinical effectiveness in human adults with ADHD, whereas both atomoxetine and methylphenidate induced improvement (Weisler et al., 2012).
6.6 Alzheimer's disease

Alzheimer’s disease (AD) is a progressive neurodegenerative brain disorder resulting in the loss of memory and cognitive functions that is often accompanied by behavioral disturbances like aggression and depression (Querfurth and LaFerla, 2010). The pathological hallmarks of AD include the accumulation of the protein β-amyloid (Aβ), which leads to the production of extracellular Aβ plaques and hyper-phosphorylation of the protein tau. This in turn results in the formation of intracellular neurofibrillary tangles and the massive loss of cholinergic neurons (Brioni et al., 2011).

In AD patients, neurofibrillary tangles occur in the TMN along with a significant loss (~50%) of the histaminergic neurons (Nakamura et al., 1993, Shan et al., 2015), but the latter effect is not matched by the reduction (-24%) in HDC mRNA expression, suggesting that enhanced histamine production by the remaining neurons compensates the cell loss. Some reports indicate that brain histamine levels are reduced in hypothalamus, hippocampus and temporal cortex from AD patients, although others show an increase in the same cerebral regions together with frontal, parietal and occipital cortices. Furthermore, H₃R mRNA levels seem to be higher in the prefrontal cortex only in female AD patients, indicating a gender-dependent change (Shan et al., 2015). In this regard, differences in histamine levels in AD and normal brains may originate from methodological issues, genetic background, time elapsed between death and analysis, and proper diagnosis. However, measurement of histamine content with a sensitive HPLC fluorimetric method in brains from controls and AD patients, with matched age and post-mortem time, found a significant decrease in the hypothalamus (-58% of control values), hippocampus (-57%), and temporal cortex (-47%) in AD brains (Panula et al., 1998).
H₃R activation could account for the diminished release of noradrenaline and ACh in the prefrontal cortex, both transmitters with an important role in cognition (Blandina et al., 1996; Schlicker et al., 1999). Based on studies with transgenic mice, it may be hypothesized that neurotransmitter release evoked by H₃R antagonists leads to postsynaptic effects such as the phosphorylation of the cAMP response element binding protein (CREB), a transcription factor related to cognitive function, or the inhibition of GSK3β, responsible for tau hyper-phosphorylation in AD (Hooper et al., 2008; Bitner et al., 2011). These *in vivo* studies raise the possibility that H₃R antagonists indirectly modulate signaling pathways resulting in symptomatic alleviation in AD patients. H₃R antagonists like PF-03654746, GSK189254, MK-0249, ABT-239, and ABT-288 bind the hH₃R with high affinity (Ki 0.2 to 3.2 nM) and some have advanced to clinical trials (Brioni et al., 2011; Sadeq et al., 2016).

6.7 Sleep disorders

The brain circuitry that regulates sleep-wake cycle in mammals comprises cell groups in the thalamus, brainstem, hypothalamus and basal forebrain. The histaminergic system plays a key role in the maintenance of cortical activation and wakefulness (Lin, 2000). The histaminergic neurons fire tonically, are more active during wakefulness, and send widespread inputs to areas crucially implicated in sleep-wake control, for instance the cerebral cortex and thalamus. In the latter region, H₁R activation decreases a resting leak K⁺ conductance (I_{KL}), which results in prolonged depolarization of thalamo-cortical neurons leading to inhibition of burst firing and the promotion of single-spike firing. This action abolishes sleep-related activity in thalamo-cortical networks and facilitates the single-spike activity typical of the waking state (McCormick and Bal, 1997).
Impaired wakefulness and increased sleep are observed after the inhibition of histamine synthesis and in HDC-KO mice (Partmentier et al., 2002). The wake-promoting activity of histamine is shared by a variety of H₃R antagonists/inverse agonists (Gao et al., 2013). H₃Rs pre-synaptically modulate the release of histamine and other neurotransmitters that participate in the wake-sleep cycle and post-synaptically reduce the excitability of MCH-producing neurons that are also involved in the cycle (Parks et al., 2014). H₃R-KO mice present a wide-range of apparently contradictory wake-sleep alterations; the animals show wake deficiency and sleep deterioration, but also display signs of enhanced vigilance (Gondard et al., 2013).

Histaminergic neurons are activated by the neuropeptide hypocretin, and type I narcolepsy is characterized by excessive daytime sleepiness and cataplexy. The main cause of type I narcolepsy is the loss of hypocretinergic neurons; however, an increase in the number of histaminergic neurons has been reported (Valko et al., 2013). Although histamine dysregulation is not central to the pathophysiology, H₃R antagonists/inverse agonists such as pitolisant are useful for the treatment of excessive sleepiness disorders, namely narcolepsy and idiopathic hypersomnia by improving alertness (Lin et al., 2008; Inocente et al., 2012; Dauvilliers et al., 2013). Furthermore, pitolisant reduces sleepiness in PD patients without interfering with anti-parkinsonian drugs (Schwartz, 2011). On March 2016, pitolisant (Wakix™) was approved in the European Union for the treatment of narcolepsy with or without cataplexy in adults, becoming thus the first drug acting at H₃Rs that reaches the market (Syed, 2016).
7. Final remarks

The histaminergic system exerts a significant modulatory effect on different brain functions, from movement to cognitive processes. The H₃R plays a key role in this effect through its wide expression and action as a modulator of several neurotransmitter systems. Although the main effects of H₃R activation are known for some brain areas, such as the striatum and the hippocampus, its actions in other areas have yet to be fully explored. Furthermore, determining the precise H₃R expression in distinct cells of several neuronal circuits, such as the cortico-striato-thalamo-cortical and meso-cortico-limbic circuits, will help to understand the participation of the histaminergic system in aspects such as the control of motor activity, cognition and the sleep-wake cycle, and certain disorders, including PD, AD, and addiction. A deeper knowledge of the functional and molecular interactions of H₃Rs with other receptors is also important for the design of novel therapeutic approaches targeting the histaminergic system, and for this purpose the elucidation of the H₃R crystal structure will represent an important discovery. Finally, more clinical studies are needed to evaluate or confirm the usefulness of H₃R ligands in several neuro-psychiatric disorders.
8. Acknowledgements

G. Nieto-Alamilla, R. Márquez-Gómez, A.-M. García-Gálvez and G.-E. Morales-Figueroa received Conacyt scholarships. G. Nieto-Alamilla is a fellow of Mexico State Council for Science and Technology (Comecyt). We offer our apologies to all the authors whose work was not included in this review.
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9. Authorship contribution

Wrote or contributed to the writing of the manuscript: Nieto-Alamilla, Márquez-Gómez, García-Gálvez, Morales-Figueroa and Arias-Montaño
10. References


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Footnotes

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Legends for figures

Figure 1. The histaminergic system in the rat brain. Histamine-synthesizing neurons are located in the hypothalamus tuberomammillary nucleus and these neurons send projections to the Central Nervous System through three major pathways, two ascending bundles that innervate the forebrain structures and one descending bundle reaching the spinal cord. Thal, thalamus; Str, striatum.

Figure 2. Alignment of the amino acid sequence of the human, monkey, guinea-pig, rat and mouse H3Rs. Sequences were obtained from Uniprot and correspond to the full length H3R (445 aa). Alignment was performed with the Clustal Omega server from the European Bioinformatic Institute. The highly conserved sequences in the seven transmembrane domains are shown in grey. The DRF and NPVLY motifs and the glycosylation site (Asn11) are well-conserved among species (green). Residues in blue are responsible for the inter-species differences in H3R pharmacology. Residues in red are likely to be involved in the receptor high constitutive activity on the basis of their identity with a mutated β2-adrenoceptor. The third intracellular loop (ICL3) possesses a region (residues 236 to 300; italics) that is highly variable among species, followed by a highly conserved sequence. The hH3R has ≥ 93% sequence identity with the other species, and the same identity can be found in the guinea-pig, mouse and rat sequences, with 99% identity between mouse and rat, and among the primate proteins. *, conserved residues; :, conservative mutations; •, non-conservative mutations.
Figure 3. Structure of the human H₃R. As a GPCR, the H₃R contains seven spanning transmembrane domains, an extracellular amino terminus (NT), an intracellular carboxyl terminus (CT), three extracellular (ECL), and three intracellular (ICL) loops, with a long ICL3 (142 aa). Potential residues for phosphorylation are shown in green. The conserved DRF and NPVLY motifs are shown in yellow. The naturally occurring mutations D18E and A280V are depicted in blue. The glycosylation site (Asn11) is shown in red and the palmitoylation site (C228) in grey. Residues Thr119 and Ala122 (purple) are responsible for the inter-species pharmacological differences. Residues indicated in black in ECL1 and ECL2 correspond to the cysteins forming a disulfide bond important for receptor trafficking. Residues important for agonist recognition are colored in pink.

Figure 4. H₃R signaling pathways. H₃R activation triggers or modulates several pathways through the Gα subunits and Gβγ complexes of Gαi/o proteins. AA, arachidonic acid; AC, adenylyl cyclases; cAMP, 3',5'-cyclic adenosine monophosphate; MAPK, mitogen-activated protein kinases; NHE, Na⁺/H⁺ exchanger; PI3K, phosphatidylinositol 3-kinase; PKA, protein kinase A; PLA₂, phospholipase A₂; PLC, phospholipase C.
Figure 5. Binding sites of agonists and antagonists at the human H₃R of 445 aa (hH₃R₄₄₅).

A. Molecular docking of the endogenous agonist histamine in a model of the hH₃R₄₄₅ in the active state. Residues Glu206 and Asp114 are pivotal for agonist binding. B. Planar view of the histamine binding pocket in the hH₃R₄₄₅. C. Proposed binding site of a protean ligand (proxyfan) in the inactive hH₃R₄₄₅ as observed by molecular docking, depicting the three functional groups of the ligand important for binding (nitrogen-containing ring, central electronegative group and aromatic ring). D. Planar view of the suggested binding residues for an antagonist at the hH₃R₄₄₅.

Figure 6. Structure of H₃R agonists.

Figure 7. Structure of H₃R antagonists.
Table 1. Brain expression and signaling of H3R isoforms from different species.

<table>
<thead>
<tr>
<th>Species</th>
<th>Isoform (aa)</th>
<th>Expression in the CNS</th>
<th>Signaling</th>
<th>References</th>
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<td>Human</td>
<td>H₃(445)</td>
<td>thalamus, striatum, cerebral cortex, cerebellum, amygdala, substantia nigra, hippocampus, hypothalamus, corpus callosum, spinal cord</td>
<td>↓ cAMP, ↑ [Ca²⁺]ᵢ, ↑ p42/p44-MAPK, ↑ [³⁵S]-GTPγS binding</td>
<td>1-10</td>
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<tr>
<td></td>
<td>H₃(431)</td>
<td>amygdala, cerebellum, striatum, thalamus, prefrontal cortex, striatum,</td>
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<tr>
<td></td>
<td>H₃(415)</td>
<td>thalamus, cerebellum, amygdala</td>
<td>↑ [Ca²⁺]ᵢ</td>
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<td></td>
<td>H₃(365)</td>
<td>cerebellum, thalamus, hypothalamus, striatum, substantia nigra, hippocampus, amygdala, prefrontal cortex</td>
<td>↑ [Ca²⁺]ᵢ, ↓ cAMP, ↑ [³⁵S]-GTPγS binding</td>
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</tr>
<tr>
<td></td>
<td>H₃(329a)</td>
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<td>NF</td>
<td></td>
</tr>
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<td></td>
<td>H₃(326)</td>
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<td></td>
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<td>NF</td>
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<td>H₃(409)</td>
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<tr>
<td></td>
<td>H₃(395)</td>
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<td>H₃(379)</td>
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<td></td>
<td>H₃(293)</td>
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<td></td>
<td>H₃(290)</td>
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<td>H₃(351)</td>
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<td>H₃(340)</td>
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<td>Monkey</td>
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<td>H₃(413)</td>
<td>frontal cortex, parietal cortex, para-hippocampal gyrus, caudate nucleus, putamen, amygdala, thalamus, cerebellum</td>
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<td>H₃(410)</td>
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<tr>
<td></td>
<td>H₃(335)</td>
<td>frontal cortex, parietal cortex, occipital cortex, para-hippocampal gyrus, hippocampus, caudate nucleus, putamen, amygdala, thalamus, cerebellum</td>
<td>NF</td>
<td></td>
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<tr>
<td>Rat</td>
<td>H₃(445)</td>
<td>cerebral cortex, hippocampus, midbrain, hypothalamus, striatum, brainstem, cerebellum, olfactory tuberculum, spinal cord</td>
<td>↓ cAMP, ↑ p42/p44-MAPK</td>
<td>13-17</td>
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<tr>
<td></td>
<td>↓ [Ca(^{2+})]</td>
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<td>↓ cAMP, ↑ p42/p44-MAPK</td>
<td>↑ [(^{3}H)] AA release</td>
<td>↑[^{35}S]-GTP(\gamma)S binding</td>
<td>ND</td>
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↑, increase; ↓, decrease; ND, not determined; NF, non-functional; [Ca\(^{2+}\)], intracellular Ca\(^{2+}\) concentration. AA, arachidonic acid. *The human H3R329a and H3R329b isoforms possess the same number of amino acids, but differ in the region where alternative splicing occurs.
Table 2. Affinities of the H₃R for agonists and antagonists.

<table>
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<th>Imidazole-containing agonists</th>
<th>pKi</th>
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<th>Dog</th>
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<tr>
<td>Histamine</td>
<td>Native</td>
<td>8.27¹</td>
<td>8.28²</td>
<td>7.92³</td>
<td>6.09 ± 0.61 (low)¹¹</td>
<td>7.92 ± 0.54 (high)¹¹</td>
<td>7.58¹</td>
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<td>8.57⁶</td>
<td>7.69⁸</td>
<td>5.70 ± 0.15⁹</td>
<td>7.89 ± 0.07¹⁰</td>
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<tr>
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<td>Native</td>
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<td>8.82³</td>
<td>8.71 ± 0.08⁴</td>
<td>8.95⁷</td>
<td>8.71 ± 0.08²¹</td>
<td>7.91 ± 0.09 (high)¹¹</td>
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<td>8.72³</td>
<td>8.44⁸</td>
<td>6.40 ± 0.20⁹</td>
<td>8.62 ± 0.07¹⁰</td>
<td>9.35 ± 0.20³¹</td>
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<td>SAMH</td>
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<td>7.33 ± 0.06¹</td>
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<td>5.93 ± 0.04¹¹</td>
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<td>7.63 ± 0.18¹</td>
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<tr>
<td>NAMH</td>
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<td>8.92³</td>
<td>8.78 ± 0.13³</td>
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<td>8.67 ± 0.27¹³</td>
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<td>10.31 ± 0.24²²</td>
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<td>Dog</td>
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<td><strong>Immepip</strong></td>
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<td>8.99 ± 0.20⁴</td>
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<td>10.00³</td>
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<td><strong>Impentamine</strong></td>
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### Table 3. Potencies of H3R agonists and antagonists.

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Abbreviations for species: gp, guinea pig; h, human; mk, monkey; r, rat.
Figure 6

- Histamine
- Nα-methylhistamine
- Rα-methylhistamine
- Sα-methylhistamine
- Immepip
- Imetit
- Impentamine
- Proxyfan
- Methimepip
- VUF-5296
- VUF-5297
Figure 7

- Thioperamide
- Ciproxyfan
- Iodoproxyfan
- Clobenpropit
- Iodophenpropit
- VUF-5861
- Burimamide
- SCH-79867
- Pitolisant
- JNJ-5207852
- ABT-239
- NNC-38-1049
- A-331440
- A-349821
- GSK-189254
- UCL-1972