REGULATION OF HISTAMINE SYNTHESIS AND RELEASE IN THE CENTRAL NERVOUS SYSTEM BY H_3 RECEPTOR TRANSDUCTION MECHANISMS

Jordi Gómez Ramírez

Universitat Autònoma de Barcelona, Juny de 2002

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Memoria presentada per Jordi Gómez Ramírez, Llicenciat en Bioquímica, per optar al grau de Doctor per la Universitat Autònoma de Barcelona.

Aquest treball ha estat realitzat a la Unitat de Bioquímica de la Facultat de Medicina del Departament de Bioquímica i de Biologia Molecular de la Universitat Autònoma de Barcelona, sota la direcció dels Doctors Isaac Blanco Fernández i Jordi Ortiz de Pablo.

Dr. Isaac Blanco Fernandez   Dr. Jordi Ortiz de Pablo

Jordi Gómez Ramírez

Universitat Autònoma de Barcelona, Juny de 2002

Three Rings for the Elven-kings under the sky,
Seven for the Dwarf-lords in their halls of stone,
Nine for Mortal Men doomed to die,
One for the Dark Lord on his dark throne
In the Land of Mordor where the Shadows lie.
One Ring to rule them all, One Ring to find them,
One Ring to bring them all and in the darkness bind them
In the Land of Mordor where the Shadows lie.

The Lord of the Rings (J.R.R. Tolkien)

Caminante, son tus huellas
el camino y nada más;
caminante, no hay camino,
se hace camino al andar.

Al andar se hace camino,
y al volver la vista atrás
se ve la senda que nunca
se ha de volver a pisar.

Caminante, no hay camino
sino estelas en la mar

(A. Machado)

A mis padres por su esfuerzo y apoyo incondicionales

A la Raquel, per donar-me el seu amor i comprenssió.

Vull expressar el meu més sincer agraïment als doctors Isaac Blanco i Jordi Ortiz, directors d'aquesta tesi, per haver confiat en mi des del primer moment, i per les seves enssenyances, suggerencies i ajudes durant els anys que hem treballat junts. També vull expressar la meva gratitut a la meva companya de grup Anna Torrent, per les hores de feina compartides, treballant colze a colze, en el laboratori, i per les llargues i profitoses converses parlant del mon de la histamina en el nostre despatx.

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ABBREVIATIONS

α-FMH	alfa-fluoromethylhistidine
ω-CTX	omega-conotoxin GVIA
AC	adenylate cyclase
cAMP	cyclic adenosine 3':5'-monophosphate
Clobenpropit	N-(4-chlorobenzyl)-S-[3-((5)-imidazolyl)propyl] isothiourea
CNS	central nervous system
db-cAMP	dibutyryl-cAMP
dd-forskolin	dideoxy-forskolin
H_1, H_2, H_3, H_4	H_1, H_2, H_3 and H_4 histamine receptors
HA	histamine
HDC	histidine decarboxylase
HIS	histidine
HMT	histamine-N-methyl-transferase
HPLC	high performance liquid chromatography
IBMX	3-isobutyl-1-methylxanthine
Imetit	5-[2-(imidazol-4-yl)ethyl]isothiourea
KRM	krebs-ringer bicarbonate medium
P1, P2, P3	crude nuclear, crude mithocondrial and microsomal fractions
P-5P	pyridoxal 5-phosphate
PKA	cAMP-dependent protein kinase
PKI_{14-22}	myristoylated cAMP-dependent protein kinase inhibor 14-22 amide
PMSF	phenyl-methyl-sulfonyl-fluoride
Rp-cAMPS	Rp-adenosine 3',5'-cyclic monophosphothioate triethylamine
Thioperamide	N-cyclohexyl-4-(imidazol-4-yl)-1-piperidinecarbothioamide
TMn	tuberomammillary nuclei

I. INTRODUCTION

I. INTRODUCTION

1. NEUROCHEMISTRY OF HISTAMINE

Histamine [or 2-(4-imidazolyl)ethylamine] was firstly recognized as a messenger molecule in cell-to-cell communication at the beginning of the XX century by Sir Henry Dale (Barger and Dale, 1910). Since then, many studies have demonstrated its important role in the regulation of several physiological processes such as neurotransmission (Schwartz et al., 1991; Brown et al., 2001), gastric secretion (Kahlson et al., 1971), local vasomotor control (Beaven et al., 1978) and inflammation responses (Hirasawa et al., 1987).

This compound belongs to the family of biogenic amines such as dopamine, norepinephrine and serotonin. All of them present a common feature which is the presence of an ethylamine “backbone” in their chemical structure. In the case of histamine, the imidazole nucleus, absent from other amines, endows histamine with several distinct chemical properties. Among these properties there is its prototypic tautomerism, a property which permits histamine to exist in two different chemical forms, is thought to be critical in the ability of this molecule to activate some of its receptors (Figure 1).

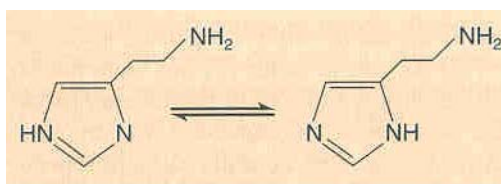


Figure 1. Chemical structure of histamine, illustrating the two tautomeric forms.

Brain Histamine, similarly to other neurotransmitters is subordinated to the processes of: biosynthesis, storage, release, uptake and metabolism.

1.1. Biosynthesis

Because of its poor penetration through the blood brain barrier, the whole of brain histamine is synthetized in the Central Nervous System (CNS). The most important cellular stores correspond to histaminergic neurons (Schayer et al., 1957) and mast cells (Snyder and Taylor, 1972; Picatoste et al., 1977). Nevertheless, histamine

synthesis has also been described in cultured microglial cells (Katoh et al., 2001) and microvascular endothelial cells (Yamakami et al., 2000). Indeed, in vivo formation of radioactive histamine in brain was detected after administration of its radioactive precursor L-histidine in rats and other animals (Schayer et al., 1957; Adam et al., 1964; Pollard et al., 1974). Histamine synthesis involve two steps: transport of His into the cell, and its decarboxylation by the enzyme histidine decarboxylase (HDC) (Figure 2).

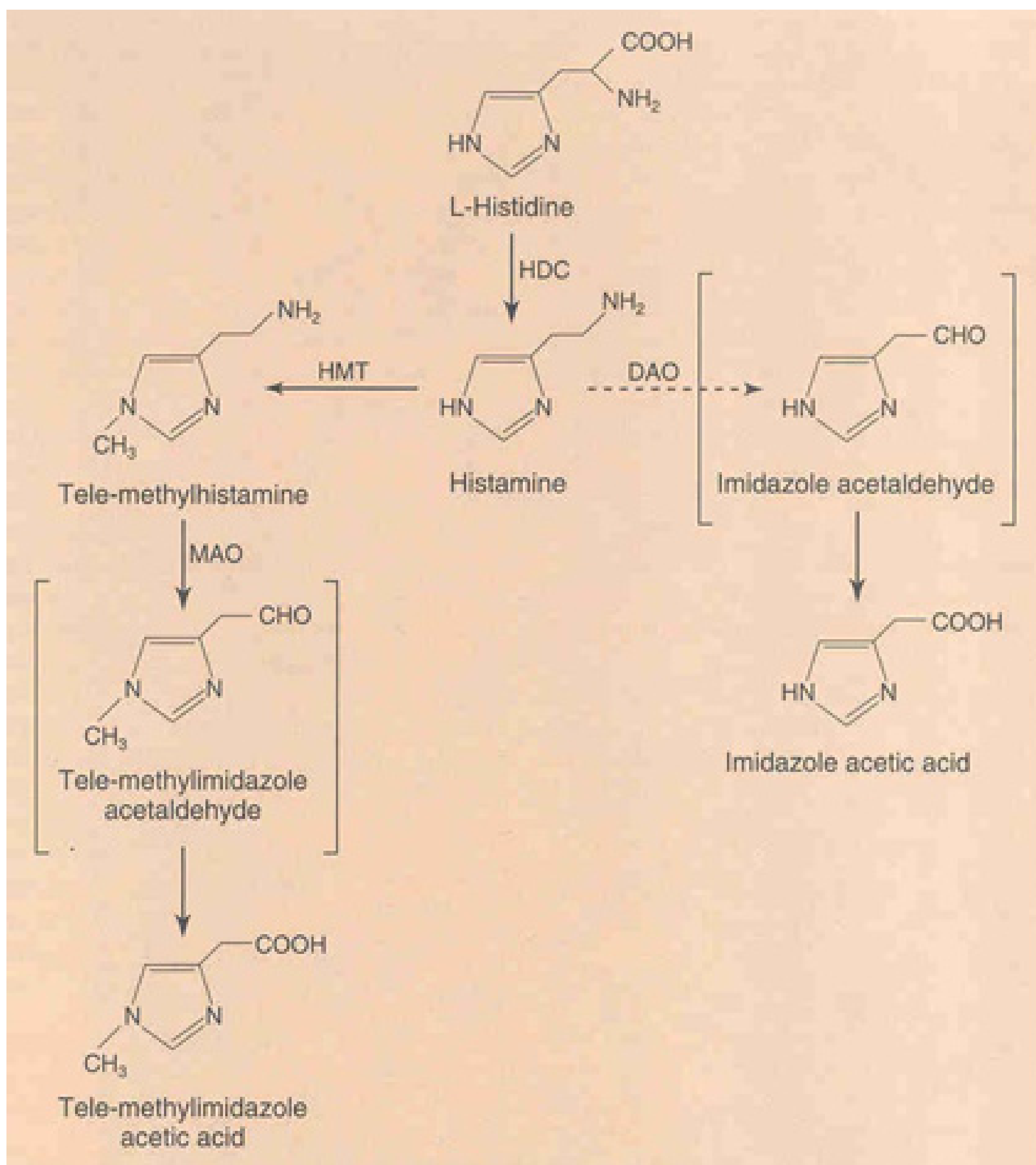


Figure 2. Synthesis and metabolism of histamine. Solid lines indicate the pathways for histamine formation and catabolism in brain. Dashed lines show additional pathways that can occur outside of the nervous system. HDC, histidine decarboxylase; HMT, histamine N-methyltransferase; DAO, diamine oxidase; MAO, monoamine oxidase. Aldehyde intermediates, shown in brackets, have been hypothesized but not isolated.

1.1.2. Histidine Transport

There is not yet evidences for the presence of a specific His transport system in histaminergic neurons. Nevertheless, several studies have been described a saturable, energy-dependent His uptake in brain slices and synaptosomes (Chudomelka and Murin, 1983; Hegstrand et al., 1985).

1.1.3. HISTIDINE DECARBOXYLASE

L-Histidine decarboxylase (EC 4.1.1.22) is responsible for the one-step HA formation in brain through decarboxylation of the natural amino acid L-Histidine. Similarly to other mammalian decarboxylases, HDC uses the pyridoxal-5'-phosphate (P-5P) as a cofactor of which approximately 50% is bound to the apoenzyme (Palacios et al., 1978).

The enzyme has been purified from various peripheral tissues such as fetal liver (Tran et al., 1981; Watanabe et al., 1982), kidney (Martin and Bishop, 1986) or gastric mucosa (Savany and Cronenberger, 1982). From most studies the enzyme appeared as a protein of 110-125 kDa constituted of two identical subunits of 55-60 kDa with an isoelectric point of 5.5. Subsequently, the complete amino acid sequence of rat HDC was deducted from the cloning, from a fetal liver library, of a cDNA encoding the enzyme (Joseph et al., 1990). The enzyme comprises 655 amino acid residues (Figure 3), corresponding to a protein of 74 kDa, a value significantly higher than the 55-60 kDa values found after purification of the subunits. Additional studies suggest that rat HDC may be produced as an enzymatically inactive proenzyme which is posttranslational processed to give rise to the active subunit of 55 kDa (Dartsch et al., 1998). Subsequent analysis of the rat HDC sequence indicated that the enzyme displays distinct homologies with other pyridoxal phosphate-dependent enzymes, such as DOPA decarboxylase, particularly in the region surrounding the putative cofactor-binding lysine residue (Lys^{307}) (Joseph et al., 1990). Moreover, the rat sequence contains two potential N-glycosilation sites and two consensus phosphorylation sites of cAMP-dependent protein kinase, suggesting that these potential modulatory sites might contribute to the regulation of HDC actvity (Figure 3). The rat HDC gene has been located to mouse chromosome 2, where it is closely located to the β2-microglobulin gene (Joseph et al., 1990).

Kinetically, L-histidine decarboxylase displays a high substrate specificity towards histidine. The enzyme decarboxylates the amino acid with a Michaelis constant (K_m)

and a maximum rate (V_{max}) that change with the pH and ionic strength of the medium (Schwartz et al., 1970; Palacios et al., 1978).

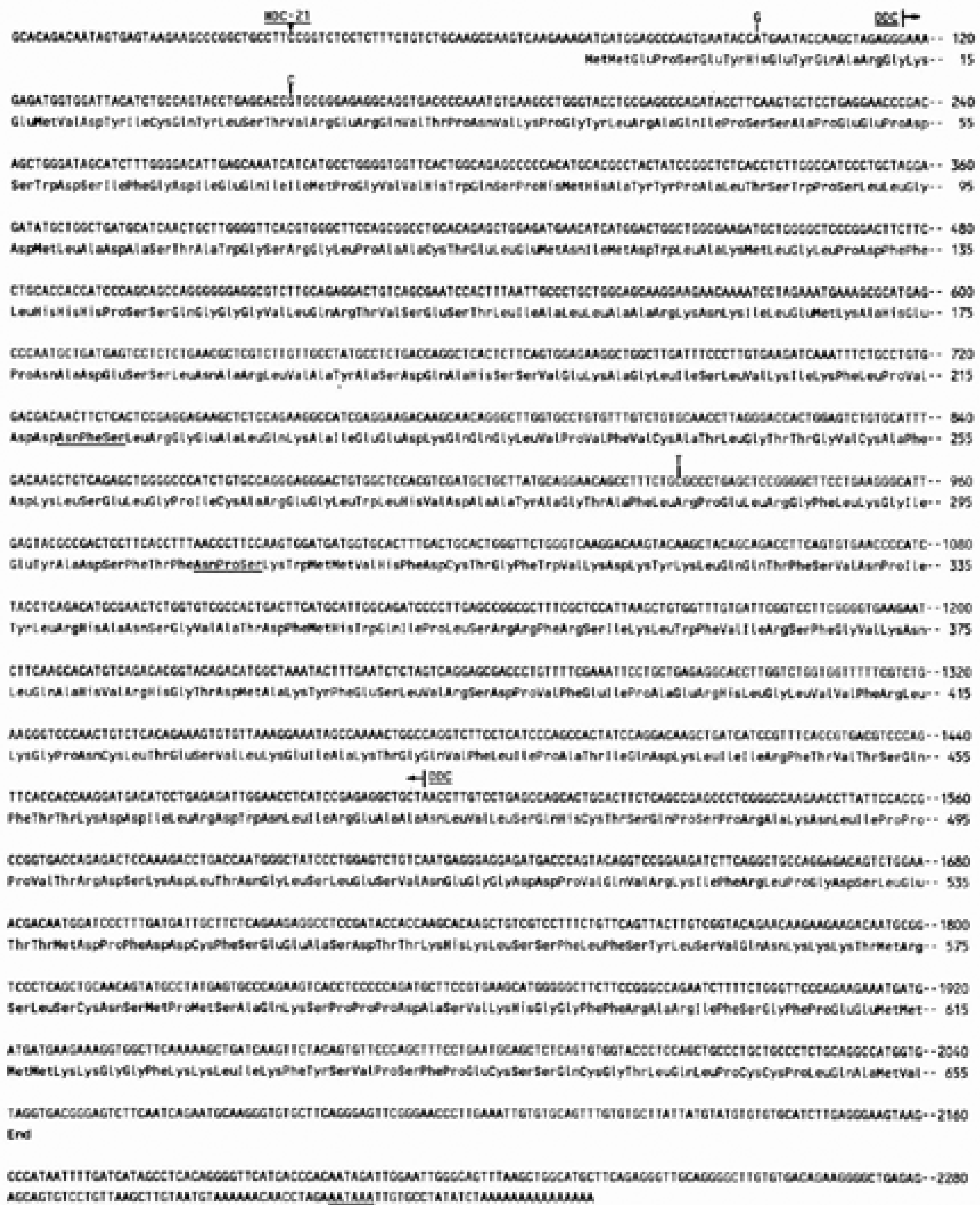


Figure 3. Nucleotide sequence and deduced amino acid sequence of clone HDC-18. The nucleotide and amino acid residue numbers are indicated at the right. The region that is homologous with the gene for *Drosophila* DDC is indicated above the nucleotide sequence. A consensus poly(A) addition signall (AATAAA) is underlined. The initial methionine is the first methionine residue following the putative transcription start site in the gene. The arrowhead marks the first residue of clone HDC-21. Clone HDC-2,3 begins at residue 118. The letters above the nucleotide sequence denote the differences in some HDC cDNA clones. Potential sites of N-glycosilation [Asn-Xaa-(Thr or Ser)] are underlined. The site at residue 304 is adjacent to the putative P-5P-binding site (Lys[307]) and would likely inactivate the enzyme if glycosilated. Two sequences fitting

consensus phosphorylation sites of cAMP-dependent protein kinse are located at residues 357-362 and 338-343. The nucleotide sequence was determined on both strands. *(figure extracted from Joseph et al., 1990)*

Biochemical and immunohistochemical studies have mainly revealed the presence of HDC in the cytoplasm of histaminergic neurons. L-Histidine decarboxylase activity is distributed in a markedly heterogeneous fashion between cerebral regions, with the highest levels being found in hypothalamus, the lowest levels in cerebellum, and intermediate levels in telencephalic areas. This regional distribution of HDC activity is generally consistent with data derived from immunohistochemical studies (Tran and Snyder, 1981).

Subcellular fractionation studies indicate that HDC is mainly found in the cytoplasm of isolated nerve endings (Toledo et al., 1988), a conclusion confirmed by immunohistochemical studies at the electron-microscopic level (Hayashi et al., 1984)

Although histaminergic neurons constitute the major localization of HDC, small fractions of cerebral enzyme are held in mast cells (Martres et al., 1975).

It have been described several kind of L-Histidine decarboxylase inhibitors. The compound which has become a useful tool to investigate the role of histamine is S-α-fluoromethylhistamine (α-FMH), which acts as a potential suicide or catalytic constant inhibitor of HDC (Kollonitsch et al., 1978). This compound potently inhibits in a stereoselective, time-dependent, concentration-dependent and irreversible manner cerebral HDC with an inhibitory constant (K_i) of approximately 10^{-5} M. Related decarboxylases such as dopa- or glutamate-decarboxylase are not significantly affected by this inhibitor (Skratt et al., 1994; Prell et al., 1996).

1.2. Storage

A variety of biochemical, histochemical and pharmacological approaches strongly indicate that the cerebral histamine is mainly held in two classes of cellular stores, neurons and mast cells.

Cerebral mast cell number changes during the animal development. In young animals, there exist an elevated number of mast cells which contributes significantly to the total histamine brain content. In adult animals, mast cells are scarce although their high histamine content also significantly contribute to the overall amine content, particularly in certain brain regions such as thalamus (Ferrer et al., 1979; Blanco et al., 1987).

Data from subcellular fractionation studies show an atypical distribution of histamine content compared to other amines (Kataoka and De Robertis, 1967; Kuhar et al., 1971). This different distribution is due to the cerebral mast cells histamine content (Picatoste et al., 1977). In mast cells, HA is located in large granules that sediment with the crude nuclear fraction (P1). In histaminergic neurons, histamine is located in vesicles from nerve endings that sediment with the crude mithocondrial (P2) and microsomal (P3) fractions which both contain synaptosomes.

Taking into account that within the P2 and P3 fractions histamine is largely bound to vesicles whereas HDC is mainly soluble, these results suggest that HA, similar to other neurotransmitters, is synthesyzed in the cytoplasm of nerve endings and thereafter bound to synaptic vesicles.

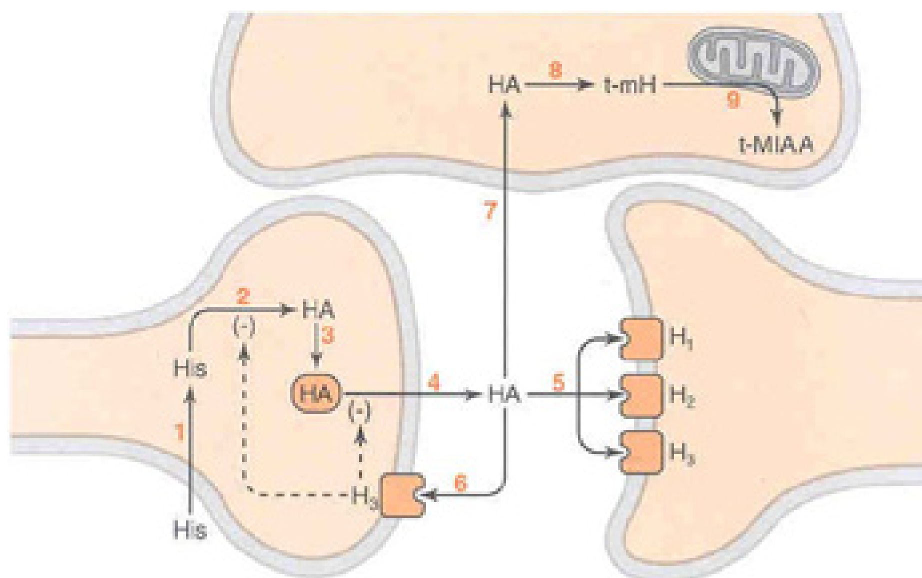


Figure 4. Dynamics of neuronal histamine. Steps in the synthesis, release and metabolism of histamine are shown: **1**, L-histidine (His) transport into nerve terminal; **2**, histamine (HA) synthesis by histidine decarboxylase; **3**, formation of histamine containing vesicles; **4**, histamine release by exocytosis; **5**, activation of post-synaptic receptors; **6**, feedback inhibition of histamine synthesis and release by H3 autoreceptors; **7**, histamine transport by astrocytes; **8**, metabolism by histamine-N-methyltransferase (HMT); **9**, oxidation of t-MH by monoamine oxidase-B. The cellular localization of steps **7-9** remain poorly understood. *t*-MH, *tele*-methylhistamine; *t*-MIAA, *tele*-methylimidazoleacetic acid.

1.3. Release

Studies on slices from various brain areas have revealed that tritiated histamine synthesized from [^{3}H]-His is released by depolarizations induced by potassium (Verdiere et al., 1975), electrical stimuli (Van der Werf et al., 1987) or veratridine (Arrang et al., 1985). This release is completely inhibited in the absence of Ca^{2+} or in the presence of 10 mM Mg^{2+}, suggesting that it results from the opening of voltage-

sensitive Ca^{2+} channels (Arrang et al., 1983). All these features are consistent with the idea that depolarization of histaminergic nerve endings induces HA secretion via mechanisms similar to those operating for other neurotransmitters.

Additional studies have revealed the modulation of histamine release by autoreceptors and heteroreceptors. It has been shown that stimulation of H3 receptors (Arrang et al., 1983), muscarinic M1 receptors (Gulat-Marnay et al., 1989A), α_2-adrenoreceptors (Hill et al., 1988), $GABA_B$ receptors (Okakura-Mochizuki et al., 1996) and opioid *k*-receptors (Gulat-Marnay et al., 1990) inhibits histamine release in slices and synaptosomes. Conversely, stimulation of μ-opiate receptors (Itoh et al., 1988) increases histamine release.

1.4. Uptake and Metabolism

Several studies with brain slices or synaptosomes suggest that no significant high-affinity uptake ocurrs at histaminergic nerve endings. However, it has been described the existence of histamine uptake in glial cells such as astrocytes (Huszti et al., 1994 and 1998). In this case, histaminergic neurons appear to be almost unique among monoaminergic neurons in that they lack a high-affinity reuptake system.

In general, histamine metabolism occurs mainly by two pathways: oxidation (Reilly et al., 1971) and methylation (Schayer R.W., 1956; Pollard et al., 1974). The oxidation is carried out by diamine oxidase (DAO, E.C. 1.4.3.6) leading to imidazole acetic acid (IAA) which can conjugate to form a riboside or ribotide complex. The methylation is produced by histamine N-methyltransferase (HMT, E.C.2.1.1.8) producing *tele*-methylhistamine (*t*-MHA). *t*-MHA is further metabolized by MAO-B, producing *tele*-methylimidazole acetic acid (*t*-MIAA). In brain, histamine is almost exclusively metabolized through methylation pathway because of DAO activity has not been detected in mammalian brains (Burkard et al., 1963).

2. HISTAMINERGIC NEURONS

2.1. Distribution

The precise localization of histaminergic neurons has been established with the advent of several reliable immunohistochemical tools such as antibodies raised against histamine (Panula et al., 1984) or its biosynthetic enzyme (Watanabe et al., 1984). High-affinity polyclonal antibodies against highly purified preparation of HDC obtained

from rat fetal liver have been used. Similarly, polyclonal antibodies to HA raised against the amine either simply mixed with serum albumin or conjugated to proteins using aldehydes or carbodiimide have also been used as histochemical tools.

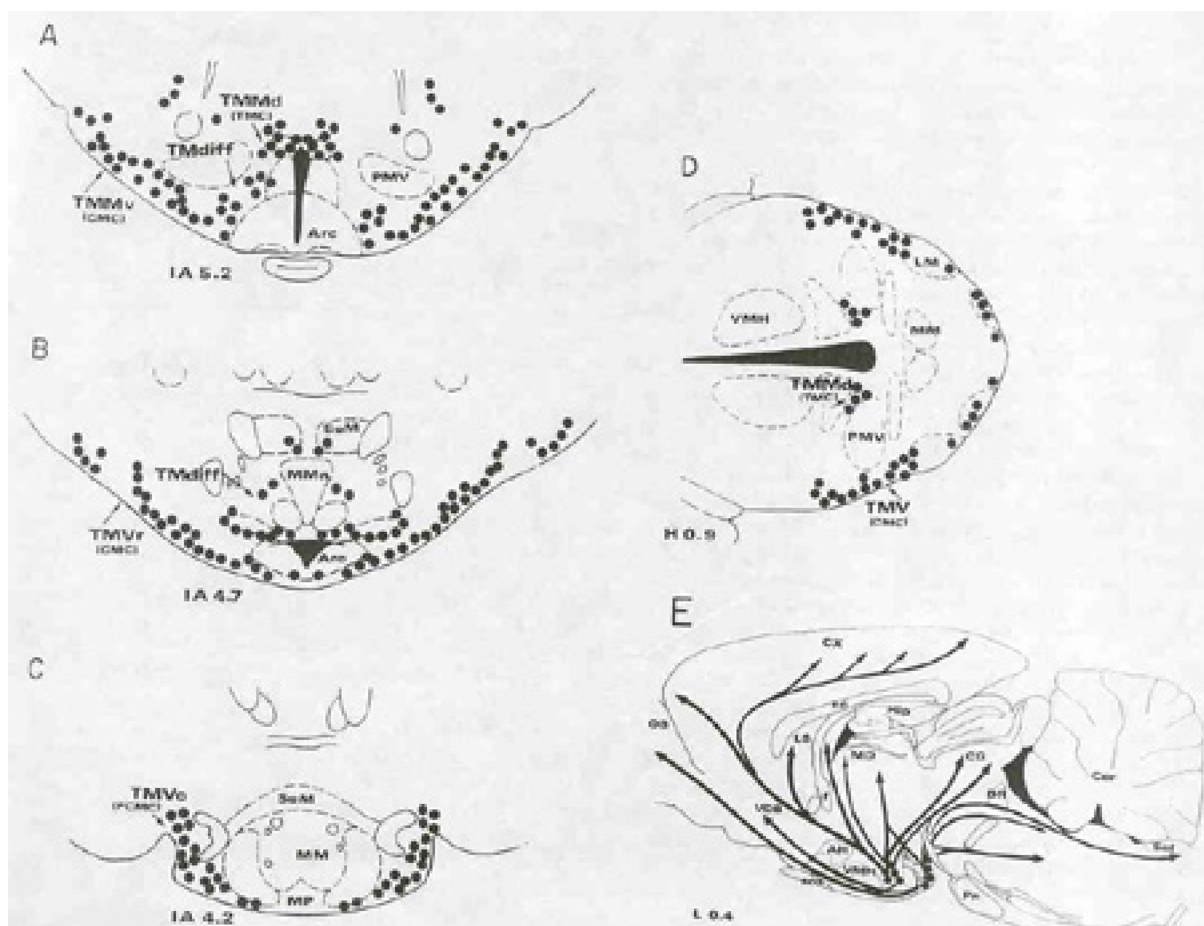


Figure 5. Localization of histaminergic perikarya (closed circles) in tuberomammillary nuclei and disposition of main histaminergic pathways (arrows) in rat brain. A, B and C: frontal sections at indicated levels of caudal hypothalamus. D: horizontal section through caudal hypothalamus. E: sagital section of brain. Abbreviation of perikarya subgroups: CMC, caudal magnocellular nucleus; PCMC, posterior caudal magnocellular nucleus; TMC, tuberal magnocellular nucleus; TMdiff, tuberomammillary nucleus diffuse part; TMMd, medial tuberomammillary subgroup dorsal part; TMMv, medial tuberomammillary subgroup caudal part; TMV ventral tuberomammillary subgroup; TMVc, ventral tuberomammillary subgroup caudal part; TMVr, ventral tuberomammillary subgroup rostral part. Other abbreviations: AH, anterior hypothalamic area; Arc, arcuate nucleus; cc, corpus callosum; Cer, cerebellum; CB, central gray; CX, cerebral cortex; DR, dorsal raphe nucleus; f, fornix; Hip, hippocampus, LM, lateral mammillary nucleus; LS, lateral septum; MD, mediodorsal thalamus; MM, medial mammillary nucleus medial part; MMn, medial mammillary nucleus medial part; MP, medial mammillary nucleus posterior part; OB, olfactory bulb; PMV, premammillary nucleus ventral part; Pn, potine nuclei; Sol, nucleus of solitary tract; Sox, supraoptic decussation; SuM, supramammillary nucleus; VDB, nucleus of vertical lib of diagonal band; VMH, ventromedial hypothalamic nucleus. *(from Schwartz et al., 1991)*

In all mammals studied, including humans, histaminergic cell bodies are found to be confined to the tuberal region of the posterior hypothalamus in an area where a group of Nissl-stained magnocellular neurons had been detected earlier and collectively named the tuberomammillary nuclei (TMn). In rats, the TM nuclei have been sudivided into five subgroups (Ericson et al., 1987; Inagaki et al., 1991). The subgroups (TMMd) and (TMMv) correspond to the dorsal and ventral parts of the medial tuberomammillary area and are constituted of approx. 600 neurons situated on each side of the mammillary recess. The subgroups (TMVr) and (TMVc) are located in the rostral and ventral parts of the ventral tuberomammillary area and include approximately 1500 mostly magnocellular neurons. Finally, the last subgroup comprises the diffuse part of the tuberomammillary nucleus (TMdiff) which is constituted by a small number of (approx. 100) HDC immunoreactive cells (Figure 5).

The histaminergic neurons of the TMn display a serie of characteristic features as revealed in the various immunohistochemical studies at the light-microscopic level or electron-microscopic level. Most of them are large (30µm) neurons, with a round unindented nucleus, a well-developed Golgi apparatus and a relatively large amount of karioplasma. These ultrastructural characteristics are very similar to those obtained in noradrenergic, serotoninergic or cholinergic cell bodies.

Histaminergic TM neurons display electrophysiological characteristics very similar to other aminergic cell groups such as the dopaminergic neurons in the substantia nigra or ventral tegmental area (Grace and Bunney, 1983; Grace and Onn, 1989). They fire spontaneously in a slow (0-3 Hz) regular, pacemaker fashion, have broad action potential (1.8 ms mid-amplitude duration) and deep (15-20 mV), long-lasting afterhyperpolarizations (Reiner and McGeer, 1987; Haas and Reiner, 1988). TM neuron firing varies across the sleep-wake cycle, being highest during the waking state, slowing down during slow-wave sleep and stopping during REM sleep (Sakai et al., 1990).

In the histaminergic cell bodies located in the hypothalamic tuberomammillary nuclei, histamine has been colocalized with several neurotransmitters and/or their synthesizing enzymes (Kohler et al., 1985; Yamamoto et al., 1990; Ericson et al., 1991B; Onodera et al., 1994). These include glutamate decarboxylase (GAD, the GABA synthesizing enzyme), GABA, GABA-transaminase, adenosine deaminase (an enzyme responsible for the inactivation of the neurotransmitter adenosine), monoamine oxidase-B (MAO-B) and neuropeptides such as Met-Enk-Arg^{6}-Phe^{7}, substance P or thyrotropin-releasing homone (TRH).

2.2. Innervation

Histaminergic neurons constitute a long and highly divergent system projecting in a diffuse manner to many cerebral areas, with immunoreactive varicose or non-varicose fibers being detected in almost all regions of rat (Panula et al., 1989), guinea pig (Airaksinen et al., 1990) or human brain (Panula et al., 1990).

2.2.1. Efferent inputs

Two ascending and, at least, one descending efferent pathways account for the histaminergic innervation of the mammalian brain and spinal cord. The ventral ascending pathway remain on the ventral surface of the brain, providing innervation to the hypothalamus, diagonal band, septum and olfactory bulb. The dorsal ascending pathway leaves the TM following the lateral side of the third ventricle to innervate the thalamus, hippocampus, amygdala and rostral forebrain structures. The descending pathway in rats is associated with the medial longitudinal fasciculus and provides inputs to the brain stem and spinal cord. As mentioned previously, the five histaminergic cell groups can be considered as one funtional group (Wada et al., 1991). One main reason for this classification is the similarity of their projection patterns. Single neurons from each of these groups send widely divergent projections to many different areas of the brain with considerable overlap. Single neurons may even send out both descending and ascending axon collaterals (Kohler et al., 1985).

Although nearly all CNS areas contain some histaminergic fibers, the density of innervation is heterogeneous. The highest density of histaminergic fibers is found in the hypothalamus, with all nuclei receiving a strong or moderate innervation. Other structures innervated by the ventral ascending pathway also receive a powerful input including the diagonal band, septum and olfactory tubercle. Structures innervated by the dorsal ascending pathway tend to have a somewhat lower density of fibers, with the amygdala being the most prominent. The cerebral cortex has a moderate density of fibers in all areas and layers with a slightly increased level in the outer layers. The innervation of the thalamus is concentrated upon the periventricular nuclei. The hippocampal formation is most strongly innervated in the subiculum and dentate gyrus, with a low density of fibers present in CA3 and CA1; a similar low to moderate level innervation is present in the striatum and nucleus accumbens. Projections to the midbrain, brain stem, cerebellum and spinal cord tend to be of lower density than the ascending projections, with some notable exceptions. All of the other aminergic cell groups receive at least a moderate density of histaminergic fibers, with the substantia nigra and ventral tegmental area being strongly innervated. In addition, the inferior and

superior colliculi, periaqueductal gray, nucleus of the trigeminal nerve and nucleus tractus solitarius receive prominent projections.

Although some brain regions receive only a low density of histaminergic fibers, it should be noted that there is a considerable mismatch between histaminergic fiber density and the density of histaminergic receptors in different regions (Schwartz et al., 1991). Furthermore, even in regions which have a low density of fibers, prominent electrophysiological effects of histamine can be observed, e.g CA1 region of the hippocampus, suggesting that depending on the level of activity of the histaminergic neurons all brain regions can be affected. Areas with high density of fibers will probably be affected tonically during waking, whilst areas with lower fiber densities may only be affected under particular behavioural situations when histamine neurons fire more rapidly.

2.2.2. *Afferent inputs* (Figure 6)

Relatively little is known about the afferent inputs to the histaminergic tuberomammillary neurons. Nevertheless, anatomical techniques have shown that the TM receives afferent inputs from the prefrontal/limbic cortex, all septal regions and several cell groups of the hypothalamus, particularly preoptic/anterior areas (Wouterlood et al., 1987; Wouterlood et al., 1988; Ericson et al., 1991A). Moreover, monoaminergic afferents originated from the adrenergic cell groups C1-C3, the noradrenergic cell groups A1-A2 and the serotoninergic cell groups B5-B9 have been also described (Ericson et al., 1989).

Electrophysiological techniques in horizontally cut brain slices revealed excitatory inputs mediated by AMPA and NMDA receptors from the lateral preoptic area and lateral hypothalamus (Yang and Hatton, 1997). TMn neurons receive a strong GABAergic input, which is responsible for quieting them during sleep (Sherin et al., 1996; Yang and Hatton, 1997; Stevens et al., 1999). Anatomical and electrophysiological techniques have demonstrated GABAergic input from the diagonal band of Broca, lateral hypothalamus and from the important sleep centre, the ventrolateral preoptica area (Sherin et al., 1998). GABA released from these sites inhibits histaminergic neurons by acting on both $GABA_A$ and $GABA_B$ receptors (Yang and Hatton, 1997; Stevens et al., 1999). This GABAergic input seems to be presynaptically inhibited by $GABA_B$, (Stevens et al., 1999) histamine H3, μ-opiod, adenosine A1 and α2-adrenergic receptors. Additionally, galanin, which hyperpolarises

TM neurons (Schonrock et al., 1991) is colocalised in the GABAergic nerve endings arriving from the preoptic area suggesting its modulatory effect.

Also, it has been shown that histaminergic neurons receive ascending afferences, presumably cholinergic, from the mesopontine tegmentum (Ericson et al., 1991A) which is likely to be important in brain arousal mechanisms. Acutely dissociated TM neurons have been observed to possess nicotinic responses, which are sensitive to α-bungarotoxin (Uteshev et al., 1996). In addition, it has been demostrated that serotonin causes a strong depolarization via 5-HT2 receptors and modulating the sodium calcium exchanger (Stevens et al., 2001).

Finally, it has been recently shown that fibers containing the novel hypothalamic peptide, orexin, densely innervate the soma and proximal dendrites of histaminergic cells suggesting its modulatory effect (Chemelli et al., 1999).

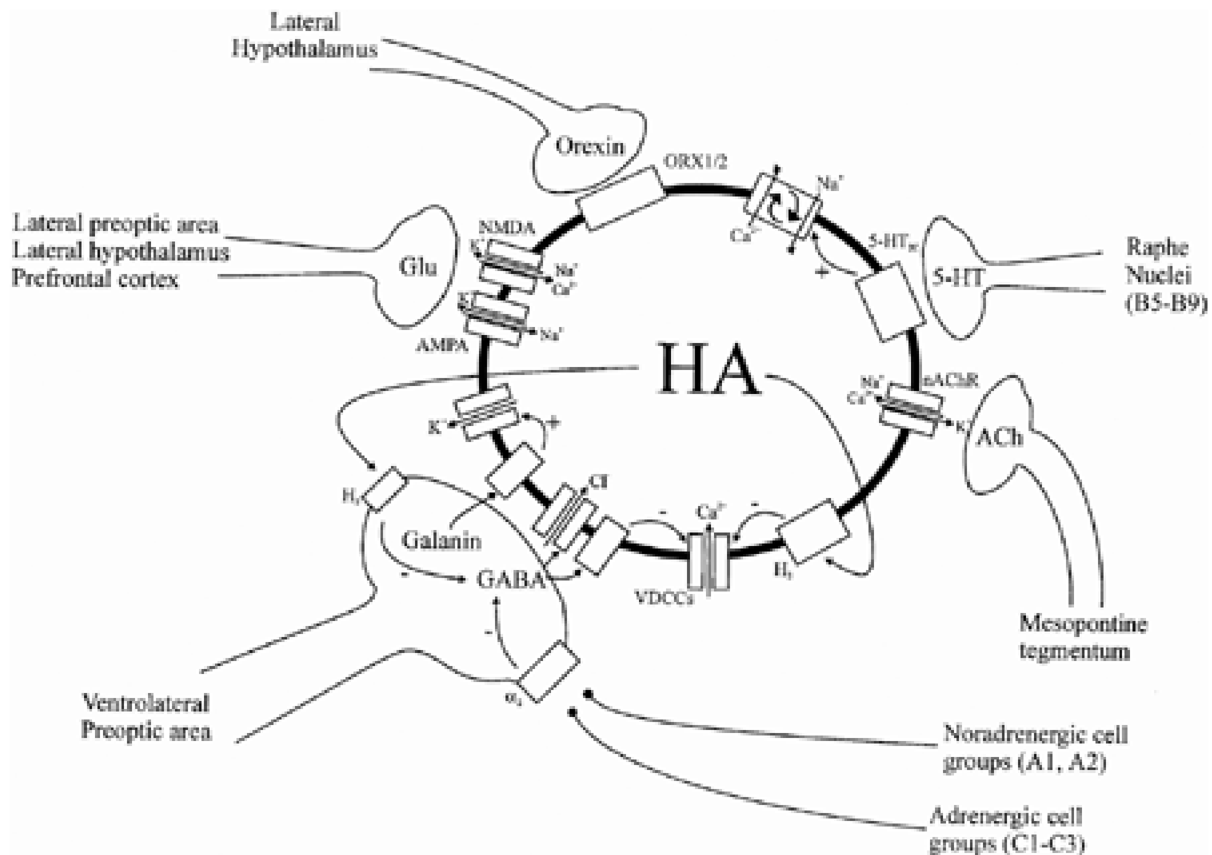


Figure 6. Afferences and regulation of histaminergic neurons. Inhibitory input arises from the ventrolateral preoptic nucleus (GABA, galanin: responsible for silencing histamine neurons during sleep) and from other histamine neurons. Excitatory inputs from the mesopontine tegmentum (ACh- acetylcholine, raphe nuclei (5-HT- 5-hydroxytryptamine), lateral hypothalamus (Glu- glutamate or orexin) and the prefrontal cortex (glutamate). Adrenergic/noradrenergic inputs seem to mainly modulate the GABAergic input. Note that nociceptin, adenosine triphosphate (ATP) and galanin can also be released from afferent or histamine neurons themselves and modulate excitability. Abbreviations: AMPA, α-Amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor; nAChRs, nicotinic acetylcholine receptors; NMDA, N-methyl-D-aspartate receptors; ORX1/2, orexin type 1 or 2 receptors; VDCCS, voltage dependent calcium channels. *(figure obtained from Brown et al., 2001)*

3. HISTAMINE RECEPTORS

Until now four histamine receptors subtypes (H_1, H_2, H_3 and H_4) have been defined by means of functional assays, design of selective agonists and antagonists or molecular cloning. All of them belong to the superfamily of receptors with seven transmembrane domains and couple to guanylyl nucleotide-sensitive proteins.

3.1. HISTAMINE H_1 RECEPTORS

Histamine H_1 receptors have been involved in several physiological processes such as allergy and inflammation in peripheral tissues and inhibition of firing and hyperpolarization (Haas et al., 1981; McCormick and Williamson, 1991; Reiner and Kamondi, 1994) in CNS. The receptor comprises a sequence of 486-487 amino acidic residues corresponding to a glycoprotein of 56 kDa. The gene encoding for the human H_1 receptor has been located on chromosome 3 (Fukui et al., 1994; Le Coniat et al., 1994). Biochemical studies indicate that the H_1 couples to a $G_{q/11}$ GTP-hydrolyzing protein which stimulates the activity of PLC (Leurs et al., 1994). In turn, PLC hydrolyzes phosphatidyl-4-5-bisphosphate to form DAG and IP3 which induce the activity of PKC and the release of stored calcium into cytoplasm, respectively (Figure 7). The receptor has been shown to have a widespread distribution in the CNS presenting highest levels in areas involved in arousal such as thalamus, cortex, cholinergic cell groups in the mesopontine tegmentum and in the basal forebrain, locus coeruleus and raphe nucleus. Pharmacologically, various selective and potent agonists (2-pyridyl-ethylamine, 2-thiazolylethylamine or 2-[3-Bromophenyl]histamine) and antagonists (mepyramine, astemizole or loratidine) have been developed .

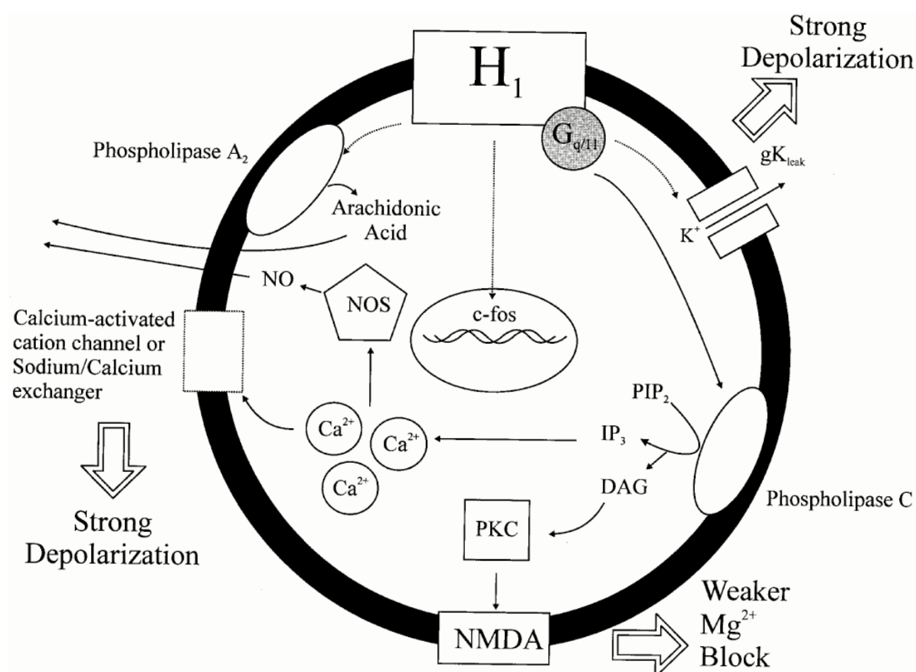


Figure 7. Summary of biochemical and electrophysiological responses following histamine H_1 receptor activation. Effects where the precise signal transduction pathway is unclear are indicated by dotted lines. The H1 receptor is coupled to stimulation of phopholipase C via the $G_{q/11}$ G-protein leading to production of the two second messengers, IP_3 and DAG from phosphatidyl-4,5-bisphosphate (PIP_2). IP_3 releases calcium from internal stores, which in some cells can lead to the activation of nitric oxide synthase (NOS), a calcium-activated cation channel or a sodium calcium exchanger. DAG potentiates the activity of PKC, which in turn can phosphorylate various proteins including the glutamate NMDA receptor, reducing the effectiveness of the magnesium block. Strong depolarisations are produced by block of leak potassium channels (gK_{leak}) or in septal neurons by activation of a calcium-activated cation channel and/or sodium/calcium exchanger. Other effects attributable to H1 receptor activation are production of arachidonic acid and *c-fos* expression. *(picture extracted from Brown et al., 2001)*

3.2. HISTAMINE H_2 RECEPTORS

Histamine H_2 receptors have been involved in several physiological processes such as gastric secretion in peripheral tissues or synaptic transmission (Kostopoulos et al., 1988) and synaptic plasticity (Brown et al., 1995) in CNS. The receptor comprises a sequence of 358-360 amino acid residues corresponding with a protein of approximately 40 kDa. The intronless gene has been located on human chromosome 5 (Traiffort et al., 1995). The H_2 receptor couples to a G_s GTP-hydrolizing protein, as demonstrated by the fact that its effects are blocked by cholera toxin and lead to stimulation of adenylyl cyclase (Hegstrand et al., 1976) (Figure 8). Like the histamine H_1 receptor, the H_2 has a widespread expression in the brain and spinal cord. Particularly, high H_2 receptor densities are found in the basal ganglia, parts of the limbic system

such as hippocampal formation and amygdala, and the superficial layers (I-III) of the cerebral cortex. Pharmacologically, the H_2 receptor has been characterized based on the development of various selective and potent agonists (i.e. dimaprit, amthamine or impromidine) and antagonists (i.e. burimamide, cimetidine, ranitidine, tiotidine, famotidine, or loratidine). Recently, it has been reported that H_2 receptors present spontaneous activity in transfected CHO cells (Smit et al., 1996), which led to reclassifying several compounds such as ranitidine and famotidine as inverse agonists, and burimamide as neutral antagonist.

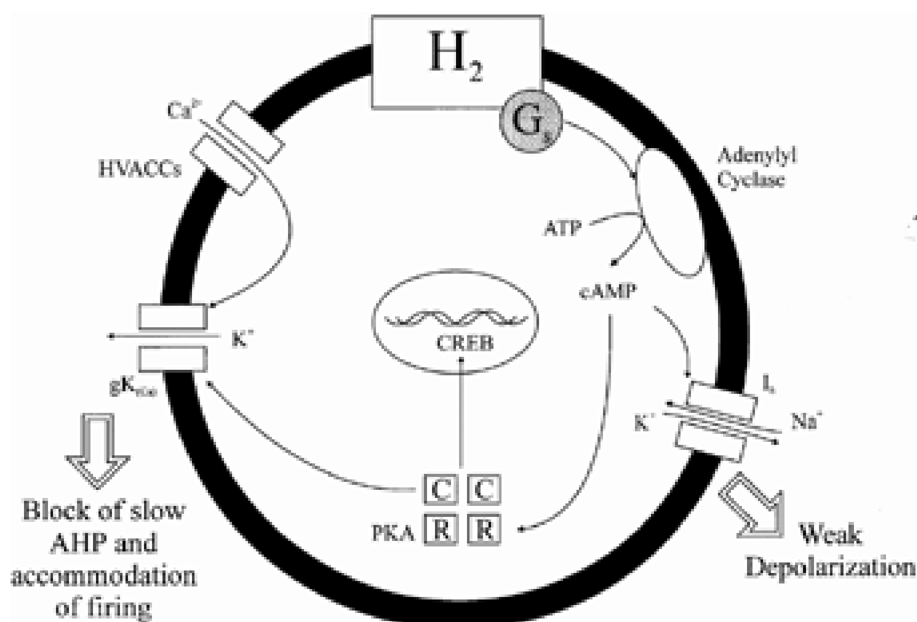


Figure 8. Summary of biochemical and electrophysiological responses following histamine H_2 receptor activation. The H_2 receptor is coupled to stimulation of adenylyl cyclase via the $G_{q/11}$ G-protein leading to production of the second messenger, cyclic adenosine monophosphate (cAMP) from adenosine triphosphate (ATP). cAMP binds to the regulatory (R) subunits of PKA, leading to their dissociation from the catalytic subunits (C). The catalytic units can phosphorylate various target proteins: (1) the CREB-activation; (2) the calcium-dependent potassium conductance $gK_{(Ca)}$- inhibition. Inhibition of $gK_{(Ca)}$, which is normally activated by calcium-influx through hihg-voltage activated calcium channels (HVACCs), leads to block of the slow afterhyperpolarization (AHP) and accomomodation of firing in pyramidal neurons. cAMP can cause a small depolarization independent of PKA by shifting the activation threshold of the hyperpolarization-activated cation current I_h. *(diagram obtained from Brown et al., 2001)*

3.3. HISTAMINE H_4 RECEPTORS

The histamine H_4 receptor has very recently been discovered for several research groups (Nakamura et al., 2000; Liu et al., 2001; Nguyen et al., 2001; Morse et al., 2001; Zhu et al., 2001). Although in each case the molecular approach to identify and clone the receptor is different, most studies converge in several conclusions. First, the

receptor belongs to the superfamily of G protein coupled receptors (GPCR) and shows a high homology to the histamine H_3 receptor cDNA. Second, the protein seems to comprise 390 amino acid residues and the gene encoding to the receptor seems to be located into the chromosome 18. Third, the histamine H_4 receptor is mainly expressed in peripheral blood mononuclear cells and in tissues which contain high concentrations of blood cells such as bone marrow, spleen, liver or lung. Conversely, its expression has not been described in brain. And finally, the H_4 receptor shows a pharmacological profile very similar to the H_3 receptor. Nevertheless, additional studies will be necessary to conclude which are the signal transduction pathways activated by this receptor as well as in which functions is involved.

3.4. HISTAMINE H_3 RECEPTORS

The histamine H_3 receptor was described in 1983 as an autoreceptor regulating the release and synthesis of histamine (Arrang et al., 1983 and 1987). Thereafter, it was shown to inhibit presynaptically the release of other monoamines in brain. In addition, the development of novel compounds with agonistic and antagonistic properties led to its pharmacological characterization and distribution. Nevertheless, the molecular and biochemical properties of the H_3 receptor remained unknown since the receptor was recently cloned (Lovenberg et al., 1999).

3.4.1. Biochemical properties

Even though no biochemical approach succeeded in purification of the protein, several studies strongly suggested that the H_3 receptor, like other histamine receptors, could belong to the superfamily of G-protein coupled receptors: firstly the binding of [^{3}H]-R-α-methylhistamine was shown to be regulated by guanylyl nucleotides (Arrang et al., 1990), and secondly the binding of [^{35}S]-GTPγ[S] in rat cerebral cortical membranes was stimulated by a H3 receptor agonist (Clark et al., 1996). In the latter, the H_3 receptor stimulatory effect was sensitive to pertussis toxin suggesting that the receptor is linked to G_i or G_0 protein subtype. These results have been subsequently confirmed when the receptor was recently cloned by Lovenberg and colleagues. In cells transfected with the H_3 receptor, the activation of the receptor lead to an inhibition of forskolin-stimulated cAMP formation (Lovenberg et al., 1999) suggesting that cAMP

could be an important messenger in the histamine H3 receptor signal transduction mechanism.

Other biochemical studies have suggested that H_3 receptor-mediated events could be dependent of additional signal transduction mechanisms. In this way, Takeshita et al (1998) reported that H_3 receptor modulates voltage-sensitive calcium channels in neurons dissociated from the rat tuberomammillary nucleus; and Drutel et al (2001) reported that activation of rat histamine H_3 receptor, in COS-7 transfected cells, led to the activation of the MAP kinase signaling cascade via pertussis toxin-sensitive G proteins.

Finally, recent studies have demonstrated that native histamine H_3 receptor presents high constitutive activity in histaminergic neurons (Morisset et al., 2000). This conclusion has been obtained by observing that proxyfan, an H3 neutral antagonists, was able to revert both imetit-(H_3 agonist) and thioperamide-(H_3 inverse agonist) effects on potassium-stimulated histamine release as well as tele-methyl histamine levels on synaptosomes. Based on these results, the pharmacological description of H_3 antagonists will have to be reconsidered.

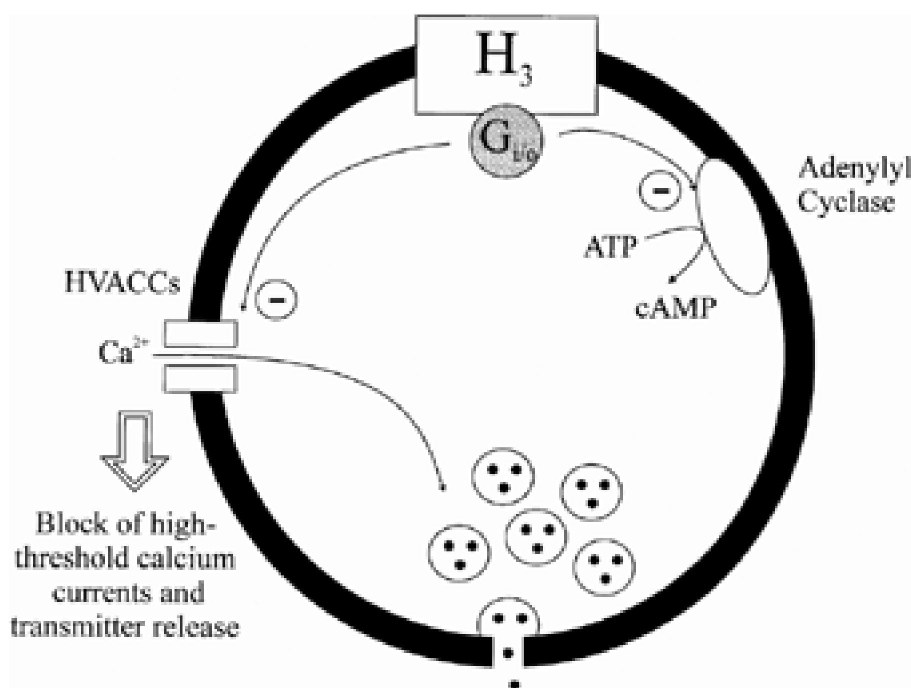


Figure 9. Summary of biochemical and electrophysiological responses following histamine H_3 receptor activation. The H_3 receptor is coupled to inhibition of adenylyl cyclase via $G_{i/0}$. The production of cyclic adenosine monophosphate (cAMP) from adenosine triphosphate (ATP) is depressed. In addition, H_3 receptor activation leads to inhibition of high-voltage activated calcium channels (HVACCs). In presynaptic terminals this inhibition reduces transmitter release. *(diagram obtained from Brown et al., 2001)*

3.4.2. Molecular properties

The histamine H_3 receptor has very recently cloned by Lovenberg and colleagues (1999) when they identified, from a private-expressed sequence tag (EST), an EST (GPCR97) that possessed 35% aminoacid homology with the seven transmembrane domain (TM7) of α_2-adrenoreceptors. Subsequently, the full-length GPCR97 sequence (GenBank AF140538) was cloned from a size-selected human thalamic cDNA library. The full-length 2.7 kb clone consisted of a 298-bp 5'-untranslated region, a 1335-bp open reading frame (encoding for a protein of 445 amino acids), and a 1100-bp 3'-untranslated region. Finally, when the receptor (GPCR97) was expressed in various cell lines, binding studies and modulation of forskolin-stimulated cAMP formation by several histamine H_3 ligands (agonists and antagonists) led to describe that the clone GPCR97 was the human H_3 receptor.

The H_3 receptor exhibits very low homology with other GPCRs (20-27%), although presents an aspartic residue in the transmembrane domain III (TM3), the putative binding site for the primary amine, which is a clear hallmark of the biogenic amine receptor subfamily. Comparison of the full amino acid sequence with other known GPCRs reveals highest homology (31%) with the α_{2A}- and α_{2C}-adrenergic receptors and the muscarinic M_1 acetylcholine receptors. Overall homology between the H_3 receptor and H_1 and H_2 receptors is only 22% and 20%, respectively and explains why the various homology-based approaches to clone the H_3 receptor gene were unsuccessful.

Analysis of the H_3 receptor protein with MOTIFFINDER have identified motifs for N-linked glycosilation at Asn^{11} of the N-terminal, cAMP-dependent protein kinase (PKA)-mediated phosphorylation of Ser^{342} in the third intracellular loop and calcium-dependent protein kinase (PKC)-mediated phosphorylation of several residues in the first (Ser^{64}), second (Ser^{141} and Thr^{149}) and third intracellular loops (Ser^{310}, Thr^{314}, Ser^{319} and Thr^{345}). These data suggest that the H_3 receptor responsiveness might be very sensitive to regulation by receptor crosstalk (Leurs et al., 2000). (Figure 10).

The expression of human H_3 receptor, using Northern blot techniques, has been confirmed only in the brain, most notably in the thalamus and caudate nucleus whereas no expression has been observed in any peripheral tissue examined (heart, placenta, lung, liver, kidney, pancreas, spleen and thymus) (Lovenberg et al., 1999; Drutel et al., 2001) (Figure 11).

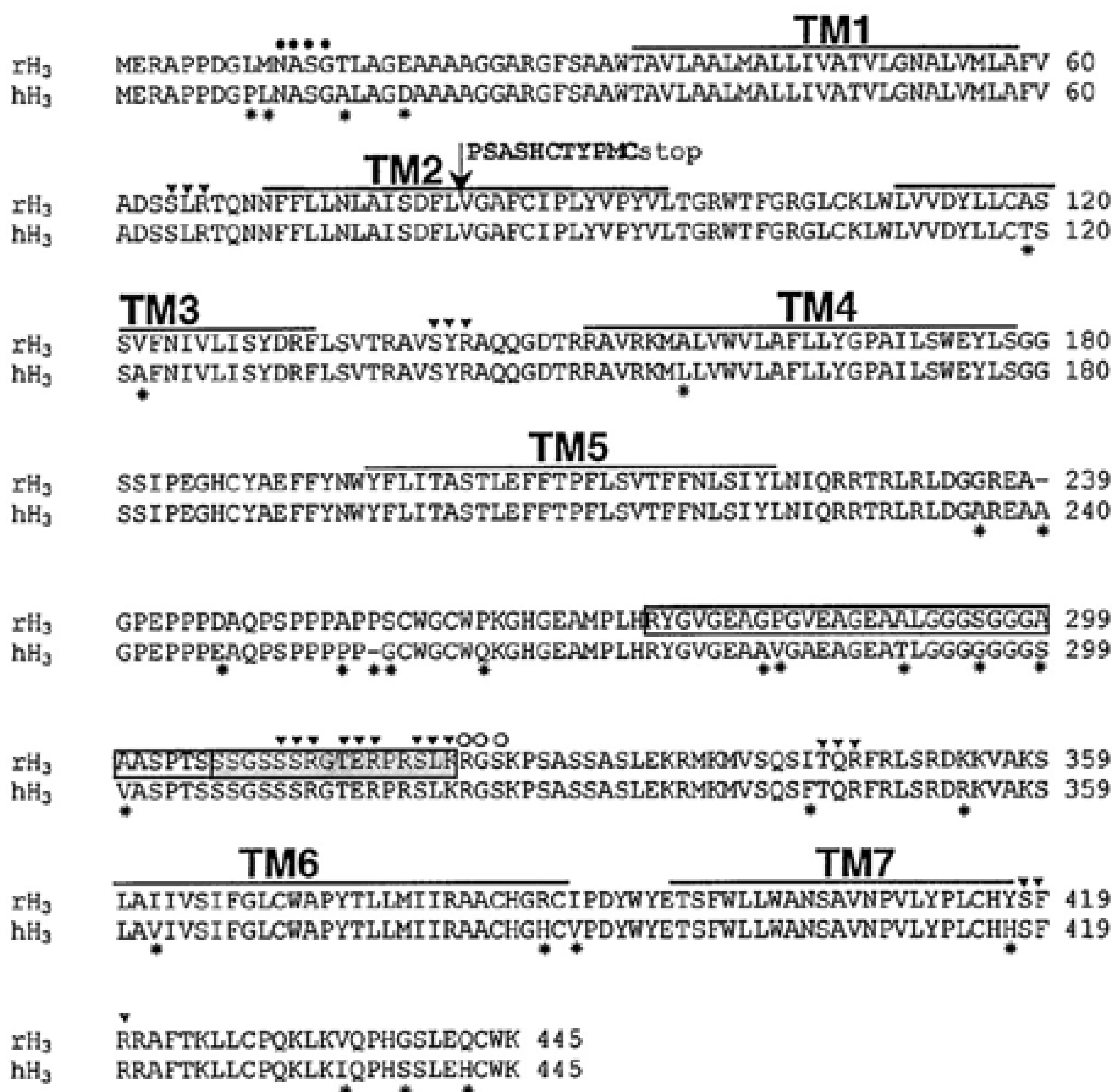


Figure 10. Alingment of the rat and human H_3 receptor amino acid sequence. The asterisks indicate the observed differences. The boxed amino acids indicate the deletions in either the H_{3B} (open box) or H_{3D} isoform (open-shaded box), whereas the arrow indicates the change in the open reading frame, resulting in the insertion of 11 additional amino acids (bold) and a stop condon $H_{3(nf1)}$. Potential glycosylation (●), PKA (○), or PKC (▲) phosphorylation sites were identified using the PROSITE database and are indicated. *(extracted from Drutel et al., 2001)*

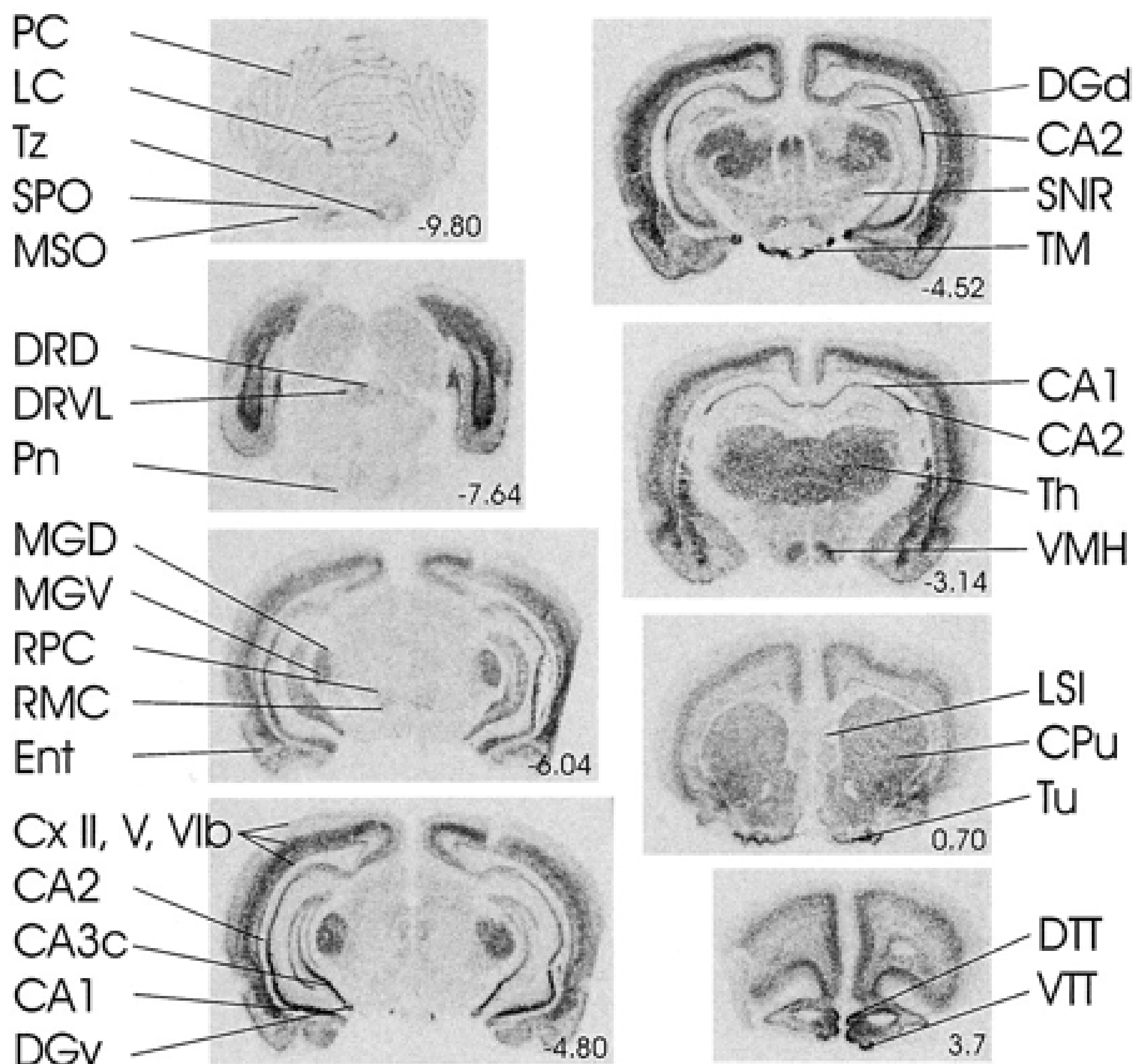
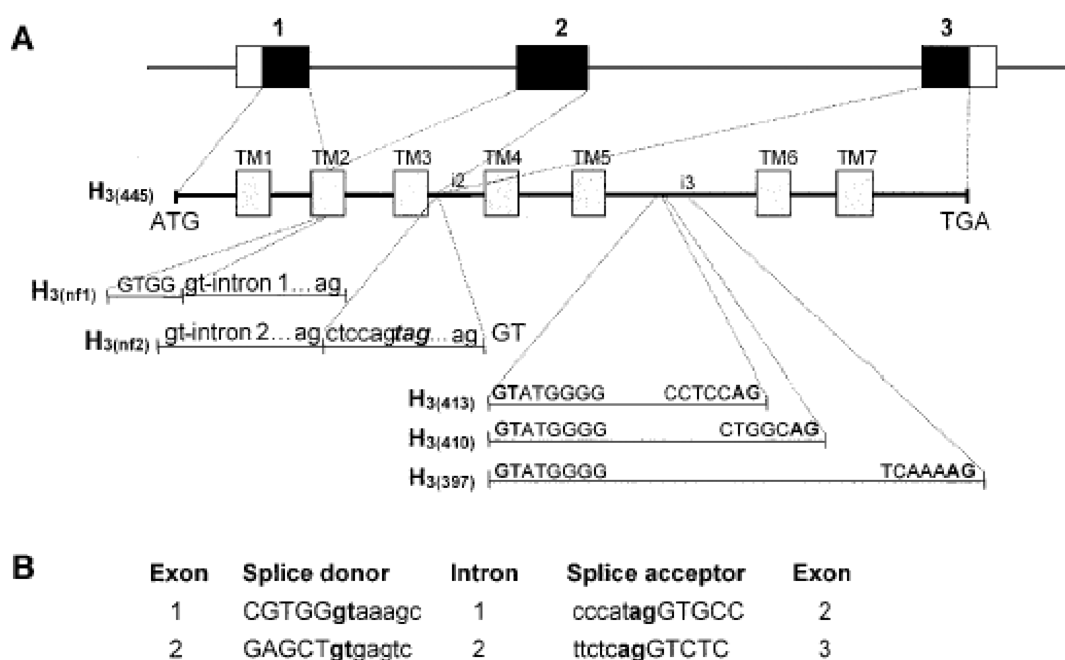


Figure 11. Histamine H_3 receptor expression in rat brain. The in situ hybridization was carried out with a H_3 probe which detects all cloned mRNA isoforms. All micrographs are scanned images from X-ray films. The stereotaxic location for each section is indicated by giving the distance to the bregma (in millimeters). CA1, CA2 and CA3, pyramidal layers of hippocampus; CA3c, CA3 caudal part; CPu, caudate putamen (striatum); Cc, cortex; DG, dentate gyrus; DGd, dentate gyrus dorsal; DGv, dentate gyrus ventral; DRD, dorsal raphe nucleus, dorsal part; DRVL, dorsal raphe nucleus, ventrolateral part; DTT, dorsal tenia tecta; Ent, entorhinal cortex; LC, locus ceruleus; LSI, lateral septal nucleus, intermediate part; MGD, medial geniculate nucleus, medial part; MGV, medial geniculate nucleus, ventral part; MSO, medial superior olive; PC, Purkinje cells; Pn, pontine nuclei; RMC, red nucleus, magnocellular part; RPC, red nucleus, parvicellular part; SNR, substantia nigra, reticular part; SPO, superior paraolivary nucleus; Th, thalamus; TM, tuberomammillary area; Tu, olfactory tubercle; Tz, trapezoid body; VMH, ventromedial hypothalamic nucleus; VTT, ventral tenia tecta. *(obtained from Drutel et al, 2001).*

Subsequent to the cloning of human H_3 receptor, it has also been reported the cloning of rat and guinea-pig H_3 receptor (Lovenberg et al., 2000; Tardivel-Lacombe et al., 2000). The rat cDNA clone encodes a putative 445 amino acid protein with 93% identity to the human H_3 receptor and, similarly to the human H_3 receptor, is abundantly expressed in CNS (particularly in caudate, thalamus and cortex) but not in peripheral tissues (Lovenberg et al., 1999; Drutel et al., 2001).

Genomic DNA analysis have revealed that the coding region of the human and rat histamine H_3 receptor comprises three exons interrupted by two introns of 1 kb each located in the second transmembrane domain (TM2) and second intracytosolic loop, respectively (Morisset et al., 2001; Tardivel-Lacombe et al., 2001) (Figure 12).



Exon	Splice donor	Intron	Splice acceptor	Exon
1	CGTGG**gt**aaagc	1	cccat**ag**GTGCC	2
2	GAGCT**gt**gagtc	2	ttctc**ag**GTCTC	3

Figure 12. Exon/intron structure of the rH_3-receptor gene and rH_3 isoforms generated by retention/deletion of pseudo-introns. (A) Top, diagram of the rH_{3R} genomic DNA. Exons indicated by the boxes are numbered. open boxes indicate nontranslated sequence. Bottom, structure of the rH_{3A} receptor cDNA. Regions encoding transmembrane domains are represented by hatched boxes (TM1-TM7), I2 and I3 indicate the second and third intracytosolic loops, respectively. Deletion/insertions leading to two presumably non-functional rH_3 receptor isoforms, $H_{3(nf1)}$ and $H_{3(nf2)}$, and three functional shorter isoforms H_{3B}, H_{3C} and H_{3D} are indicated. (B) Exon/itron junctions within the rH_3 receptor gene. (picture obtained from Morisset et al., 2001)

Additional studies have demonstrated the existence of several rat H_3 receptor isoforms which varies in the length of their third intracytosolic loop and in the brain expression (Morisset et al., 2000; Liu et al., 2000; Drutel et al., 2001; Morisset et al., 2001). At the moment, four functional isoforms of rat histamine H_3 receptor have been

described corresponding to a proteins of 445 (H_{3A}), 413 (H_{3B}), 410 (H_{3C}) and 397 (H_{3D}) amino acids. The H_{3A} receptor isoform corresponds to the whole/intact protein that shows 93% identity with the corresponding human H_3 receptor. The 32-, 35- and 48-amino acid deletions of the H_{3B}, H_{3C} and H_{3D} isoforms are located in the middle of the third intracytosolic (I3) loop, resulting in the deletion of potential PKC and PKA phosphorylation sites in the H_{3D} isoform. In addition, it have also been identified two additional and presumably nonfunctional isoforms corresponding to a 94- and 141-amino acid proteins, respectively. The first non-functional isoform, termed $H_{3(nf1)}$, was generated by a deletion of 4 nucleotides in TM2 which results in a shift of the open reading frame indroducing a down-stream stop codon. The second non-functional isoform, termed $H_{3(nf2)}$, was generated by an insertion of 396 nucleotides located in the second intracellular loop and containing a stop codon (Drutel et al., 20001; Morisset et al., 2001) (Figure 12).

In addition, it has been described that three of these isoforms (H_{3A}, H_{3B}, H_{3D}) differentially couple to the Gi protein-dependent inhibition of adenylate cyclase or stimulation of p44/p452 mitogen activated protein kinase (MAPK) (Drutel et al., 2001). Whereas the H_{3A} receptor is less effective in inhibiting forskolin-induced cAMP production in transfected cells as compared with the H_{3B} or H_{3D}, this isoform is more effective in the stimulation of p44/p42 MAPK.

3.4.3. Distribution (Figure 11)

Autoradiography of H_3 receptors in rat (Pollard et al., 1993) and monkey brain (Martinez-Mir et al., 1990) suggests that the number of H_3 receptors varies among species. The H_3 receptor distribution, established from either membrane binding or autoradiographic studies, using [^{3}H]-R-α-methylhistamine, is highly heterogeneous.

In cerebral cortex, H_3 receptors are rather dense in all areas and layers with, however, highest abundance in rostral areas and in laminae IV and V. In the hippocampal formation, they are moderately to highly abundant, with their density being the highest in the dentate gyrus, moderate in subiculum, and very low in the fimbria. In the amygdaloid complex high densities are found as well as in the bed nucleus of the stria terminalis, which contains a dense histaminergic innervation (Schwartz et al., 1991 for Review).

In the basal forebrain, numerous H_3 receptors are present in anterior olfactory nuclei, nucleus accumbens, and olfactory tubercles, as well as in striatum, particularly in its dorsomedial part. In the thalamus, H_3 receptors are mainly detected in various

midline, intralaminar, and lateral nuclei. In the hypothalamus, their moderate density contrasts with the high density of histaminergic axons, but they are detectable at the level of the tuberomammillary nucleus where the may reside on perikarya or dendrites. Finally, low densities are present in all layers of the cerebellum and in external layers of the dorsal horn in the spinal cord.

This distribution of H_3 receptors is not strictly parallel to that of histaminergic axons, which suggests that the majority of these receptors are not autoreceptors. This is confirmed by the functional identification of H_3 heteroreceptors on serotoninergic (Schlicker et al., 1988), noradrenergic (Schlicker et al., 1989), acetylcholinergic (Prast et al., 1999), glutamatergic (Brown et al., 1996; Molina-Hernandez et al., 2001), GABAergic (Garcia et al., 1997) and dopaminergic (Schlicker et al., 1993; Molina-Hernandez et al., 2000) nerve terminals in several areas of the brain.

3.4.4. Electrophysiology

Three main electrophysiological actions of receptors coupled to pertussis toxin sensitive G-proteins have been described: inhibition of transmitter release, inhibition of high-threshold calcium currents and hyperpolarizations caused by activation of inwardly-rectifying potassium channels. For histamine H_3 receptors the first two actions have convincingly been demonstrated but no evidence is available suggesting a coupling to inwardly-rectifying potassium channels. Since the demostration by Arrang et al. (1983) that histamine H_3 receptors inhibit histamine release and synthesis, histamine has been also found to inhibit the release of many other transmitters, including glutamate (Brown et al., 1996), GABA (Garcia et al., 1997) noradrenaline (Schlicker et al., 1989), dopamine (Schlicker et al., 1993), acetylcholine (Arrang et al., 1995), serotonin (Schlicker et al., 1988) and various peptides (Hill et al., 1997). A direct, G-protein mediated inhibition of presynaptic calcium channels appears to be the most likely mechanism to explain this inhibition. The maximum inhibition of transmitter release varies between 20 and 60%, dependent on the extracellular calcium concentration (Schlicker et al., 1994, Brown et al., 1999B).

Activation of H_3 autoreceptors on TM neurons leads to an inhibition of multiple high-threshold calcium channels (Takeshita et al., 1998) and an inhibition of firing (Haas et al., 1992). However, in contrast to other cell body autoreceptors, the H_3 receptors do not appear to be coupled to inwardly-rectifying potassium channels. The maximum inhibition of high-threshold calcium currents is quite small, but given the power

relationship between calcium concentration and transmitter release it can easily account for the observed depression of release.

3.4.5. Pharmacology

At the histamine H_3 receptor, histamine itself is a highly active agonist. Methylation of the terminal amino function results in compounds such as R-(α)-methylhistamine that are more active and selective H_3 receptors agonists than histamine. This agonist has been very useful for the pharmacological characterization of H_3 receptor-mediated effects (Leurs et al., 1995A). Additional H_3 agonists have been produced by replacing the amine function of histamine with an isothiourea group (imetit) or a ring structure (immepip) (Vollinga et al., 1994). Because of its early availability, R-(α)-methylhistamine has been extensively used in vitro and in vivo. Recently, high affinity of this compound for the histamine-metabolizing enzyme histamine-N-methyltransferase (HMT) was observed (Rouleau et al., 1997). Methylation of the imidazole ring of R-(α)-methylhistamine by HMT results in a very short half-life compound in human plasma which, combined with the high polarity of R-(α)-methylhistamine, strongly limits its brain penetration. In these sense, the development of novel H_3 agonists such as imetit and immepip has overcome this problem (Krause et al., 1995).

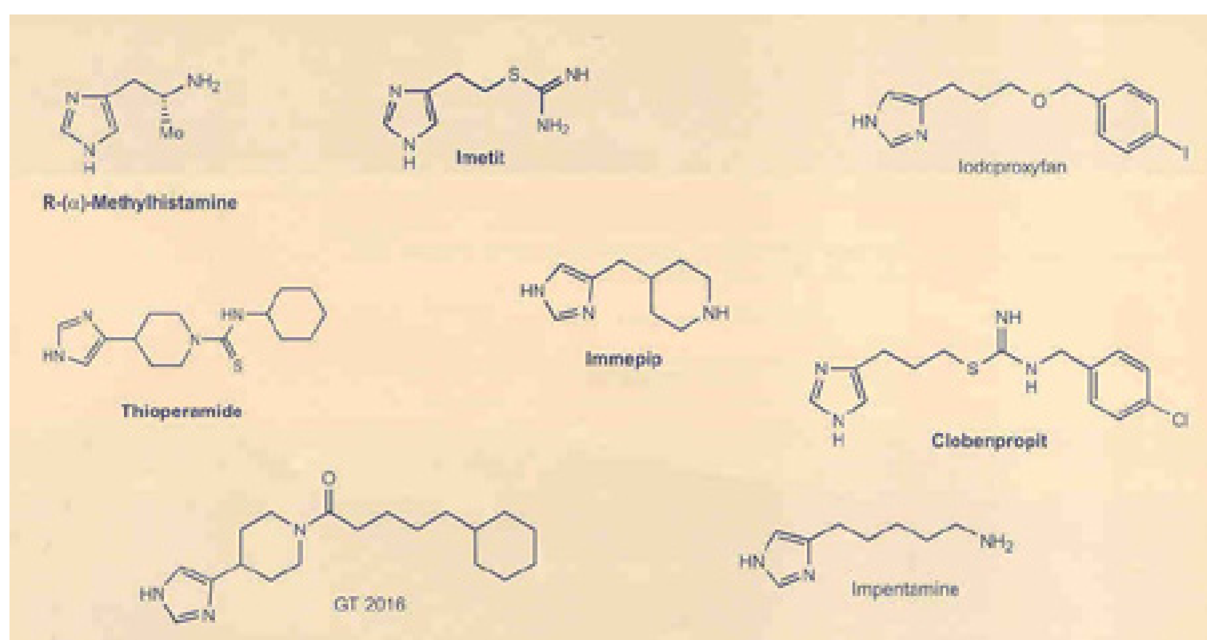


Figure 13. Chemical structure of some histamine H_3 receptors agonists and antagonists.

Many potent H_3 receptor antagonists have also been developed. The prototypic H_3 receptor antagonis, thioperamide (Arrang et al., 1987A), has a nanomolar affinity for the

H_3 receptor and crosses fairly well the blood-brain barrier. Despite its widespread use as a selective antagonist, thioperamide also has some affinity for the 5-HT_3 receptor (Leurs et al., 1995B) and probably interacts with neuronal GABA transport (Yamamoto et al., 1997). Incorporation of an isothiourea moiety in the side chain of thioperamide gives the highly potent H_3 receptor antagonist clobenpropit. This antagonist is tenfold more potent than thioperamide in vitro, but has a reduced brain penetration. To increase central effectiveness and to overcome potential toxicological problems connected with the thiourea and isothiourea moieties, these groups have been replaced by several side-chains with other polar groups (e.g. ureas, amines, amides, ethers, carbamates, and oxadiazoles) to produce the H_3 receptor antagonists iodoproxyfan, impentamine and GT2016. Many of these ligands show an improved brain penetration and high efectiveness in vivo in rodents. Consequently, various antagonists have been described as good radioligands for the H3 receptor such as [^{125}I]-iodophenpropit and [^{125}I]-iodoproxyfan (Ligneau et al., 1994).

Recently and similarly to H_2 receptor, it has been reported the high constitutive activity of native H_3 receptor regulating histaminergic neurons in brain (Morisset et al., 2000; Wieland et al., 2001). Based on this concept, thioperamide, clobenpropit and ciproxifan are inverse agonists, whereas proxyfan and VUF4904 (a newly developed impentamine derivative) act as neutral antagonists.

3.4.6. Potencial therapeutical properties

The H_3 receptor can modulate a variety of functions in CNS such as sleep and wakefulness, cognition and memory processes. Disorders related with such functions may be improved by treatment with H3 ligands. Similarly they could also be helpful in attention-deficit hyperactive disorder, epilepsy or obesity.

3.4.6.1. Sleep and wakefulness

The presence of histamine-containing cell bodies in the tuberomammillary nucleus of the posterior hypothalamus (an area involved in the maintenance of wakefulness) and their projections to the cerebral cortex suggest a role of histamine in the modulation of the arousal state and sleep-wake cycle. Lesions of the posterior hypothalamus are known to produce sleep in rats, cats and monkeys (Monti et al., 1993) and neurochemical and electrophysiological studies indicate that the activity of histaminergic neurons is maximal during periods of wakefulness and is suppressed by barbiturates and other hypnotics (Vanni-Mercier et al., 1984). Moreover, histamine release in the rat hypothalamus in vivo shows a circadian rhythm, with higher histamine release in

periods with high locomotor activity (Mochizuki et al., 1992). Modulation of histamine-mediated neurotransmission with H_3 receptor agonists results in an increase of the slow-wave sleep in rat (Monti et al., 1993). On the contrary, systemic application of H_3 receptor antagonists produce increased wakefulness (drecreases in rapid eye movement (REM) and slow-wave sleep) and increased locomotion. All these results strongly suggest that H_3 ligands could be useful in the treatment of sleep and wakefulness disorders.

3.4.6.2. Cognition and memory processes

Dysfunctions of acetylcholine-mediated neurotransmission are considered to underlie the cognitive decline associated with ageing and Alzheimer's disease. However, changes typical of ageing and Alzheimer's disease course with alterations of other neurotransmitter systems, including histamine (Panula et al., 1998). There is direct evidence that histaminergic neurotransmission plays an important role in learning and memory. Administration of histamine enhances cognitive performance of rats in an active avoidance task, while H_1 receptor antagonists impair memory retention (Kamei et al., 1990; Kamei et al., 1991). Neurochemical studies show that histamine modulates the activity of cholinergic neurons (Mochizuki et al., 1994; Blandina et al., 1996). In addition, thioperamide improves significantly the response latency in a passive-avoidance response in senescence-accelerated mice (Meguro et al., 1995). Flood et al (1998) showed that H3 ligands modulated memory retention when injected into the septum. Other studies report that administration of thioperamide or clobenpropit to mice attenuates the amnesic effects of scopolamine in the elevated plus-maze test and the step-through passive-avoidance test (Miyazaki et al., 1995; Miyazaki et al., 1997). More recently, Orsetti et al (2001) showed that thioperamide improved memory in a recognition task. All these studies suggest that H_3 receptor antagonists / inverse agonists may provide a novel approach to improve cognitive deficits.

3.4.6.3. Attention-deficit hyperactive disorder (ADHD)

ADHD is a developmental disorder with underlying motor, emotional, attentional and learning alterations. Underlying abnormalities in monoamine neurotransmitters appear to significantly contribute to disturbances in ADHD patients (Oades et al., 1987). The use of H_3 receptor antagonists can be useful in attentional disorders on the basis of the previously described wake- or vigilance-promoting effects of these drugs. In addition, H_3 antagonist treatments have procognitive properties in animal models of learning and memory, as mentioned before, as well as direct effects on neurotransmitter release. Developmental models have been described in which impairments in cognitive

processes or motor patterns are similar to those observed in ADHD (Dumery et al., 1987). In one of these models, administration of the selective H3 receptor antagonist GT2016 improved the rate of acquisition of a multi-trial passive-avoidance response. These data suggest that H_3 receptor antagonists / inverse agonists may be of use in the treatment of ADHD. These drugs not only improve the cognitive deficits, but they also normalize motor disturbances.

3.4.6.4. Epilepsy

The involvement of histamine-mediated neurotransmission acting as an endogenous anticonvulsivant in epilepsy has been reported. As mentioned before, presynaptic control via the H_3 receptor is an important regulatory mechanism of histamine-mediated neurotransmission. Recent data indicate that various H_3 receptor antagonists (thioperamide and clobenpropit) decrease the seizure susceptibility of electrically induced convulsion in mice (Yokoyama et al., 1993; Yokoyama et al., 1994; Murakami et al., 1995) by increasing endogenous release of histamine in the brain. These findings suggest that H_3 receptor antagonists could represent a new approach to the development of antiepileptic drugs.

3.4.6.5. Obesity

A number of studies suggest that histamine can suppress appetite and that histamine-containing neurons in the hypothalamus participate in the endogenous suppression of food intake. Intracerebroventricular injections of histamine depress feeding in rats, whereas the application of H_1 receptor antagonists or depletion of endogenous histamine elicits a feeding response (Sakata et al., 1995). It is considered that histamine activates postsynaptic H_1 receptors in the ventromedial nuclei of the hypothalamus to suppress food intake. In addition, H_3 receptors have been identified in that region (Pollard et al., 1993). In line with these observations, administration of thioperamide i.c.v. suppresses food intake, which is consistent with a drug-induced increase of histamine release. Finally, dysfunctions in histamine-mediated neurotransmission have been identified in the obese Zucker rat, a genetic model for obesity (Machidori et al., 1992). All these results support the clinical use of H_3 receptor antagonists in the treatment of obese conditions.

II. OBJECTIVES

II. OBJECTIVES

The importance of histaminergic neurotransmission on several neurophysiological and neuropathological processes is well established. However, little knowledge is available on the transduction mechanisms controlling histamine synthesis and release in neurons. It is known that histamine H_3 autoreceptors have a main role on these processes. Nevertheless, the signal transduction mechanisms used by H_3 autoreceptors are poorly understood.

The main purpose of this work has been the elucidation of the signal transduction pathways involved in the modulation of histamine release and synthesis specifically by histamine H_3 autoreceptors. To this end, the three objectives of this work were:

1). To develop a method to purify radiolabelled histamine by HPLC in order to measure histidine decarboxylase activity in homogenates as well as [^{3}H]-histamine synthesis and release in rat brain cortical miniprisms.

2). To study the signal transduction pathway by which histamine H_3 autoreceptors modulate [^{3}H]-histamine synthesis in rat brain cortex miniprisms. In particular, we wanted to investigate the involvement of AC-PKA in these processes because of the coupling of H_3 receptors to G_i proteins (Clark and Hill, 1996).

3). To investigate how histamine release is modulated in rat brain cortical miniprisms. In particular, we wanted to study the involvement of intracellular cAMP levels and calcium entry in depolarization-stimulated [^{3}H]-histamine release.

In addition to its scientific interest for the comprehension of histaminergic function, the knowledge of the transduction mechanisms of H_3 autoreceptors may also help the development of a new drugs that may become useful for the treatment of several classes of diseases of the nervous system.