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ACCELERATED COMMUNICATION

Identification of Rat H_3 Receptor Isoforms with Different Brain Expression and Signaling Properties

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ABSTRACT

We identified the cDNAs of three functional rat H_3 receptor isoforms (H_{3A}, H_{3B}, and H_{3C}) and one nonfunctional truncated H_3 receptor (H_{3T}). The H_{3A}, H_{3B}, and H_{3C} receptor isoforms vary in the length of their third intracellular loop; the H_{3B} and H_{3C} receptor lack 32 and 48 amino acids, respectively. Transient expression of the H_{3A}, H_{3B}, and H_{3C} receptors in COS-7 cells results in high affinity binding for the H_3 antagonist [^{125}I]iodophenpropit, which is displaced by selective H_3 agonists and antagonists. The three isoforms differentially couple to the G_i protein-dependent inhibition of adenylate cyclase or stimulation of p44/p42 mitogen activated protein kinase (MAPK), a new signaling pathway for the H_3 receptor. Whereas the H_{3A} receptor was less effective in inhibiting forskolin-induced cAMP production compared with the H_{3B} or H_{3C} receptor, this isoform was more effective in the stimulation of p44/p42 MAPK. The H_3 receptor isoforms also displayed differential CNS expression in key areas involved in regulation of sensory, endocrine, and cognitive functions. A differential H_3 receptor isoform expression was seen in, for example, hippocampus, where a characteristic dorsoventral distribution was revealed. Differential H_3 receptor expression was also characteristic for the cerebellum, indicating possible histaminergic regulation of motor functions. The identification of these new H_3 receptor isoforms and their specific signaling properties adds a new level of complexity to our understanding of the role of histamine, and the H_3 receptor in brain function. The heterogeneous distribution of the isoforms suggests that H_3 receptor isoform-specific regulation is important in several brain functions.

Brain histamine is involved in the regulation of arousal state, brain energy metabolism, locomotor activity, autonomic and vestibular functions, feeding, drinking, sexual behavior, and analgesia (Hough, 1988; Schwartz et al., 1991; Wada et al., 1991). Identification of molecular mechanisms used by brain histamine is therefore necessary for a better understanding of these complex physiological functions. The histamine H_3 receptor is one of the three receptors that is considered responsible for the actions of the neurotransmitter histamine (Schwartz et al., 1991; Hill et al., 1997). Originally discovered in 1983 as a presynaptic autoreceptor (Arrang et al., 1983), numerous studies have since shown that the H_3 receptor also regulates the release of other important neurotransmitters, such as acetylcholine, dopamine, glutamate, noradrenaline, and serotonin in both the central nervous system (CNS) and peripheral nervous system (Schlicker et al., 1988, 1989; Clapham and Kilpatrick, 1992; Schlicker et al., 1993; Brown and Reymann, 1996).

In vitro and in vivo studies suggest that H_3 receptor ligands have potential therapeutic use (e.g., Bowel's disease, ADHD, Alzheimer's disease, obesity) (Leurs et al., 1998), but also led to the recognition of potential receptor heterogeneity. Results from both radioligand binding and functional studies have provided evidence for the existence of H_3 receptor subtypes (West et al., 1990; Cumming and Gjedde, 1994; Jansen et al., 1994; Schworer et al., 1994; Leurs et al., 1996; Schlicker et al., 1996; Harper et al., 1999). So far, no convincing proof for receptor heterogeneity has been presented, probably because of the lack of knowledge on the genetic

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ABBREVIATIONS: CNS, central nervous system; GPCR, G protein-coupled receptor; PCR, polymerase chain reaction; MAP, mitogen-activated protein; IPP, iodophenpropit; NAMH, N^{α}-methylhistamine; RT, reverse transcription; I3, intracellular loop 3; bp, base pair(s); PTX, pertussis toxin.

information encoding the H_3 receptor protein(s). Recently, Lovenberg et al. (1999) showed that, like the H_1 and H_2 receptor, the H_3 receptor belongs to the large superfamily of G protein-coupled receptors (GPCRs). Using the genetic information of the human H_3 receptor, we set out a PCR-based strategy to establish the existence of H_3 receptor subtype(s). In this study we report the existence of at least three functional rat H_3 receptor isoforms (H_{3A}, H_{3B}, and H_{3C}) that are generated as a result of alternative splicing. The three isoforms have distinct CNS expression profiles and couple differentially to adenylate cyclase and MAP kinase signaling pathways.

Materials and Methods

Cloning of H_3 Receptor Isoform cDNAs. Total RNA (5 μg) of rat brain (CLONTECH, Palo Alto, CA) was reverse transcribed with random hexamer primers (100 ng/μl) (Invitrogen, Carlsbad, CA) and Superscript II reverse transcriptase (200 U) (Life Technologies, Gaithersburg, MD) according to the manufacturer's protocol. The cDNA was amplified by PCR using 2.6 U Expand High Fidelity DNA polymerase (Roche Diagnostics, Nutley, NJ) and 15 pmol of different couples of primers based on the human cDNA sequence (Lovenberg et al., 1999). After a 10-min denaturation step at 95°C, 35 cycles (1 min at 96°C, 40 s at 66°C, and 3 min at 72°C) were followed by a final extension for 8 min at 72°C. The use of primers overlapping the third intracellular loop of the human H_3 receptor (GenBank accession number AF140538) (5′-TGAACATCCAGAGGCGCACCC-3′ as forward primer and 5′GCAGAGCCCAAAGATGCTCAC-3′ as reverse primer corresponding to amino acids 224 to 229 and 364 to 370, respectively) resulted in the amplification of three different products, which were cloned in pCRII-TOPO and sequenced. The full-length cDNAs were isolated with primers overlapping the full H_3 sequence. The forward primer was based on the human H_3 cDNA sequence (5′-GTCCCGGAGCCGCGTGAGCCTGC-3′), whereas the reverse primer (5′-TACAAGGGCCTGGCCGTAGAAGG-3′) was based on a mouse expressed sequence tag sequence (GenBank accession number AI509395). Five clones of each of the three different cDNA isoforms were sequenced automatically (PRISM 310; ABI, Norwalk, CT) on both DNA strands. For cellular expression, cDNAs were amplified with a new rat forward primer including a Kozak sequence (underlined) (5′-CCGCCACCATGGAGCGCGCGCCGCCCGACGGGCTG-3′) and the reverse mouse primer and subcloned in pcDNA3.

Characterization of H_3 Receptor Isoforms. COS-7 cells were grown and transfected as described previously (Wieland et al., 1999). After 48 h, cells were homogenized and binding of [^{125}I]iodophenpropit (IPP) (1900 Ci/mmol; Menge et al., 1992), or [^{3}H]N^{α}-methylhistamine in 50 mM Tris, 5 mM $MgCl_2$ buffer, pH 7.4, was determined (Jansen et al., 1994). Binding data were evaluated by nonlinear regression analysis using GraphPad Prism (GraphPad Software, San Diego, CA). All binding data were analyzed according to one- and two-binding site models and evaluated statistically.

Coupling of the H_3 receptor isoforms to adenylate cyclase was measured by cotransfection of H_3 receptor cDNAs and the reporter gene plasmid pTLNC121–3, containing 21 cAMP-responsive elements (Fluhmann et al., 1998). Cells were seeded in black, 96-well plates and stimulated with the test compounds. After 48 h, the medium was removed, cells were lysed, and the luciferase activity was determined using a Wallac Victor^2 multilabel counter (Wallac Oy, Turku, Finland). Inositol phosphate accumulation was determined as described previously (Wieland et al., 1999).

For MAPK activation, growth-arrested cells were treated with immepip for 5 min at 37°C, washed twice in cold PBS, lysed in radioimmunoprecipitation assay buffer (PBS containing 1% Nonidet P-40, 0.5% sodium deoxycholate, and 0.1% SDS) with protease inhibitors (2 μg/ml aprotinin, 2 μg/ml leupeptin, 1 mM phenylmethylsulfonyl fluoride) and sonicated. Cell extracts were separated on a 10% SDS-polyacrylamide gel and transferred to nitrocellulose. Phospho-p44/p42 and total p44/p42 MAPK was detected using a mouse monoclonal phospho-p44/p42 specific antibody and a rabbit polyclonal p44/p42 antibody (New England Biolabs, Beverly, MA) respectively. Phospho-p44/p42 and total p44/p42 levels were visualized via chemiluminescence and quantified using an Imagestation (NEN, Boston, MA).

Experimental Animals and Preparation of Tissue. All experiments were approved by the Åbo Akademi Animal Care and Use Committee. Adult male Wistar rats (weight, 200–250 g) were decapitated, tissues were rapidly dissected, frozen in precooled isopentane, and stored at −70°C. All tissues were cut to 15-μm cryosections, thaw-mounted onto poly-L-lysine slides, and stored at −70°C until used.

In Situ Hybridization. The oligonucleotides used for in situ hybridization were designed so that they specifically recognized the different H_3 receptor isoform mRNAs. The sequences are indicated in Fig. 3, except for oligo X, which detects all characterized H_3 receptor isoforms and spans the nucleotides 496 to 540 (5′-GCCACCAGACAGGTACTCCCAACTCA GGATGGCAGGCCCATACAG-3′). As a control probe, we used a *Staphylococcus aureus* chloramphenicol acetyltransferase-specific oligonucleotide. As an additional control, we routinely used a normal hybridization mixture with a 100-fold excess of unlabeled specific probes. The hybridization procedure used has been described before and was used with minor modifications (Dagerlind et al., 1992; Lintunen et al., 1998). All probes were labeled with [^{35}S]deoxyadenosine 5′-α(-thio) triphosphate (NEN) at their 3′ ends using terminal deoxynucleotide transferase (Promega, Madison, WI). Nonincorporated nucleotides were removed by purification through Sephadex G-50 columns.

Before hybridization, the cryosections were taken from the −70°C environment and kept at room temperature for 10 min and treated with UV light for 5 min. The hybridization (10^7 cpm/ml) was carried out at 52°C for 16 to 20 h in a humidified chamber. Posthybridization washes were carried out as described previously (Lintunen et al., 1998). Sections were exposed to Kodak BioMax X-ray films, and after that dipped in Kodak NTB2-emulsion (Kodak, Rochester, NY). Exposure times on film were 1 to 2 weeks and on emulsion, 12 to 18 weeks.

Results

Cloning of cDNAs Encoding Rat H_3 Isoforms. Using RT-PCR on rat whole-brain total RNA with primers based on the human H_3 cDNA sequence (Lovenberg et al., 1999), we obtained evidence for the existence of receptor isoforms. RT-PCR with a primer pair overlapping the nucleotide sequences, encoding the ends of the intracellular loop 3 (I3), resulted in the amplification of three different DNA products. One fragment showed 85% identity with the I3 loop of the human H_3 receptor cDNA and represented part of the rat H_3 receptor homolog. The other two sequences were identical to the first PCR product, but contained deletions of 96 and 144 bp, corresponding to potential in-frame deletions of 32 and 48 amino acids, respectively. Subsequent RT-PCR on rat whole-brain total RNA resulted in the isolation of full-length cDNAs, encoding three isoforms of the H_3 receptor. The open reading frames of the different cDNAs encode for proteins with 445 (H_{3A}), 413 (H_{3B}), or 397 (H_{3C}) amino acids. The H_{3A} receptor isoform shows 93% identity with the corresponding human H_3 receptor (Fig. 1). The 32- and 48-amino-acid deletions of the H_{3B} and H_{3C} isoform are located in the middle of the I3 loop, resulting in the deletion of potential PKC and PKA phosphorylation sites in the H_{3C} isoform (Fig. 1). In addition to the H_{3A}, H_{3B}, and H_{3C} isoforms, sequence anal-

ysis of the full-length cDNA clones revealed a deletion of 4 bp of the cDNA sequence corresponding to transmembrane domain 2. This 4-bp deletion results in a shift of the open reading frame, resulting in a truncated H_3 receptor isoform (H_{3T}) of 94 amino acids (amino acids 1–83 and 11 new amino acids) (Fig. 1).

Pharmacological Characterization of the Rat H3 Receptor Isoforms. The H_3 receptor isoforms were transiently expressed in COS-7 cells and assayed for [^{125}I]IPP or [^{3}H]NAMH binding. Except for the H_{3T} receptor, all H_3 receptor isoforms specifically bound the agonist and antagonist radioligands (Table 1, data not shown). High-affinity binding of [^{125}I]IPP to the H_{3A}, H_{3B}, or H_{3C} receptor did not differ importantly for the three isoforms and was displaced by a variety of selective H_3 receptor agonists and antagonists. The agonists histamine, immepip, and (*R*)-α-methylhistamine show a 3- to 5-fold difference in affinity for the H_{3A} compared with the H_{3B} or H_{3C} receptor (Table 1). A similar difference was observed for impentamine, which behaves as an agonist at the three isoforms (see below). For the H_3 antagonists clobenpropit and thioperamide, only slight differences in affinity were noticed (Table 1). Coexpression of the H_{3T} receptor with each of the other isoforms affected neither the expression of the respective H_3 receptor isoform nor the affinity of the agonist immepip (data not shown).

All three isoforms inhibited the forskolin-induced production of cAMP in a thioperamide- and PTX-sensitive manner (Fig. 2A, inset; data not shown) as measured by a cAMP-responsive element-luciferase reporter gene assay. Cells cotransfected with the H_3 isoforms and the H_{3T} receptor did not respond differently to immepip (data not shown). The potencies of the full H_3 agonists immepip and (*R*)-α-methylhistamine were significantly higher at the H_{3B} and H_{3C} receptor than at the H_{3A} receptor (Table 2). Interestingly, impentamine, a compound known as H_3 antagonist in the periphery and a partial agonist in the rat brain (Leurs et al., 1996) also displayed full agonism at the three isoforms (Fig. 2A); again, the potency at the H_{3A} receptor was lower compared with the H_{3B} or H_{3C} receptor (Fig. 2A, Table 2). As observed for other GPCRs, for the three full agonists, the pD_2 values were considerably higher than their respective pK_i values.

None of the H_3 receptor isoforms coupled to phospholipase C as determined by the accumulation of [^{3}H]inositol phosphates (data not shown). Yet, H_3 receptor activation resulted in stimulation of the MAP kinase cascade. Treatment of transfected COS-7 cells with the agonist immepip resulted in the rapid activation of p44/p42 MAPK. Within 5 min of H_3 receptor stimulation, MAP kinase activation was detected by an increase in phosphorylation of p44/p42 MAPK by using an antibody against the phosphorylated forms of p44/p42 MAPK. In mock-transfected cells, no increase in p44/p42 MAPK activity was observed upon immepip treatment (data not shown). The H_{3A} receptor coupled considerably better to p42/p44 phosphorylation compared with the other two isoforms (Fig. 2B). For all isoforms, the immepip-induced p44/p42 phosphorylation was blocked by pretreatment with 1 μM thioperamide or treatment with 100 ng/ml PTX (data not shown).

CNS Expression of Rat H_3 Receptor Isoforms. Application of four different histamine H_3-receptor specific probes revealed the receptor isoform expression patterns in the rat brain. The signal intensities for the probes were generally highest for H_{3X}, followed by H_{3C}, H_{3A}, and H_{3B} ($H_{3X} > H_{3C} > H_{3A} \gg H_{3B}$; Fig. 3). In situ hybridization with the H_{3X} probe gave the strongest signal, because it detects the unspliced RNA message as well as all the isoforms. H_{3C} receptor isoform expression pattern resembled that seen with the H_{3X} probe, but the signal intensity was weaker. It is important to note that the signal intensity does not directly and reliably indicate expression levels, although probes were designed for similar conditions. Comparisons are possible between various brain regions when each probe is applied. The H_{3C} signal

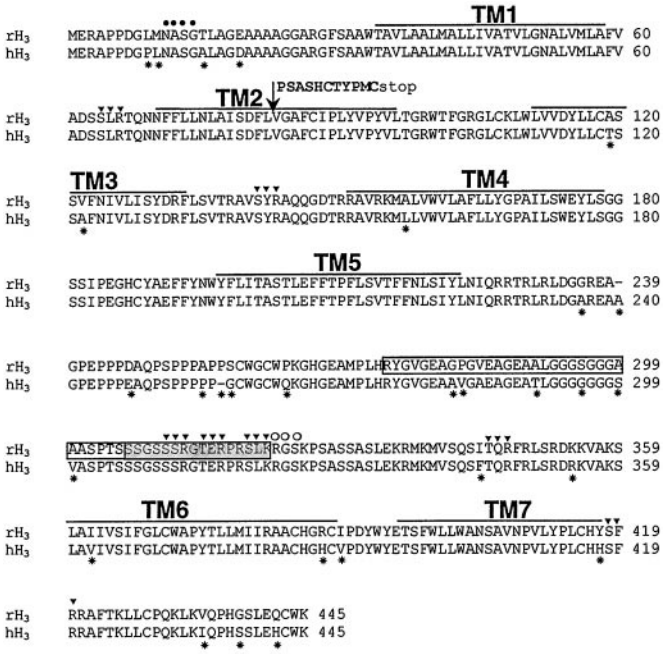

Fig. 1. Alignment of the rat and human H_3 receptor amino acid sequence. The asterisks indicate the observed differences. The boxed amino acids indicate the deletions in either the H_{3B} (open box) or H_{3C} isoform (open shaded box), whereas the arrow indicates the change in the open reading frame, resulting in the insertion of 11 additional amino acids (bold) and a stop codon (H_{3T}). Potential glycosylation (●), PKA (○), or PKC (▲) phosphorylation sites were identified using the PROSITE database (Hofmann et al., 1999) and are indicated.

TABLE 1

Affinity of various H_3 agonists and antagonists for the different rat H_3 receptor isoforms as determined by [^{125}I]IPP binding studies. Data are the means ± S.E.M. from at least three independent experiments

Receptor subtype	[^{125}I]IPP		pK_i Values					
	K_D	B_{max}	Histamine	Immepip	(*R*)-α-methyl histamine	Impentamine	Clobenpropit	Thioperamide
	nM	*pmol/mg of protein*						
H_{3A}	2.1 ± 0.2	1.2 ± 0.07	5.7 ± 0.15	7.5 ± 0.08	6.4 ± 0.2	6.9 ± 0.06	8.3 ± 0.02	7.2 ± 0.08
H_{3B}	1.4 ± 0.1	1.2 ± 0.09	6.2 ± 0.1	8.0 ± 0.06	6.9 ± 0.08	7.4 ± 0.05	8.6 ± 0.1	7.5 ± 0.15
H_{3C}	1.3 ± 0.1	1.3 ± 0.1	6.3 ± 0.07	8.0 ± 0.1	7.1 ± 0.14	7.6 ± 0.04	8.5 ± 0.03	7.6 ± 0.06

was strong in the striatum, olfactory tubercle, cortical laminae V and VIb, pyramidal layers of hippocampal fields CA1 and CA2, dorsal thalamic nuclei, ventromedial hypothalamic nucleus, locus ceruleus, tuberomamillary nucleus, trapezoid body, and the cerebellar Purkinje cell layer (Figs. 3 and 4). Moderate expression was also evident in layer II of the cerebral cortex, but it was low in, for example, medial septum, diagonal band, and substantia innominata (data not shown)

H_{3A} receptor signal intensity was weaker than that of H_{3C}; the Purkinje cells did not express it significantly, but the cerebellar granule cells were instead positive. The expression of H_{3A} in the dorsal part of the dentate gyrus was, in proportion to overall expression, more prominent than that of the other isoforms (Fig. 5A), and expression in CA1 area was strongest in the ventral hippocampus (Fig. 3).

The signal intensity of the H_{3B} isoform was the weakest of all expression patterns tested (Fig. 3). Characteristically, expression of the H_{3B} isoform was very weak in cerebellar Purkinje cells and granule cells (Fig. 5C), the red nucleus did not express detectable signal, the dentate gyrus was devoid of detectable signal (Figs. 3 and 5B), and the cortical expression was limited to layers V and Vib, whereas layer II expression was not detected. Very low expression patterns were seen in the striatum, thalamus, and dorsal raphe (Fig. 5C). The strongest expression was seen in the ventral and ventrolateral tuberomamillary neurons (Fig. 3). Hybridization with a nonrelated control probe yielded no signal in the brain sections.

Discussion

In this study, we report the cloning, CNS expression, and functional characterization of three rat H_3 receptor isoforms, named H_{3A}, H_{3B}, and H_{3C}, that vary in I3 length, with H_{3B} and H_{3C} lacking 32 and 48 amino acids, respectively. Moreover, we identified a 4-bp deletion variant that would give rise to a truncated receptor protein with only one transmembrane domain (H_{3T}). The rat H_{3A} receptor protein is 93% homologous to its human counterpart (Lovenberg et al., 1999) and corresponds to the rat variant that was reported by Lovenberg et al. (2000) (GenBank accession number AF237919) while this work was in progress. Also, the sequences encoding the H_{3B} and H_{3C} isoforms are already known in GenBank, although they are characterized as cDNAs for orphan GPCRs (BAA88767 and BAA88768).

The H_3 receptor isoforms are likely to be generated by alternative splicing. Submission of the human H_3 receptor cDNA sequence to the GenBank database led us to locate the human H_3 receptor gene at human chromosome 20 (GenBank accession number 7263900). Comparison of the reported human cDNA (Lovenberg et al., 1999) and the genomic sequence reveals that the human H_3 receptor gene consists of at least three exons separated by two introns. Exon 1 encodes the first 84 amino acids of the human H_3 receptor. In the human gene, the first intron of 1063 bp is exactly located at the position of the identified 4-bp deletion, suggesting that the rat H_3 receptor gene has a similar organization. The rat H_{3T} variant is probably the result of alternative splicing of intron 1. Exon 2 encodes for the amino acids 85 to 139 and is separated from exon 3 by a second intron of 1564 bp, which encodes for the rest of the human H_3 receptor (amino acids 140 to 445). If we assume a similar genomic organization of the rat gene, the H_{3B} and H_{3C} variants are generated by an alternative splicing mechanism without the obvious presence of an intron. The presence of potential splice donor and acceptor sites in exon 3 of the rat cDNA sequence (see Fig. 3) are apparently responsible for the generation of these isoforms, as has been shown previously for the P_{2X2} receptor (Simon et al., 1997), for example. These

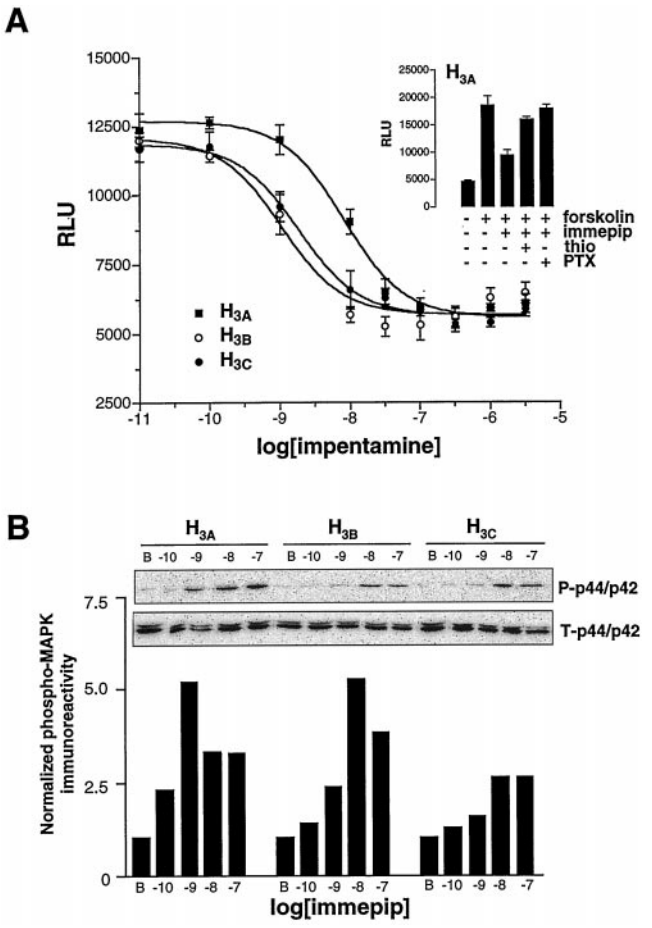

Fig. 2. Signal transduction of the H_3 isoforms after expression in COS-7 cells. A, transfected cells were stimulated with 10 μM forskolin in the absence or presence of impentamine. cAMP was determined as the amount of luciferase expression and expressed as relative light units (RLU). The inset shows the effect of 0.1 μM immepip at the H_{3A} receptor on forskolin-induced cAMP production in the absence or presence of 0.1 μM thioperamide or after PTX treatment (100 ng/ml). Similar data were obtained for the H_{3B} and H_{3C} isoforms. Data shown are mean ± S.E.M. of four determinations. B, effect of different concentrations of immepip on the phosphorylation of p44/p42 MAPK as determined by Western blot analysis using specific anti-phospho-p44/p42 (P-p44/p42) antibodies. Phosphorylation was quantified by chemiluminescence imaging and corrected for total MAPK (T-p44/p42) expression on stripped blots. Data shown are expressed as fold basal and are from a representative experiment. Similar data were obtained in two other independent experiments.

TABLE 2

Potencies (pD_2-values) of H_3 receptor agonists at the three H_3 receptor isoforms, as determined by the inhibition of forskolin-induced cAMP production in transfected COS-7 cells. Data shown are the mean ± S.E.M. of four independent experiments

Receptor subtype	(*R*)-α-methylhistamine	Immepip	Impentamine
H_{3A}	8.5 ± 0.1	9.4 ± 0.1	8.1 ± 0.1
H_{3B}	9.1 ± 0.2	9.7 ± 0.1	9.0 ± 0.05
H_{3C}	9.0 ± 0.2	9.8 ± 0.1	8.8 ± 0.1

putative splice donor and acceptor sites of the rat H_3 receptor gene are conserved in the human cDNA (Lovenberg et al., 1999) and genomic sequence, suggesting the presence of human H_3 receptor isoforms as well.

The general distribution of the H_3 receptor mRNA as revealed with the H_{3X} probe resembled that described previously in some brain areas (Lovenberg et al., 1999). All isoforms were expressed in the tuberomamillary histamine neurons. Further studies are needed to find out whether the H_3 receptor-mediated effects on histamine synthesis (Arrang et al., 1987) and release (Arrang et al., 1983) are regulated by the same or different isoforms. It is likely that the H_{3C} isoform is important in regulation of striatal, thalamic and cortical functions. The relatively strong expression of the H_{3A} isoform in the hippocampus renders it a likely candidate for regulation of hippocampal functions. Histamine has been shown to depress synaptic transmission in the dentate gyrus through an H_3 receptor-mediated mechanism (Brown and Reymann, 1996). The current results demonstrate differential expression of H_3 receptor isoforms in the dentate gyrus and hippocampal subfields. Based on current results, it is obvious that H_3 receptors in the hippocampal formation are located on pyramidal neurons of CA1–3, dentate granule cells, and multiple long-axon afferent pathways. The low expression of H_3 receptor isoforms in dorsal CA3 areas was in contrast with the strong expression in the basal CA3 and CA1 area. Binding of [^{3}H]NAMH is also low in dorsal hippocampus, whereas it is moderate in ventral hippocampus (Cumming et al., 1991). This, together with the heavier innervation of ventral compared with dorsal hippocampus by histaminergic afferents (Panula et al., 1989), suggests that the ventral hippocampal areas are primarily regulated by tuberomamillary histaminergic neurons through H_{3A} and H_{3C} receptor isoforms. Strong expression of H_{3B} and H_{3C} isoforms in the locus ceruleus and dorsal raphe nucleus suggests that these isoforms may be responsible for inhibition of noradrenaline (Schlicker et al., 1989) and serotonin (Schlicker et al., 1988) release, respectively. Lack of all isoforms in the pars compacta of the substantia nigra suggests that the H_3 receptor-mediated inhibitory effect on dopamine release as observed in the mouse (Schlicker et al., 1993) may be indirect. This is supported by the abundant presence of H_3 receptor mRNA in striatal cells, which may thus mediate the inhibitory effect on dopamine release from striatal dopaminergic terminals. In agreement with evidence that suggests that H_3 receptors are not located on cholinergic terminals (Arrang et al., 1995), very low expression of all isoforms was characteristic of the medial septum, diagonal band, and substantia innominata, areas in which cholinergic projection neurons are located. Regulation of acetylcholine release in

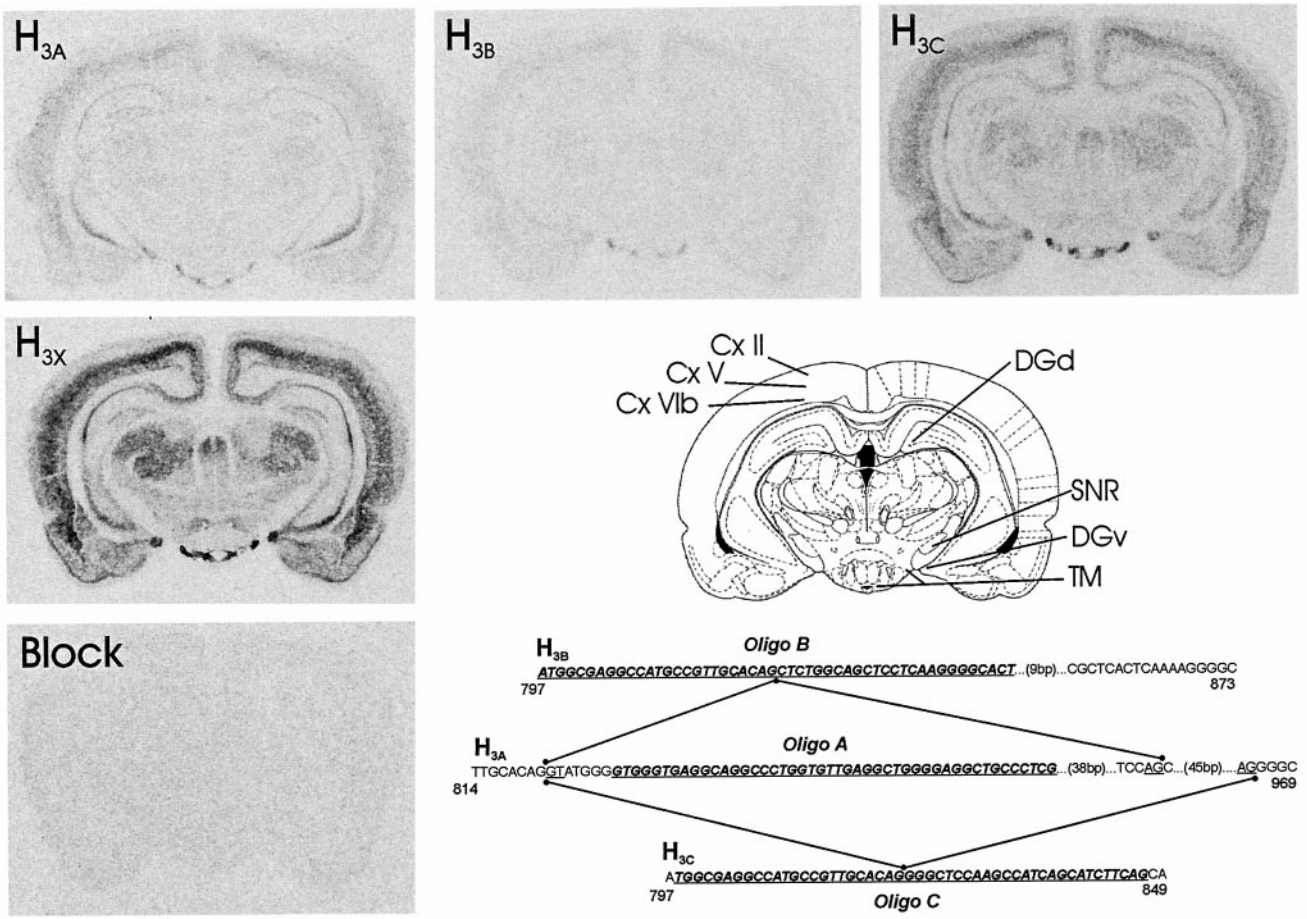

Fig. 3. Histamine H_3 receptor isoform expression in rat brain. The expression pattern seen with the H_{3X} probe resembles that seen with the H_{3C} probe. The H_{3A} and H_{3B} expression pattern clearly differs from that of H_{3C} and H_{3X}. Control hybridization with a 100-fold excess of unlabeled H_{3X} probe results in a completely abolished signal (Block). The brain map indicates some of the relevant areas, and a schematic picture of part of the nucleotide sequence shows the positions for the isoform-specific oligonucleotides (oligo A, B, and C, respectively). The positions for the mRNA splicing are also indicated on the H_{3A} sequence (underlined). The micrographs with in situ hybridization pictures are images from exposed X-ray films.

vitro (Clapham and Kilpatrick, 1992) or in vivo (Blandina et al., 1996) by histamine through H_3 receptor may thus also involve indirect mechanisms. The cortical neurons in several cortical laminae that express H_3 receptor mRNA may mediate the effect. These cells, together with cholinergic terminals, are present in slice preparations used in some experiments, which makes it difficult to evaluate the release site in perfusion experiments. Cerebellar Purkinje cells expressed strongly the H_{3C} isoform, and H_{3A} suptype was found in granule cells. A direct hypothalamo-cerebellar pathway consists of long histaminergic axons that pass through the granule cell and Purkinje cell layer and enter the superficial portion of the molecular layer also in human brain (Panula et al., 1993). Histamine may thus participate in regulation of motor functions in cerebellum through H_{3A} and H_{3C} receptor isoforms.

Differences in H_3 receptor isoform expression were found in many other brain areas as well. The functions of H_3 receptor in many of these areas are in general poorly known. The heterogeneous distribution of the isoforms suggests that H_3 receptor isoform-specific functional histaminergic regulation may be important in several areas. The H_3 receptors

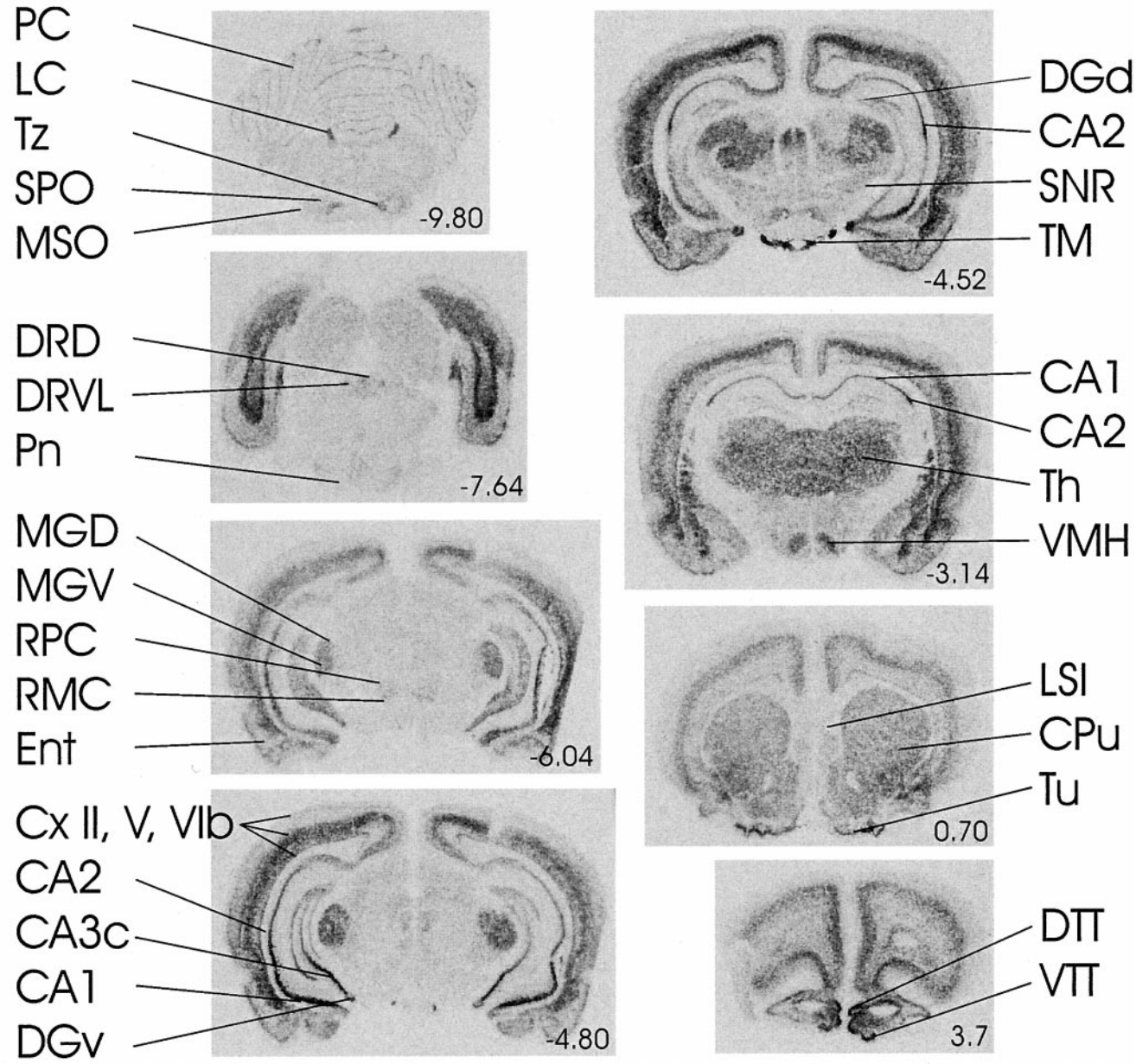

Fig. 4. Histamine H_3 receptor expression in rat brain. The in situ hybridization was carried out with the H_{3X} probe which detects all the cloned mRNAs. All micrographs are scanned images from X-ray films. The stereotaxic location for each section is indicated by giving the distance to the bregma (in millimeters). CA1, 2, and 3, CA1, CA2, and CA3 pyramidal layer of hippocampus; CA3c, CA3 caudal part; CPu, caudate putamen (striatum); Cx, 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, tuberomamillary area; Tu, olfactory tubercle; Tz, trapezoid body; VMH, ventromedial hypothalamic nucleus; VTT, ventral tenia tecta.

displayed differential expression in key areas involved in regulation of the sensory, endocrine, and cognitive functions in the brain. Robust changes also occur in the brain histamine system during the hibernation cycle, in which the turnover is high during the hibernation bout when other transmitter systems are generally inactive (Sallmen et al., 1999). Hence, histamine may modulate many general functions (Hough, 1988; Schwartz et al., 1991; Wada et al., 1991) through the H_3 receptor isoforms.

As found for the human H_3 receptor (Lovenberg et al., 1999), the rat isoforms bind H_3 selective agonists and antagonists with high affinity and inhibit the production of cAMP via PTX-sensitive G proteins. In line with the idea that the I3 loop is important for GPCR-G protein coupling (Wess, 1997), reduced potencies for various H_3 receptor agonists at the H_{3A} receptor were observed in comparison with the H_{3B} or H_{3C} isoform. The histamine homolog impentamine, which has been reported as an antagonist at the H_3 receptor in the guinea pig jejunum and an agonist for the H_3 receptor in the rat cerebral cortex, showed full agonism at all three isoforms. Again, activity at the H_{3A} receptor was reduced. For all H_3 receptor isoforms, the inhibition of adenylate cyclase was completely PTX-sensitive, (i.e., $G_{i/o}$-mediated). The observed differences in agonist potency at the three isoforms point to differences in coupling efficiencies to the same $G\alpha_{i/o}$-subunit but can also be explained by an isoform-specific coupling to distinct $G\alpha_{i/o}$-subunits, as previously reported for the isoforms of the D_2 receptor (Monsma et al., 1989).

Interestingly, activation of the H_3 receptor isoforms also leads to activation of the MAP kinase signaling cascade via PTX-sensitive G proteins. The H_{3A} isoform seems to be more effectively coupled to the p44/p42 MAPK activation, further stressing the differences in G protein coupling of the different isoforms. This is the first report linking the H_3 receptor to the MAPK pathway, which is believed to be important in neuronal plasticity and is activated in hippocampal long term potentiation (English and Sweatt, 1996; Bhalla and Iyengar, 1999). The H_3 receptor is known to be involved in learning and memory processes (see Leurs et al., 1998) and histamine has also been implicated in long-term potentiation (Brown et al., 1995). The strong expression of the H_{3A} isoform in the hippocampus and the preferential linkage to the MAP kinase cascade will add a new level of complexity to our understanding of the role of the H_3 receptor(s) and histamine in this process.

In conclusion, in contrast to the H_1 and H_2 receptor (Hill et al., 1997) the presence of introns in the H_3 receptor gene gives rise to various H_3 receptor isoforms via alternative splicing. The three identified rat H_3 receptor isoforms have a distinct CNS distribution and show some differences in pharmacology and signaling. Moreover, all three isoforms couple to the MAPK cascade, a newly identified signaling pathway for the H_3 receptor. Our data are supported by the recent report of Tardivel-Lacombe et al. (2000). While this article was in preparation, the cDNAs of the guinea pig H_3 receptor and one shorter isoform (Tardivel-Lacombe et al., 2000) were reported. The shorter guinea pig H_3 isoform corresponds to the rat H_{3B} variant, but no radioligand binding data, signal

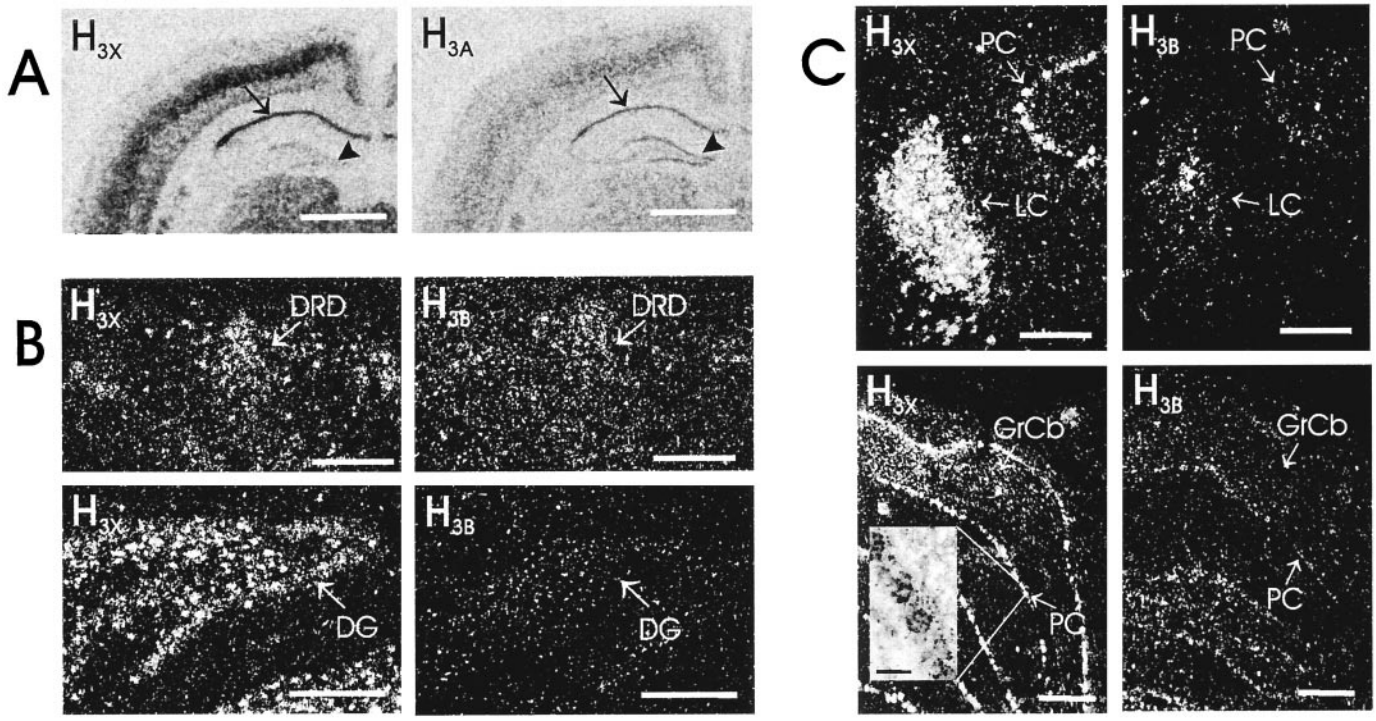

Fig. 5. Histamine H_3 receptor isoform expression in rat brain. A, the differential expression patterns of H_{3X} and H_{3A} probes in rat hippocampus. The pyramidal layer of the CA1 area is indicated with arrows, and dentate gyrus with arrowheads. B, expression pattern with the H_{3X} probe in the dorsal raphe nucleus, dorsal part, is identical to the pattern seen with H_{3B}. In dentate gyrus, the H_{3X} expression is also obvious, whereas the H_{3B} expression is below the detection level. C, a prominent expression signal with the H_{3X} probe is revealed in the locus ceruleus and in Purkinje cells, but in adjacent sections, the H_{3B} expression is faint. The micrographs are X-ray film images from adjacent brain sections and the film exposure times are similar for both probes (A), darkfield images taken from emulsion-coated and exposed slides (B and C), except for the inset picture, which is a bright-field image from a hybridized and counterstained section. DRD, dorsal raphe nucleus, dorsal part; DG, dentate gyrus; LC, locus ceruleus; PC, Purkinje cells; GrCb, granular layer of cerebellum. [Scale bars, 2 mm (A), 500 μm (B and C), 30 μm (inset micrograph)].

transduction, or isoform-selective expression was reported. Because the identified rat H_{3A}, H_{3B}, and H_{3C} subtypes do not explain all pharmacological findings that gave rise to suggestions of H_3 receptor heterogeneity, it is possible that the complex genomic organization of the H_3 receptor can result in further isoforms.

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