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# Identification of zebrafish histamine $H_1$, $H_2$ and $H_3$ receptors and effects of histaminergic ligands on behavior

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ABSTRACT

Neuronal histamine regulates several functions in the vertebrate brain. The zebrafish brain contains a widespread histaminergic system and $H_3$ receptor ligand binding has been reported. In this study we provide evidence for the existence of histamine $H_1$, $H_2$ and $H_3$ receptor genes in zebrafish. Single copies of putative histamine $H_1$, $H_2$ and $H_3$ receptors were identified and cloned from the zebrafish brain. Expression analysis suggested that they are expressed in the brain and a few other tissues. Widespread distribution of zebrafish $H_2$ receptor binding sites was detected with [$^{125}$I]iodoaminopotentidine in brain sections. Zebrafish larvae were exposed to 1, 10 or 100 μM of the $H_1$ ligand pyrilamine, the $H_2$ ligand cimetidine and the $H_3$ ligands thioperamide and immepip for 5 days. Significant decreases in swimming distance were observed with the highest dose of all ligands, whereas cimetidine gave a significant decrease also with 1 and 10 μM doses. These results provide the first molecular biological evidence for the presence of histamine receptors in zebrafish. These histamine receptors resemble those of higher vertebrates and they provide a useful model for pharmacological and behavioral studies for characterizing the functions of histamine in more detail.



## 1. Introduction

Histamine is involved in several regulatory mechanisms in the brain, including alertness and sleep, seizure threshold, hormone secretion and pain [1–4], but the detailed mechanism behind these functions are poorly established. It acts through at least four types of characterized G-protein-coupled receptors (GPCR) in mammals: histamine $H_1$, $H_2$, $H_3$ and $H_4$ receptors. Of these receptors only the $H_4$ receptor has not been consistently found in the brain [5]. Mammalian histaminergic neurons are located in the tuberomamillary nucleus (TMN) and their fibers innervate the whole brain [6–8], supporting the concept that this is a phylogenetically well preserved system.

The histaminergic system in zebrafish resembles that of other vertebrates [9,10]. Expression of L-histidine decarboxylase has been identified only in the caudal part of the hypothalamus in areas corresponding to the TMN in the

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*Abbreviations:* α-FMH, α-fluoromethylhistidine; [$^{125}$I]APT, [$^{125}$I]iodoaminopotentidine; dpf, day post fertilization; GPCR, G-protein-coupled receptor; hpf, hour post fertilization; IC, intracellular loop; TM, transmembrane region; TMN, tuberomamillary nucleus

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mammalian hypothalamus [9]. Histamine $H_3$-like receptor binding and $H_3$-related G-protein activation have been described in the zebrafish brain [11]. However, no molecular characterization of this receptor has been done in zebrafish or any other fish species. The $H_1$ receptor has earlier been mapped using in vivo [$^{14}$C]2-deoxyglucose autoradiography and in vitro receptor-binding methods in *Tilapia nilotica* [12]. Recently it was shown that both zolantidine and chlorpheniramine give inhibitory avoidance responses in goldfish *Carassius auratus*, suggesting the potential presence of both $H_1$ and $H_2$ receptor in fish [13]. To date there is no evidence of the molecular identity of these receptors in fish, including the most commonly used species, zebrafish (*Danio rerio*). The zebrafish brain histamine content can be reduced by α-fluoromethylhistidine (α-FMH), and this reduction is associated with changes in the exploratory behavior and in T-maze performance. These changes might be due to reduced anxiety and some memory-related mechanisms after histamine depletion [14].

The gene for $H_1$ receptor, as for several other GPCR, lacks introns [15–18]. It has long been known that, in addition to the well known, important function in allergic and inflammatory conditions [19], the $H_1$ receptor is connected to the sleep-wakefulness cycle. More recent reports suggest that the arousal effect of orexin A is due to activation of histaminergic neurotransmission mediated by $H_1$ receptor [20–22]. The $H_1$ receptor expression pattern in the CNS is rather widespread [23,24]. The expression in the hypothalamus suggests the involvement in regulation of feeding, and a role in mediating the effects of leptin has been documented [25]. Activation of the $H_1$ receptor is coupled to the stimulation of phospholipase C via $Gq_{/11}$ G-proteins [26] the important transcription factor NF-κB [27].

The canine $H_2$ receptor [28] was the first cloned intron-lacking histamine receptor. It was soon followed by the cloning of the $H_2$ receptor from several species including human, rat and mouse [29,30]. The $H_2$ receptor signals through the $G_s$-protein, leading to stimulation of adenylyl cyclase and activation of CREB [31,32]. The $H_2$ receptor binding pattern has been well characterized in guinea pig brain using [$^{125}$I]iodoaminopotentidine ([$^{125}$I]APT) [33]. The involvement of $H_2$ receptors in defensive/escape behavior as a response to fear has been suggested [34–36].

The histamine $H_3$ receptor was first characterized as an autoreceptor which mediates inhibition of histamine synthesis and release [37,38]. It is a modulator of the release of several transmitters [39–42]. The cloning of $H_3$ receptor by Lovenberg et al. [43] was followed by identification of receptor subtypes [44–46]. The $H_3$ receptor signaling couples to the inhibitory $G_{i/o}$ type of G-protein [43,47]. The activation of $H_3$ receptor causes an inhibition of adenylyl cyclase [43,45]. Distinct pharmacology and expression pattern has been described for some of the isoforms of $H_3$ receptors. The stimulation of isoforms $H_{3A}$, $H_{3B}$ and $H_{3C}$ induces phosphorylation of mitogen activated protein kinase (MAPK) [45]. Recently the isoforms $H_{3D}$, $H_{3E}$ and $H_{3F}$ with an alternative extracellular C terminus from rat were described [48].

The interest in the histamine receptors as drug targets has been increasing, particularly for the $H_3$ receptor [49,50], because histamine regulates important central functions and the receptors are abundantly expressed in important brain areas. Existing data on the zebrafish histaminergic system indicates that histamine is mostly present in the CNS of the zebrafish [9], and manipulation of histamine levels alters behavior [14]. Earlier binding studies indicate that the zebrafish has a $H_3$-like receptor, however, no molecular biological evidence for the presence of any of the histamine receptors is available. The purpose of this study was to identify the genes for the histamine receptors, and find out if histaminergic ligands produce significant behavioral effects in developing zebrafish.

## 2. Methods

### 2.1. Experimental animals

Zebrafish (*D. rerio*) larvae and adults, from outbred (originate from local fish shop) and AB strains maintained in the laboratory for several generations, of both sexes were used for the study. The fish were kept at 28.5 °C with a light/dark cycle of 14 h/10 h and fed twice daily. Breeding and raising was done according to Westerfield [51]. The permit to carry out these studies was obtained from the Committee for Animal Experiments of Abo Akademi University and the Office of the Regional Government of Western Finland.

### 2.2. Cloning of zebrafish $H_1$, $H_2$ and $H_3$ receptor

Sequences showing high similarity to the human $H_1$, $H_2$ and $H_3$ receptor were identified using the Sanger Institute zebrafish blast pages (http://www.ensembl.org/Danio_rerio). The predicted coding sequences for the receptors, based on homology analysis with known histamine receptors, were amplified using PCR. cDNA reverse transcribed (First-Strand Synthesis Kit, Amersham Biosciences, Uppsala, Sweden or SuperScript™ First-Strand Synthesis System for RT-PCR, Invitrogen, BV, Groningen, The Netherlands) from RNA extracted (RNAwiz™, Ambion Inc., Austin, TX) from zebrafish brains was used as template in the reactions. For the $H_1$ receptor (Genbank accession no. DQ647806) the following primers were used: forward 5′-TGT CTC TCC TCA CCG TCA TC-3′ and reverse 5′-CAA TCC CTT CAT TTA CCC GCT CT-3′. The $H_2$ receptor coding sequence was amplified using (Genbank accession no. DQ647808), the forward primer 5′-ATG CAA TTT ATA TTC AGC GAT-3′ and reverse primer 5′-ACT ACC TTT TAC TCC CAT TAG C-3′ were used. The corresponding primers for the $H_3$ receptor (Genbank accession no. DQ647807) coding sequence were 5′-ATG GAG AGA GAA AAC GCG-3′ and 5′-AAT ACT TCT GGT CAA TGT TC-3′. Amplified PCR products were sequenced from both directions by automatic sequencing (PRISM 310; ABI, Norwalk, CT).

### 2.3. mRNA expression by RT-PCR

RNA was extracted from different developmental stages (3, 6, 11, 24, 48 and 72 hpf, 5, 7 and 10 dpf) and from fresh isolated brains, eyes, gills, intestine, heart, liver, muscle and spleen from 10 adult fish and reverse transcribed as described above. cDNA originating from 0.25 μg total RNA was used for the PCR

reactions which were carried out with the same primer pairs as above for $H_1$, $H_2$ and $H_3$ receptor, respectively.

### 2.4. Sequence comparisons and analysis

Multisequence alignments were performed using ClustalW on the Web pages provided by the European Bioinformatics Institute (EMBL-EBI), sequence analyses were carried out with GENSCAN Web Server at MIT. Sequence comparisons were carried out with ALIGN provided by (EMBL-EBI) using the needle method.

### 2.5. [$^{125}$I]iodoaminopotentidine binding to histamine $H_2$ receptors

[$^{125}$I]iodoaminopotentidine ([$^{125}$I]APT) was synthesized as described [26] and was received as a kind gift from Professor Rob Leurs and Professor Henk Timmerman (Vrije Universiteit, Amsterdam, The Netherlands). Brain sections were incubated for 3 h at room temperature with 50 mM $Na_2$/K phosphate buffer, pH 7.5, containing 0.2% gelatin and 0.2 nM [$^{125}$I]APT, 2000 Ci/mmol. To determine non-specific binding, adjacent sections were incubated in the presence of [$^{125}$I]APT and 10 μM tiotidine. The sections were rapidly rinsed in ice-cold 50 mM $Na_2$/K phosphate buffer, pH 7.5 and then washed 3× 10 min in cold, fresh buffer. The slides were dipped into ice-cold water and rapidly dried under cold airflow. The sections were apposed to Kodak BioMax MR film (Kodak, Rochester, NY) for 1 day.

### 2.6. Treatment of zebrafish with histamine receptor ligands

Ligand treatment of young fish was done in 6-well plates. Thirty-five fish were kept in 3 ml E3 (5 mM NaCl, 0.17 mM KCl, 0.4 mM $CaCl_2$, and 0.16 mM $MgSO_4$) with 1, 10 or 100 μM, pyrilamine, cimetidine, thioperamide or immepip. The control groups were kept in E3. The treatment was started at 2–3 h post fertilization (hpf). At 1-day post fertilization (dpf) the fish were dechorionated to ease the penetration of ligand into embryo. The E3 and ligands were changed once every day to fresh solutions. The behavior was tracked at 5 dpf.

Thioperamide and immepip were kind gifts from Professor Rob Leurs and Professor Henk Timmerman (Vrije Universiteit, Amsterdam, The Netherlands). Pyrilamine and cimetidine were purchased from Sigma (St. Louis, MO).

### 2.7. Video recording of behavior

Young zebrafish exposed to histaminergic ligands for 5 dpf were used for studies of behavioral changes.

In order to minimize effects on social behavior the fish were individually tracked in separate observation tanks. In the test the swimming performance of young fish were recorded simultaneously [14]. The experiments were done in a calm sealed-off area.

The swimming performance of young fish was recorded with a digital video camera connected to a standard PC computer system running the EthoVision Pro 3.1 software (Noldus Information Technology, Wageningen, The Netherlands). Due to the size of young fish they were placed individually in 48-well plates where they were tracked for 10 min using sample rate 5 samples/s. The coordinates of the swimming performance were stored and used for further software analysis of the behavior. Analysis was performed with input filter 0.2 cm for minimum distance moved to get rid of system noise. One exclusion criterion were used; when the sample size did not exceed 90% of the maximum sample size the tracks were excluded. Statistical analysis was preformed with Student's t-test (GraphPad Prism, GraphPad Software Ink, CA).

## 3. Results

### 3.1. Cloning and expression of zebrafish $H_1$, $H_2$ and $H_3$ receptors

The zebrafish histamine receptors were cloned to provide evidence of the molecular identity. The zebrafish putative $H_1$ receptor gene is located on chromosome 8. The intronless gene codes for a 534 aa long peptide. This is 57 aa longer than the human $H_1$ receptor and 2 aa shorter than the predicted canine $H_1$ receptor. The zebrafish $H_1$ has a 20 aa extension in the 5′ end and a 12, 3 and 5 aa inserts in the intracellular loop (IC) 3 compared to human $H_1$. The zebrafish peptide has a second methionine 17 aa downstream from the first, based on Clustal W multiple sequence analysis this is more likely the real translation start site (Figure 1 in supplementary data). Peptide comparisons display 40–46% identity with the corresponding mammalian receptor (Table 1).

The putative zebrafish $H_2$ receptor is an intronless gene located on chromosome 15, which displays 43–47% identity on the peptide level with the corresponding peptides from dog, human and mouse (Figure 2 in supplementary data). The zebrafish $H_2$ is 51 aa longer than the corresponding receptor from human, with 27 aa more in the 5′ end, a 6 aa longer IC3 and 13 aa longer 3′ end.

Sequence comparisons with the corresponding mammalian receptor reveal highest identities in the transmembrane (TM) regions for both zebrafish $H_1$ and $H_2$ receptor. The aspartic acid in TM2, conserved in all studied aminergic receptors, exists also in the zebrafish $H_1$ and $H_2$ receptor (Figures 1 and 2 in supplementary data).

In the zebrafish $H_2$ receptor the alanine-lysine-arginine (AKR) and glutamic acid-histidine-lysine-alanine (EHKA) motifs in IC3 suggested to be involved in $G_s$ binding are conserved.

The putative zebrafish $H_3$ receptor gene contains three exons and is located on chromosome 7. The first exon contains the start site and continues to the middle of TM2, the second exon runs to the beginning of IC2. The third exon contains the rest of the coding sequence. Peptide comparisons reveal 42–50% identities with the corresponding sequences from mouse, human and dog (Table 1, Figure 3 in supplementary data). The 1422 nucleotide long sequence has two possible translation start sites. The correct ATG is most likely the later (nucleotide 103–105, Genbank accession no. DQ647807). When compared to rat histamine $H_3$ receptor isoforms A, B, C, D and E, the zebrafish peptide shows highest identities to rat $H_{3A}$. As for the $H_1$ and $H_2$ receptor, the highest identities compared to other $H_3$

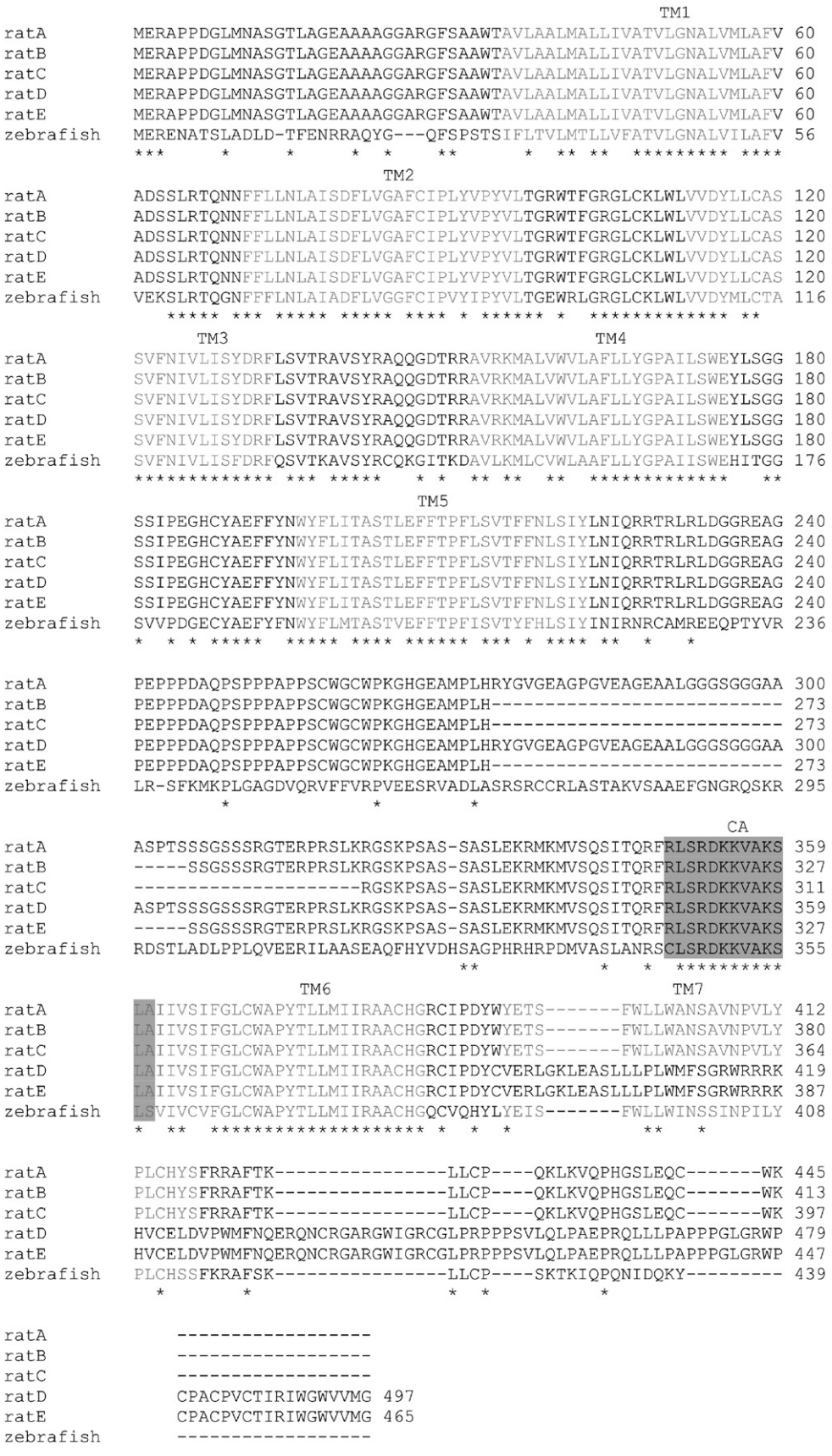


**Fig. 1 – Sequence identities. Peptide sequence comparisons of the zebrafish $H_3$ receptor with the different isoforms of $H_3$ receptor in rat. Amino acids identical in all peptides are marked by an asterisk. The transmembrane regions 1–7 are marked TM1-7 and printed with gray letters. The area involved in constitutive activity of the receptor is marked CA and shaded.**

**Table 1 – Peptide comparison of the zebrafish $H_1$ (GenPept accession no. ABF71708), $H_2$ (GenPept accession no. ABF71710), and $H_3$ (GenPept accession no. ABF71709) peptide to the corresponding sequence from human ($H_1$ GenPept accession no. NP_000852, $H_2$ GenPept accession no. NP_071640, $H_3$ GenPept accession no. NP_009163), mouse ($H_1$ GenPept accession no. NP_032311, $H_2$ GenPept accession no. AAM47009, $H_3$ GenPept accession no. NP_598610), dog ($H_1$ GenPept accession no. AAD14167, $H_2$ GenPept accession no. AAD14163, $H_3$ GenPept accession no. AAO63755), predicted sequence from chick ($H_1$ GenPept accession no. XP_425153, $H_2$ GenPept accession no. XP_425153, $H_3$ GenPept accession no. XP_425117) and urchin ($H_1$ GenPept accession no. AAW88352, $H_2$ GenPept accession no. XP_794263, $H_3$ GenPept accession no. XP_794616)**

| $H_1$ | Human | Mouse | Dog | Chick | Urchin | Zebrafish | zf $H_2$ | zf $H_3$ |
|---|---|---|---|---|---|---|---|---|
| Human | 100 | | | | | | 19 | 25 |
| Mouse | 80 | 100 | | | | | 18 | 26 |
| Dog | 79 | 70 | 100 | | | | 18 | 24 |
| Chick | 46 | 46 | 43 | 100 | | | 20 | 24 |
| Urchin | 27 | 28 | 29 | 28 | 100 | | 15 | 16 |
| Zebrafish | 40 | 46 | 40 | 40 | 30 | 100 | 17 | 17 |
| $H_2$ | Human | Mouse | Dog | Chick | Urchin | Zebrafish | zf $H_1$ | zf $H_3$ |
| Human | 100 | | | | | | 16 | 16 |
| Mouse | 85 | 100 | | | | | 17 | 17 |
| Dog | 87 | 80 | 100 | | | | 15 | 16 |
| Chick | 56 | 58 | 57 | 100 | | | 16 | 16 |
| Urchin | 22 | 22 | 21.4 | 21 | 100 | | 14 | 15 |
| Zebrafish | 44 | 47 | 43 | 45 | 20 | 100 | 17 | 20 |
| $H_3$ | Human | Mouse | Dog | Chick | Urchin | Zebrafish | zf $H_1$ | zf $H_2$ |
| Human | 100 | | | | | | 18 | 17 |
| Mouse | 94 | 100 | | | | | 18 | 17 |
| Dog | 75 | 75.5 | 100 | | | | 18 | 16 |
| Chick | 59 | 59 | 48 | 100 | | | 14 | 15 |
| Urchin | 24 | 24 | 26 | 14 | 100 | | 16 | 16 |
| Zebrafish | 50 | 49 | 42 | 30 | 21 | 100 | 17 | 20 |

zf: zebrafish.

receptors are found in the TM regions and the aspartic acid (D) in TM2 is conserved. The 3′ end of IC3, suggested to be involved in constitutive activity, is well conserved in the zebrafish $H_3$ receptor (Fig. 1). The rest of the IC3 shows low homologies compared to the corresponding receptor from human and rat. The aa 119 and aa 122 are known to be involved in the species differences of the $H_3$ pharmacology (REF). The zebrafish has threonine as aa 119 and valine as aa 122, this is similar to the canine receptor.

### 3.2. *Histamine receptor expression*

A signal in the RT-PCR reaction for the $H_1$ and $H_2$ receptor was detected in all developmental stages tested, indicating that mRNA for these receptors is expressed throughout the development (Fig. 2A). The product in the $H_3$ receptor RT-PCR reaction from 3 hpf was very weak, at 6 hpf the signal was more clearly visible.

RT-PCR was done on RNA from adult zebrafish brain, eye, gill, heart, intestine, liver, muscle and spleen for the histamine receptors (Fig. 2B). A product in the brain was detected for all three receptors. In addition, primers for the $H_1$ receptor gave clear products in the RNA from intestine, liver and spleen. The $H_2$ receptor product was also visible in gills, heart and spleen. The $H_3$ receptor expression was strong, in addition in the

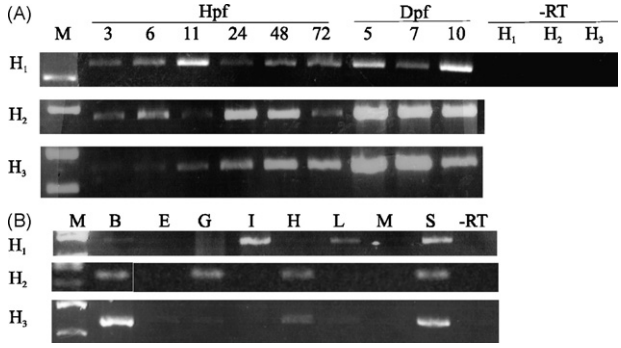


**Fig. 2 – Expression of histamine receptors during development and in different adult tissues. RT-PCR the $H_1$, $H_2$ and $H_3$ receptor. (A) Developmental stages 3, 6, 11, 24, 48, 72 hpf and 5, 7 and 10 dpf showing PCR products for the $H_1$, $H_2$ and $H_3$ receptor. (B) RT-PCR products for $H_1$, $H_2$ and $H_3$ receptor from different adult tissues. Brain (B), eye (E), gill (G), intestine (I), heart (H), liver (L), muscle (Mu), spleen (S). RT controls are processed as the samples, contains RNA but lacks reverse transcriptase. In (B) the $H_2$ panel is reordered by cutting and pasting to have the samples in the same order for all three panels.**

**GenPept accession numbers for the rat receptors; AF237919 ($H_{3A}$), BAA88767 ($H_{3B}$), BAA88768 ($H_{3C}$), AAZ93637 ($H_{3D}$), AAZ93638 ($H_{3E}$).**

brain, in heart and spleen, weaker bands were seen in liver, eyes and gills.

### 3.3. $H_2$ receptor binding

Binding sites for [$^{125}$I]APT were identified in most parts of the zebrafish brain. High densities of binding were observed in optic tectum, hypothalamus, locus coeruleus and the superior reticular formation (Fig. 3).

### 3.4. Changes in behavior after ligand exposure

The swimming behavior of young fish was analyzed 5 dpf. Parameters analyzed were total distance moved (cm/recording time), velocity (cm/s), turn angle (°), angular velocity (°/s) and meander (°/cm). The $H_2$ receptor antagonist cimetidine decreased the total distance moved at all three concentrations used. The distance moved compared to the control group was decreased to 75% with 1 μM, 75% with 10 μM and 62% with 100 μM cimetidine. For pyrilamine, thioperamide and immepip a significant decrease in swimming behavior was detected only with the highest, 100 μM, dose at 5 dpf (Fig. 4A). Pyrilamine was the ligand giving the biggest effect, at 100 μM concentration the fish swam only 14% of the control distance, the 1 and 10 μM concentration decreased the distance moved to 75% compared to the control. Thioperamide, at the concentrations 1, 10 and 100 μM decreased the swimming distance to 89%, 95% and 58%, correspondingly. Fish treated with immepip swam 88% (1 μM), 89% (10 μM) and 73% (100 μM) of the control distance. The turning behavior of the fish was affected only by the 1 μM concentration of pyrilamine, which gave a slightly decreased turning behavior

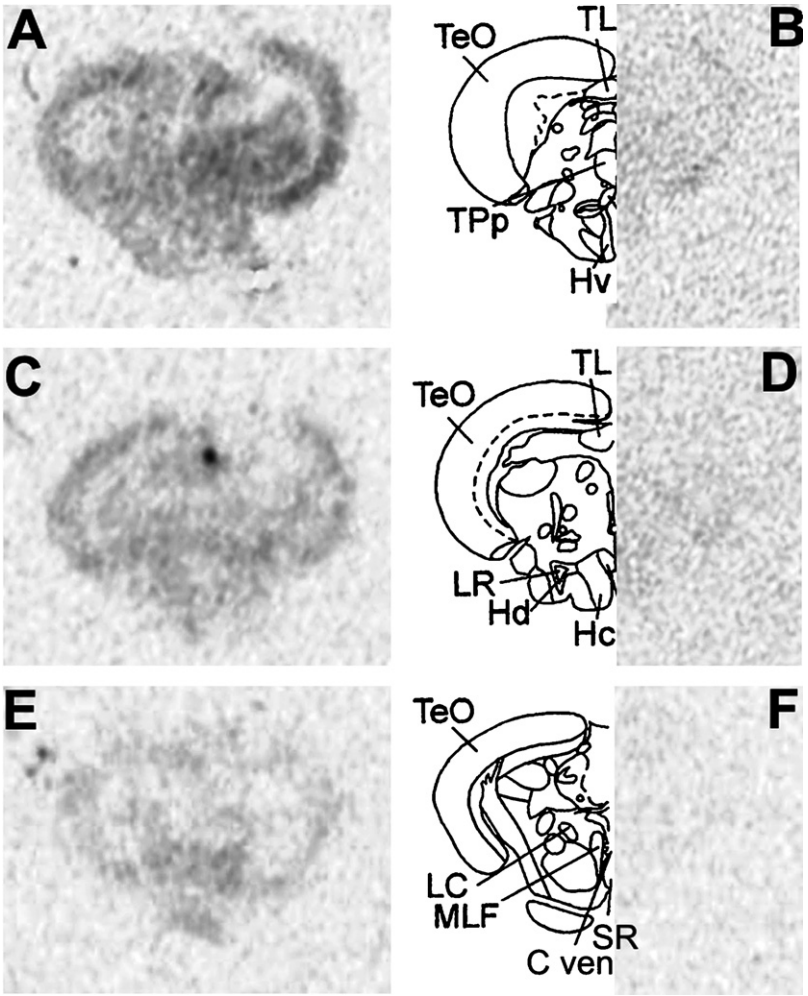


**Fig. 3 – $H_2$ receptor binding sites in zebrafish brain revealed with [$^{125}$I]iodoaminopotentidine. (A) Binding sites in optic tectum (TeO) and the central of diencephalon are shown. (B) The corresponding section blocked with 10 mM tiotidine and a schematic drawing of the corresponding level. (C) A more caudal section compared to (A) shows the high binding in TeO and hypothalamic regions. (D) The signal is almost completely blocked in sections treated with 10 mM tiotidine. (E) Caudal part of the hindbrain revealing binding in locus coeruleus (LC) and the superior reticular formation (SR). (F) A corresponding section with the signal blocked with tiotidine is displayed together with a schematic drawing of the section level. C ven, commissura ventralis rhombencephali; Hc, caudal zone of periventricular hypothalamus; Hd, dorsal zone of periventricular hypothalamus; Hv, ventral zone of periventricular hypothalamus; LR, lateral recess of diencephalic ventricle; MLF, medial longitudinal fascicle; TL, torus longitudinalis; TPp, periventricular nucleus of posterior tuberculum.**

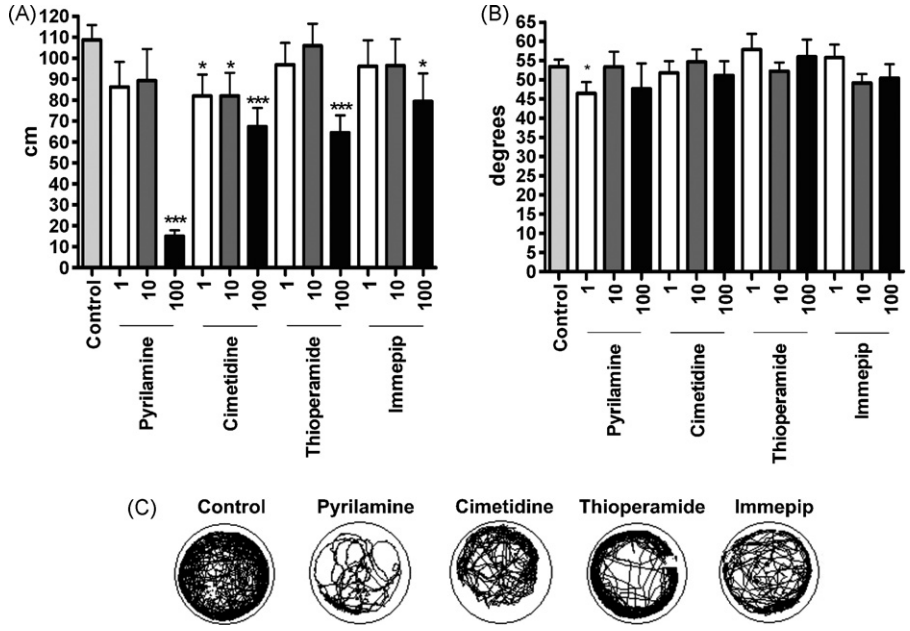


**Fig. 4 – Swimming patterns of young zebrafish after treatment with histamine receptor ligands. (A) Total distance moved of young fish at 5 dpf. Bars indicate means and standard deviation. Statistical analysis preformed with Student's *t*-test, $^{***}P < 0.001$, $^{*}P < 0.05$. *N* varies in the different groups; at 5 dpf control = 81, pyrilamine 1 μM = 34, 10 μM = 35, 100 μM = 36, cimetidine 1 μM = 38, 10 μM = 37, 100 μM = 37, thioperamide 1 μM = 38, 10 μM = 42, 100 μM = 45, immepip 1 μM = 38, 10 μM = 41, 100 μM = 36. (B) Turning angel, the data represents the turning behavior of the same group of fish as in (A). (C) Typical swimming patterns for the different treatments at 100 μM ligand concentration at 5 dpf.**

(turn angle) in the 5-day-old fish (Fig. 4B). Typical swimming tracks for the 100 μM treated groups at 5 dpf are shown in Fig. 4C. The pattern of the swimming did not change significantly after larvae were treated with histamine ligands. Thioperamide-treated fish tend to swim along the walls of the well, while other histamine ligand treated larvae exhibited similar patterns of middle crossing/wall circling as the control group.

## 4. Discussion

Cloning of the sequences for the putative $H_1$, $H_2$ and $H_3$ receptors in zebrafish based on homology searches of the genbanks suggests that behavioral effects observed in fish after treatment with histamine receptor ligands are potentially mediated through specific receptors. No putative zebrafish $H_4$ sequences were found at the time of the searches, using the human and rat $H_4$ sequence information. No other closely related sequences were identified in the databases.

Amino acids known to be of importance for ligand binding to the $H_2$ receptor are an aspartic acid in TM3 and aspartic acid and threonine in TM5 [52]. Aspartic acid-arginine-tyrosine (DRY) residues in the IC2 have been reported to be of importance for the receptor stability [53], and this motif is conserved in zebrafish. The AKR and EHKA motifs in IC3 are suggested to be involved in $G_s$ G-protein binding [54]. Both the residues are crucial for ligand binding and $G_s$ binding are conserved in the putative zebrafish $H_2$ receptor. The results in this study on zebrafish $H_2$ receptor indicate that the pharmacology and signaling mechanisms resemble those of higher vertebrates, although agonist/inverse agonist properties of ligands are difficult to predict.

A single form of putative zebrafish $H_3$ receptor was identified in this study. This resembles the isoform $H_{3A}$ from rat and isoform $H_3$ 1 from human, the so called full-length receptor. Further studies are needed to clarify if there are isoforms of zebrafish $H_3$ receptor.

The constitutive activity of the $H_3$ receptor is ensured by the end of the IC3 and the two first amino acids of TM6 [55]. This area is almost completely conserved in zebrafish, indicating the possibility of a similar constitutive activity, as in rodents, of histamine $H_3$ receptor. This would further strengthen the proposed involvement of zebrafish histamine $H_3$ receptor in modulation of higher behaviors. Furthermore, the aspartic acid residue in TM3 known to be conserved in aminergic receptors [56], is conserved in the three zebrafish histamine receptors $H_1$, $H_2$ and $H_3$ receptor.

Using autoradiography and cloning of the putative peptide we indicate here that the zebrafish has a histamine $H_2$ receptor, which has not been characterized earlier in fish. Earlier pharmacological studies suggest the presence of $H_2$ receptors in peripheral tissues of cod [57]. A putative $H_3$ receptor, which may correspond to the receptor ligand binding published earlier [11], was cloned and found to display 42–50% identity with the corresponding mammalian receptor and 30% to the predicted chick $H_3$ receptor. All three zebrafish histamine receptors were expressed in the brain as revealed with RT-PCR, suggesting involvement in CNS regulation. In addition to this the $H_1$ receptor is expressed in intestine, liver

and spleen. Expression of $H_2$ receptor was found in the periphery in gills, heart and spleen and the $H_3$ receptor clearly in heart and spleen. Interestingly, all three receptors were also expressed very early during development, which may indicate developmental regulatory roles.

Binding sites for the $H_2$- and $H_3$-ligands are identified in the zebrafish brain in corresponding areas as in mammals. The aspartic acid residue in TM3 known to be conserved in aminergic receptors [56], is conserved in the three zebrafish histamine receptors $H_1$, $H_2$ and $H_3$ receptor. This data indicates that the $H_1$, $H_2$ and $H_3$ receptors have remained highly conserved between zebrafish and mammals.

The binding pattern for the $H_3$-like receptor was described earlier [11]. Attempts to map the binding of the $H_1$-ligand [$^3$H]mepyramine in adult zebrafish brain sections failed due to the low intensity of the signal/small size of the tissue sections and long exposure times, making it difficult to distinguish the specific signal from background. $H_2$ receptor binding with [$^{125}$I]APT revealed autoradiography signals throughout the brain with high densities in optic tectum, hypothalamic areas, locus coeruleus and the superior reticular formation in a pattern, comparable with the widespread, distribution of this receptor in mammals [33]. $H_2$ receptor ligand binding signals were abolished with tiotidine, indicating that the detected signal is specific for the $H_2$ receptor. We have earlier identified the binding sites for an $H_3$-like receptor in zebrafish brain [11]. Binding data for both $H_2$ and $H_3$ receptors indicated that these receptors regulate integrated functions in several areas of zebrafish brain, e.g. optic tectum.

To assay possible behavioral involvement of these receptors young zebrafish were exposed to ligands for the $H_3$ receptor through water. The drug concentration in the water was 1, 10 or 100 μM. The exact amounts that the fish take up from water are difficult to determine, which may affect the behavioral results obtained. The young fish in this study showed a declined distance moved after exposure to 100 μM of all histamine receptor ligands studied. The $H_1$ antagonist pyrilamine decreased the distance moved to 14% compared to the control group. This is in agreement with the data from $H_1$ knock-out mice, which display a reduced exploratory behavior and decreased locomotor behavior [18]. Classical $H_1$ antagonists to which pyrilamine belongs, or antihistamines, are well known to cause a sedative effect, leading to a decreased movement. Interestingly, since the $H_2$ knock-out mice seem to lack a CNS phenotype [58], the $H_2$ receptor antagonist cimetidine gave rise to a significant decline in movement already at the concentration 1 μM. The $H_3$ receptor agonist immepip and inverse agonist thioperamide induced similar changes in fish behavior with the highest dose used. The decrease seen with thioperamide was, however, to 60% of control, whereas that of immepip showed a smaller decline to 74% of the control group value. This similar type of behavior may be due to agonist/inverse agonist properties of $H_3$ receptor ligands that differ from those at mammalian receptors, or regulation of unrelated phenomena that give similar results.

The behavioral changes in zebrafish after exposure to agonist/antagonist for the receptors have, to some extent been identified in mammals. Our data indicate that histamine receptors are functional in zebrafish brain and we provide here the first, to our knowledge, molecular evidence of zebrafish histamine receptor genes. These results on the zebrafish histaminergic system is involved in measurable behaviors and further examination of its genomic organization will help reveal the evolutional functions of histamine. The results also suggest that zebrafish larvae could be a feasible model to test pharmacological compounds in vivo, affecting the locomotor activity. However, the functional properties of the zebrafish $H_1$, $H_2$ and $H_3$ receptor need to be elucidated, with e.g. in vitro cell system with zebrafish signaling properties.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bcp.2007.01.014.

## REFERENCES

[1] Schwartz JC, Arrang JM, Garbarg M, Pollard H, Ruat M. Histaminergic transmission in the mammalian brain. Physiol Rev 1991;71:1–51.
[2] Hough LB.Histamine In: Siegel GJ, editor. Basic neurochemistry: molecular, cellular and medical aspects. Philadelphia: Lippincott-Raven Publishers; 1999. p. 293–313.
[3] Brown RE, Stevens DR, Haas HL. The physiology of brain histamine. Prog Neurobiol 2001;63:637–72.
[4] Haas H, Panula P. The role of histamine and the tuberomamillary nucleus in the nervous system. Nat Rev Neurosci 2003;4:121–30.
[5] Liu C, Wilson SJ, Kuei C, Lovenberg TW. Comparison of human, mouse, rat, and guinea pig histamine H4 receptors reveals substantial pharmacological species variation. J Pharmacol Exp Ther 2001;299:121–30.
[6] Panula P, Yang HY, Costa E. Histamine-containing neurons in the rat hypothalamus. Proc Natl Acad Sci USA 1984;81:2572–6.
[7] Watanabe T, Taguchi Y, Shiosaka S, Tanaka J, Kubota H, Terano Y, et al. Distribution of the histaminergic neuron system in the central nervous system of rats; a fluorescent immunohistochemical analysis with histidine decarboxylase as a marker. Brain Res 1984;295:13–25.
[8] Steinbusch HW, Sauren Y, Groenewegen H, Watanabe T, Mulder AH. Histaminergic projections from the premammillary and posterior hypothalamic region to the caudate-putamen complex in the rat. Brain Res 1986;368:389–93.
[9] Eriksson KS, Peitsaro N, Karlstedt K, Kaslin J, Panula P. Development of the histaminergic neurons and expression of histidine decarboxylase mRNA in the zebrafish brain in the absence of all peripheral histaminergic systems. Eur J Neurosci 1998;10:3799–812.

[10] Kaslin J, Panula P. Comparative anatomy of the histaminergic and other aminergic systems in zebrafish (*Danio rerio*). J Comp Neurol 2001;440:342–77.
[11] Peitsaro N, Anichtchik OV, Panula P. Identification of a histamine H(3)-like receptor in the zebrafish (*Danio rerio*) brain. J Neurochem 2000;75:718–24.
[12] Choich JA, El-Nabawi A, Silbergeld EK. Evidence of histamine receptors in fish brain using an in vivo [14C]2-deoxyglucose autoradiographic method and an in vitro receptor-binding autoradiographic method. Environ Res 2004;94(1):86–93.
[13] Cofiel LP, Mattioli R. Involvement of histamine receptors in the acquisition of inhibitory avoidance in *Carassius auratus*. Prog Neuropsychopharmacol Biol Psychiatry 2006;30(7):1246–50.
[14] Peitsaro N, Kaslin J, Anichtchik OV, Panula P. Modulation of the histaminergic system and behaviour by alpha-fluoromethylhistidine in zebrafish. J Neurochem 2003;86:432–41.
[15] Fujimoto K, Horio Y, Sugama K, Ito S, Liu YQ, Fukui H. Genomic cloning of the rat histamine H1 receptor. Biochem Biophys Res Commun 1991;190:294–301.
[16] Horio Y, Mori Y, Higuchi I, Fujimoto K, Ito S, Fukui H. Molecular cloning of the guinea-pig histamine H1 receptor gene. J Biochem (Tokyo) 1993;114:408–14.
[17] Fukui H, Fujimoto K, Mizuguchi H, Sakamoto K, Horio Y, Takai S, et al. Molecular cloning of the human histamine H1 receptor gene. Biochem Biophys Res Commun 1994;201:894–901.
[18] Inoue I, Yanai K, Kitamura D, Taniuchi I, Kobayashi T, Niimura K, et al. Impaired locomotor activity and exploratory behavior in mice lacking histamine H1 receptors. Proc Natl Acad Sci USA 1996;93:13316–20.
[19] Hill SJ, Ganellin CR, Timmerman H, Schwartz JC, Shankley NP, Young JM, et al. International Union of Pharmacology. XIII. Classification of histamine receptors. Pharmacol Rev 1997;49:253–78.
[20] Huang ZL, Qu WM, Li WD, Mochizuki T, Eguchi N, Watanabe T, et al. Arousal effect of orexin A depends on activation of the histaminergic system. Proc Natl Acad Sci USA 2001;98:9965–70.
[21] Shigemoto Y, Shinomiya K, Mio M, Azuma N, Kamei C. Effects of second-generation histamine H1 receptor antagonists on the sleep-wakefulness cycle in rats. Eur J Pharmacol 2004;494:161–5.
[22] Jorgensen EA, Knigge U, Watanabe T, Warberg J, Kjaer A. Histaminergic neurons are involved in the orexigenic effect of orexin-A. Neuroendocrinology 2005;82:70–7.
[23] Traiffort E, Leurs R, Arrang JM, Tardivel-Lacombe J, Diaz J, Schwartz JC, et al. Guinea pig histamine H1 receptor. I. Gene cloning, characterization, and tissue expression revealed by in situ hybridization. J Neurochem 1994;62:507–18.
[24] Lintunen M, Sallmen T, Karlstedt K, Fukui H, Eriksson KS, Panula P. Postnatal expression of H1-receptor mRNA in the rat brain: correlation to L-histidine decarboxylase expression and local upregulation in limbic seizures. Eur J Neurosci 1998;10:2287–301.
[25] Masaki T, Chiba S, Yasuda T, Noguchi H, Kakuma T, Watanabe T, et al. Involvement of hypothalamic histamine H1 receptor in the regulation of feeding rhythm and obesity. Diabetes 2004;53:2250–60.
[26] Leurs R, Smit MJ, Menge WM, Timmerman H. Pharmacological characterization of the human histamine H2 receptor stably expressed in Chinese hamster ovary cells. Br J Pharmacol 1994;112:847–54.
[27] Bakker RA, Schoonus SB, Smit MJ, Timmerman H, Leurs R. Histamine H(1)-receptor activation of nuclear factor-kappa B: roles for G beta gamma- and G alpha(q/11)-subunits in constitutive and agonist-mediated signaling. Mol Pharmacol 2001;60:1133–42.
[28] Gantz I, Schaffer M, DelValle J, Logsdon C, Campbell V, Uhler M, et al. Molecular cloning of a gene encoding the histamine H2 receptor. Proc Natl Acad Sci USA 1991;88:5937.
[29] Gantz I, Munzert G, Tashiro T, Schaffer M, Wang L, DelValle J, et al. Molecular cloning of the human histamine H2 receptor. Biochem Biophys Res Commun 1991;178:1386–92.
[30] Ruat M, Traiffort E, Arrang JM, Leurs R, Schwartz JC. Cloning and tissue expression of a rat histamine H2-receptor gene. Biochem Biophys Res Commun 1991;179:1470–8.
[31] Baudry M, Martres MP, Schwartz JC. H1 and H2 receptors in the histamine-induced accumulation of cyclic AMP in guinea pig brain slices. Nature 1975;253:362–4.
[32] Tanimoto A, Murata Y, Nomaguchi M, Kimura S, Arima N, Xu H, et al. Histamine increases the expression of LOX-1 via H2 receptor in human monocytic THP-1 cells. FEBS Lett 2001;508:345–9.
[33] Vizuete ML, Traiffort E, Bouthenet ML, Ruat M, Souil E, Tardivel-Lacombe J, et al. Detailed mapping of the histamine H2 receptor and its gene transcripts in guinea-pig brain. Neuroscience 1997;80:321–43.
[34] Santos NR, Huston JP, Brandao ML. Escape behavior under tonic inhibitory control of histamine H(2)-receptor mediated mechanisms in the midbrain tectum. Behav Brain Res 2001;124:167–75.
[35] Santos NR, Husto JP, Brandao ML. Further evidence for the involvement of histamine H2 receptors in the control of defensive behaviour generated in the midbrain tectum. Behav Pharmacol 2002;13:73–80.
[36] Santos NR, Huston JP, Brandao ML. Blockade of histamine H2 receptors of the periaqueductal gray and inferior colliculus induces fear-like behaviors. Pharmacol Biochem Behav 2003;75:25–33.
[37] Arrang JM, Garbarg M, Schwartz JC. Auto-inhibition of brain histamine release mediated by a novel class (H3) of histamine receptor. Nature 1983;302:832–7.
[38] Arrang JM, Garbarg M, Schwartz JC. Autoinhibition of histamine synthesis mediated by presynaptic H3-receptors. Neuroscience 1987;23:149–57.
[39] Schlicker E, Betz R, Gothert M. Histamine H3 receptor-mediated inhibition of serotonin release in the rat brain cortex. Naunyn Schmiedebergs Arch Pharmacol 1998;337:588–90.
[40] Schlicker E, Fink K, Hinterthaner M, Gothert M. Inhibition of noradrenaline release in the rat brain cortex via presynaptic H3 receptors. Naunyn Schmiedebergs Arch Pharmacol 1998;34:633–8.
[41] Schlicker E, Fink K, Detzner M, Gothert M. Histamine inhibits dopamine release in the mouse striatum via presynaptic H3 receptors. J Neural Transm Gen Sect 1993;93:1–10.
[42] Van Der Werf JF, Timmerman H. The histamine H3 receptor: a general presynaptic histaminergic regulatory system? Trends Pharmacol Sci 1989;10:159–62.
[43] Lovenberg TW, Roland BL, Wilson SJ, Jiang X, Pyati J, Huvar A, et al. Cloning and functional expression of the human histamine H3 receptor. Mol Pharmacol 1999;55:1101–7.
[44] Tardivel-Lacombe J, Rouleau A, Heron A, Morisset S, Pillot C, Cochois V, et al. Cloning and cerebral expression of the guinea pig histamine H3 receptor: evidence for two isoforms. Neuroreport 2000;11:755–9.
[45] Drutel G, Peitsaro N, Karlstedt K, Wieland K, Smit MJ, Timmerman H, et al. Identification of rat H3 receptor isoforms with different brain expression and signaling properties. Mol Pharmacol 2001;59:1–8.

[46] Morisset S, Sasse A, Gbahou F, Heron A, Ligneau X, Tardivel-Lacombe J, et al. The rat H3 receptor: gene organization and multiple isoforms. Biochem Biophys Res Commun 2001;280:75–80.
[47] Clark EA, Hill SJ. Sensitivity of histamine H3 receptor agonist-stimulated [35S]GTP gamma[S] binding to pertussis toxin. Eur J Pharmacol 1996;296:223–5.
[48] Bakker RA, Lozada AF, van Marle A, Shenton FC, Drutel G, Karlstedt K, et al. Discovery of naturally occurring splice variants of the rat histamine H3 receptor that act as dominant-negative isoforms. Mol Pharmacol 2006;69: 1194–206.
[49] Leurs R, Bakker RA, Timmerman H, De Esch IJ. The histamine H3 receptor: from gene cloning to H3 receptor drugs. Nat Rev Drug Discov 2005;4:107–20.
[50] Passani MB, Lin JS, Hancock A, Crochet S, Blandina P. The histamine H3 receptor as a novel therapeutic target for cognitive and sleep disorders. Trends Pharmacol Sci 2004;25:618–25.
[51] Westerfield M. The zebrafish book. A guide for laboratory use of the zebrafish (*Brachydanio rerio*). University of Oregon Press; 1995.
[52] Birdsall NJ. Cloning and structure-function of the H2 histamine receptor. Trends Pharmacol Sci 1991;12: 9–10.
[53] Alewijnse AE, Timmerman H, Jacobs EH, Smit MJ, Roovers E, Cotecchia S, et al. The effect of mutations in the DRY motif on the constitutive activity and structural instability of the histamine H(2) receptor. Mol Pharmacol 2000;57:890–8.
[54] Strader CD, Dixon RA, Cheung AH, Candelore MR, Blake AD, Sigal IS. Mutations that uncouple the beta-adrenergic receptor from Gs and increase agonist affinity. J Biol Chem 1987;262:16439–43.
[55] Morisset S, Rouleau A, Ligneau X, Gbahou F, Tardivel-Lacombe J, et al. High constitutive activity of native H3 receptors regulates histamine neurons in brain. Nature 2006;408:860–4.
[56] Fraser CM, Wang CD, Robinson DA, Gocayne JD, Venter JC. Site-directed mutagenesis of m1 muscarinic acetylcholine receptors: conserved aspartic acids play important roles in receptor function. Mol Pharmacol 1998;36:840–7.
[57] Holstein B. Characterization with agonists of the histamine receptors mediating stimulation of gastric acid secretion in the Atlantic cod, *Gadus morhua*. Agents Actions 1986;19: 42–7.
[58] Kobayashi T, Tonai S, Ishihara Y, Koga R, Okabe S, Watanabe T. Abnormal functional and morphological regulation of the gastric mucosa in histamine H2 receptor-deficient mice. J Clin Invest 2000;105:1741–9.