Genomics Meets Histamine Receptors: New Subtypes, New Receptors

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Received January 16, 2001; accepted January 16, 2001

Whether the job is waking the brain after a peaceful sleep, initiating gastric secretion when dinner is served or orchestrating the elements of inflammation after a mosquito bite, histamine has been a known biological messenger for decades (Green, 1964; Eichler and Farah, 1966). At the end of the twentieth century, in the midst of the genomics and bioinformatics revolution, researchers in this field knew of the existence of only three histamine receptors (H1, H2, and H3). But histamine receptors are catching up! Not only have multiple forms of the H3 receptor recently been described but also a new histamine receptor, H4, has now been identified.

The presently-known histamine receptors (H1, H2, and H3) are all G protein-coupled molecules and they transduce extracellular signals via Gq, Gs, and Gi/o, respectively (Hill et al., 1997; Lovenberg et al., 1999). Not surprisingly, classic pharmacology studies (Ash and Schild, 1966; Black et al., 1972; Arrang et al., 1983) argued for their existence decades before they were cloned (Gantz et al., 1991; Yamashita et al., 1991; Lovenberg et al., 1999). Likewise, heterogeneity among H2 receptors had long been suspected based on agonist kinetics (West et al., 1990), radioligand binding characteristics (Cumming et al., 1991; Alves-Rodrigues et al., 1996), peripheral versus central nervous system pharmacology (Leurs et al., 1996; Harper et al., 1999), and other functional studies (Schlicker et al., 1992; Schwerer et al., 1994), but the absence of subtype-selective compounds prevented firm classification.

Although the H1 and H2 receptors were cloned nearly a decade ago (Gantz et al., 1991; Yamashita et al., 1991), the H3 receptor was not cloned until 1999 (Lovenberg et al., 1999). However, this elucidation of the H3 receptor structure in man and other species (Lovenberg et al., 1999, 2000; Tardivel-Lacombe et al., 2000; Drutel et al., 2001) quickly led to discoveries of the H3 receptor subtypes and the closely related H4 receptor, which are discussed presently. Recent molecular studies have shown that a single form of the H3 gene can give rise to multiple mRNA isoforms, named H3A, H3B, and H3C in the rat (Drutel et al., 2001), and H3L and H3S in the guinea pig (Tardivel-Lacombe et al., 2000). The variants all are known to differ in the structure of their third cytoplasmic loops, although the relevant splicing mechanisms remain uncertain (Tardivel-Lacombe et al., 2000; Drutel et al., 2001). Thus far, similar variants in human samples have not been identified (Liu et al., 2000), although the existence of multiple, somewhat different H3 isoforms in humans was reported recently (Wellendorf et al., 2000). The H3 receptor isoform that seems to be most predominant in human brain corresponds to the rat H3A and the guinea pig H3L. In the January 2001 issue of this journal, pharmacological differences in the H3 receptor subtypes, as well as evidence for a differential distribution of the subtypes in rat brain, were presented (Drutel et al., 2001). Considering the current interest in the H3 autoreceptor (Morisset et al., 2000), the ability of the H3 heteroreceptor to regulate the activity of many brain transmitters (Hill et al., 1997; Hough, 1999) and the potential for developing new H3 pharmacotherapies [e.g., in attention deficit/hyperactivity disorder, Alzheimer's disease, obesity, and others (Leurs et al., 1998; Tedford, 1998)], the characterization of the H3 receptor subtypes is of considerable significance.

Phylogenetic (Leurs et al., 2000) and homology analysis (Lovenberg et al., 1999) of the H3 receptor showed it to be surprisingly different from the previously cloned H1 and H2 receptors, a likely explanation for the delay in its discovery. Indeed, at the time of the H3 receptor cloning, its homology to any other known G protein-coupled receptor was only 31% (Leurs et al., 2000). Because of this, the search for new receptors in a family more closely related to the H3 receptor seemed promising. As described in the accompanying articles (Liu et al., 2001; Nguyen et al., 2001; Zhu et al., 2001) and in other recent (Oda et al., 2000) and concurrent (Morse et al., 2001) articles, screening of libraries and public databases for H3-like fragments succeeded and led to the cloning and preliminary characterization of what is now referred to as the H4 receptor. This receptor is a 390-amino-acid, 7-transmembrane G protein-coupled receptor, with a 37 to 43% homology to the H3 (58% in transmembrane regions). All of the current

This work was supported by Grant DA03816 from the National Institute on Drug Abuse.
studies report identical amino acid sequences for the receptor (Liu et al., 2001; Morse et al., 2001; Nguyen et al., 2001; Zhu et al., 2001); this sequence varies slightly from that of the original H₄ receptor (Oda et al., 2000). The human H₃ and H₄ receptors possess very similar genomic structures; both have two introns and three exons (Liu et al., 2001; Zhu et al., 2001), although the receptors are localized on different chromosomes (20 and 18, respectively). In addition, like the H₃ receptor, the H₄ receptor seems to couple to Gi/o [and possibly to other pathways (Oda et al., 2000)], thereby inhibiting forskolin-activated cAMP formation (Zhu et al., 2001). Evidence for a plasma membrane localization and agonist-stimulated internalization of H₄ has also been presented (Nguyen et al., 2001). Notably, the distribution of the H₄ receptor is quite different from that of the H₃ receptor. In contrast to a nearly exclusive brain localization for the H₃ receptor, the H₄ receptor shows highest levels in bone marrow and leukocytes (particularly eosinophils and neutrophils), with moderate levels in spleen and small intestine. Mast cells may also contain the H₄ receptor (Zhu et al., 2001). Northern analyses and other preliminary expression studies reported the absence of the H₄ receptor in the central nervous system (Oda et al., 2000; Morse et al., 2001; Nguyen et al., 2001). However, in situ hybridization studies in mouse (Zhu et al., 2001) and RNase protection assays in human samples (Liu et al., 2001) yielded evidence for a brain localization.

In general, the H₄ studies show excellent agreement on the preliminary pharmacology of the new receptor. Reported potencies of histaminergic compounds in competing against [³H]histamine binding to the various H₄ clones are highly correlated across four laboratories (Fig. 1). However, results with [³H]pyrilamine binding on another H₄ clone are discrepant (Fig. 1). These results, along with the lack of activity of pyrilamine on the H₄ receptor reported by other labs (Table 1), raise a question regarding the suitability of pyrilamine as a radioligand for studying the H₄ receptor. Although the reasons for this discrepancy are not clear, it should be noted that [³H]pyrilamine (also known as mepyramine) has been used as a radioligand for the H₁ receptor, but was later shown to also bind specifically to certain cytochrome isozymes, thus yielding false positives for the H₁ assay (Leurs et al., 1989; Liu et al., 1994).

Given the structural similarities of the receptor, it is not surprising that the pharmacologies of the H₃ and H₄ receptors overlap (Table 1; Fig. 2). The high-affinity H₃ agonists also have H₄ agonist activity, but with a reduced potency. Most notable is (R)-α-methylhistamine, which shows several hundred-fold weaker activity at H₄ versus H₃ receptors. Thioperamide, the prototypical H₃ antagonist, also has appreciable H₄ antagonist activity (Table 1; Fig. 2). Some data (Liu et al., 2001) even suggest that this drug may be an inverse agonist at H₄ receptors, similar to recent results showing this effect on H₃ receptors (Morisset et al., 2000). Most of the results suggest that thioperamide has a 5- to 10-fold lower potency at the H₄ receptor than at the H₃ receptor (Table 1; Fig. 2). The H₃ antagonists clobenpropit and burimamide also have a lower affinity for the H₄ receptor, but these compounds show partial agonist activity at the new receptor. Most promising for pharmaceutical development are data showing the existence of potent, non-imidazole H₄ antagonists (e.g., compound 17 in Table 1 and Fig. 2) that lack activity at the H₄ receptor (Table 1). Taken together, these results suggest that H₄ responses are activated by low doses of histamine, but not by (R)-α-methylhistamine, and are blocked by large doses of thioperamide (an imidazole) but not by non-imidazole-containing H₃ antagonists. Although compounds capable of selectively acting at the new receptor have not yet been described, the atypical antipsychotic drug clozapine (discussed further below) shows moderate H₄ and no H₃ activity (Fig. 2), and thus may be a lead in this direction.

The above characteristics suggest that the H₄ receptor has been with us longer than we realized. Raible et al. (1994) reported a histamine-activated increase in cytosolic calcium in human eosinophils; the effect was sensitive to thioperamide and partially mimicked by burimamide but not by low concentrations of (R)-α-methylhistamine. Similarly, the histamine-induced inhibition of serotonin release in intestinal enterochromaffin cells resembles an H₄ response with respect to pharmacology and tissue expression (Schwerer et al., 1994). It is also likely that the “histamine uptake” discovered in bone marrow hematopoietic cells (Corbel et al., 1997) represents in-fact binding of [³H]histamine and other ligands to the H₄ receptor, based on the pharmacology. In some of these studies, the potency of thioperamide can be difficult to interpret because of a large species difference (up to 10-fold) in the affinity of thioperamide for the human versus the rat H₃ receptor (Lovenberg et al., 2000); the difference is controlled by only two amino acid substitutions (Ligneau et al., 2000). There are other reported effects of thioperamide that are not reversed by H₃ agonists, and the H₄ receptor must now be considered in these cases. For example, thioperamide increases extracellular levels of both histamine and γ-aminobutyric acid in brain, but only the former effect is reversed by H₃ agonists (Yamamoto et al., 1997). Of course, thioperamide actions are not restricted to the H₃ and H₄ receptors; it has some affinity at other sites as well [e.g., 5-HT₃ (Leurs et al., 1995)] and may even be found to have activity at additional, unknown histamine receptors. Although the new H₄ work accounts for the existence of some novel histamine receptors previously suggested to exist, it cannot account for others. For example, HTMT [6-(2-(4-imidazolyl)ethylamino)-N-(4-trifluoromethylphenyl)heptanecarboxamide], the histamine...
derivative that suppresses lymphocyte function by a novel receptor (Khan et al., 1986), is not active at the H₄ (Table 1). Similarly, improgan, a cimetidine congener that induces anaglogs by a mechanism distinct from known histamine receptors (Hough et al., 2000), also had low affinity for the H₄ site (Table 1).

The newly discovered effects of clozapine on the H₄ receptor (Table 1, Fig. 2) add a new chapter to the longstanding relationship between psychosis, antipsychotic drugs, and brain histamine (Green et al., 1977; Raucher et al., 1977). Chlorpromazine, the first neuroleptic, was developed from brain histamine (Green et al., 1977; Raucher et al., 1977). The newly discovered effects of clozapine on the H₄ receptor is an intriguing question which remains to be answered; it is tempting to speculate that the eosinophilic rolepetic clozapine was reported to have moderate activity on the rat brain H₃ receptor (Rodrigues et al., 1995), an effect confirmed on the rat (Kathmann et al., 1994) but not on the human receptor (Table 1). Although the Kᵢ value for clozapine on the H₄ receptor is relatively high (500–700 nM, Table 1), plasma and brain concentrations associated with clinical responses meet or exceed these values (Baldessarini and Frankenburg, 1991). Even more interesting is that clozapine seems to be an agonist at H₄ receptors (Oda et al., 2000; Liu et al., 2001). Although we do not yet know the consequences of H₄ receptor stimulation in the hippocampus (Zhu et al., 2001) or in eosinophils, it seems quite possible that patients taking clozapine are recipients of both actions. Whether this receptor participates in either the therapeutic or toxic effects of this drug is an intriguing question which remains to be answered; it is tempting to speculate that the eosinophilic

**TABLE 1**

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<th>Potencies of histaminergic drugs on four histamine receptors.</th>
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a Guinea pig ileum (Hill et al., 1997).

b Guinea pig atrium (Hill et al., 1997).

Kᵢ values for competition against [³H]N-methylhistamine binding on the human recombinant H₃ receptor (Liu et al., 2001).

Kᵢ values for competition against [³H]histamine binding on the human recombinant H₄ receptor (Liu et al., 2001).

Kᵢ values for competition against [³H]histamine binding on the human recombinant H₅ receptor (Zhu et al., 2001).

Kᵢ values for competition against [³H]histamine binding on the human recombinant H₆ receptor (Morse et al., 2001).

T. Lovenberg, unpublished observations.

Radioligand binding (Tran et al., 1978).

Adenylate cyclase (Green et al., 1977).

Bioassay (Ganellin, 1982).

Highly selective H₃ agonists (Hill et al., 1997).

Highly selective H₄ agonists (Hill et al., 1997).

Clozapine has up to a 10-fold higher potency on the rat H₃ receptor (Lovenberg et al., 2000).

Abcissa values (as in the studies identified. When more than one laboratory studied the same compound, a single H₃ Kᵢ value is plotted against more than one H₄ Kᵢ value. Compounds numbers correspond with those in Table 1. Values plotted as 10,000 nM were reported to be inactive at that concentration.

![Fig. 2. Relationship between H₃ and H₄ receptor potency.](https://example.com/fig2.png)
agranulocytosis, which often limits clozapine effectiveness, might be related to the H4 receptor (Oda et al., 2000). Much additional work on the H4 system is needed. H4 receptor subtypes may be found based on similarities to H3. The activities of the histamine metabolites need to be assessed on this receptor, because several of these metabolites have biological activity (Phillis et al., 1968; Thomas and Prell, 1995), and histamine metabolism is highly regulated in some cases (Haddock et al., 1990). Finally, H4-selective drugs will need to be developed that can further define the biological roles for this receptor and lead to unique pharmacotherapies. All indications suggest that many more receptors for histamine remain to be discovered.

Acknowledgments

I thank Dr. Tim Lovenberg for valuable discussions.

References


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