Constitutive Activity of Histamine H3 Receptors Stably Expressed in SK-N-MC Cells: Display of Agonism and Inverse Agonism by H3 Antagonists

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ABSTRACT

Agonist-independent activity of G-protein-coupled receptor, also referred to as constitutive activity, is a well-documented phenomenon and has been reported recently for both the histamine H1 and H2 receptors. Using SK-N-MC cell lines stably expressing the human and rat H3 receptors at physiological receptor densities (500–600 fmol/mg of protein), we show that both the rat and human H3 receptors show a high degree of constitutive activity. The forskolin-mediated cAMP production in SK-N-MC cells is inhibited strongly upon expression of the H3 receptor and can be enhanced by a variety of H3 antagonists acting as inverse agonists at the H3 receptor. Thioperamide, clobenpropit, and idodphenpropit raise the cAMP levels in SK-N-MC cells with potencies that match their receptor binding affinities. Surprisingly, impentamine and burimamide act as effective H3 antagonists. Modification of the amine group of impentamine dramatically affected the pharmacological activity of the ligand. Receptor affinity was reduced slightly for most impentamine analogs, but the functional activity of the ligands varied from agonist to neutral antagonist and inverse agonist, indicating that subtle changes in the chemical structures of impentamine analogs have major impact on the (de)activation steps of the H3 receptor. In conclusion, upon stable expression of the rat and human H3 receptor in SK-N-MC cells constitutive receptor activity is detected. In this experimental system, H3 receptors ligands, previously identified as H3 antagonists, cover the whole spectrum of pharmacological activities, ranging from full inverse agonists to agonists.

The histamine H3 receptor was discovered in 1983 by Arrang and coworkers as a presynaptic autoreceptor regulating the release of histamine from histaminergic neurons (Arrang et al., 1983). Since then, the H3 receptor has been shown to act as heteroreceptor as well, inhibiting the release of important neurotransmitters, e.g., acetylcholine, glutamate, noradrenaline, and serotonin (Leurs et al., 1998). With the availability of a variety of selective and potent H3 agonists and antagonists (Leurs et al., 1995; Stark et al., 1996), it has become clear that the H3 receptor is involved in the regulation of several important physiological processes. Consequently, the H3 receptor is regarded as an interesting target for the modulation of a variety of functions such as cognitive processes, epilepsy, food intake, and sleep-wakefulness (Leurs et al., 1998).

Despite the interest in H3 receptor ligands for therapeutic application, the actual therapeutic development has for a long time been hampered by the lack of information on the molecular target. Whereas the cloning of the H1 and H2 receptor genes was reported in the early 90s (Gantz et al., 1991; Yamashita et al., 1991), it was 1999 before the gene of the human H3 receptor was cloned finally by Lovenberg et al. (1999) after the identification of a partial sequence of an orphan G-protein-coupled receptor (GPCR) in the Incyte expressed sequence tags database. The H3 receptor was shown finally to be a GPCR with only limited homology (<30%) with the H1 and H2 receptor genes (Lovenberg et al., 1999).

Classical models of GPCRs require agonist occupation of receptors to activate signal transduction pathways. Yet, it is now well-documented that GPCRs can be spontaneously active, and this agonist-independent receptor activity is often referred to as constitutive receptor activity (Costa et al., 1992; Lefkowitz et al., 1993; Milligan et al., 1995). Inverse agonists reduce the constitutive GPCR activity, whereas neutral antagonists do not affect the basal GPCR activity but prevent the action of both agonists and inverse agonists. Constitutive activity has been shown recently for both the histamine H1 and H2 receptors (Smit et al., 1996; Bakker et

ABBREVIATION: GPCR, G-protein-coupled receptor.
al., 2000), and we reported that the therapeutically important H₃ and H₂ antagonists, in fact, act as inverse agonists. In the present study, we describe that the human and rat histamine H₃ receptors stably expressed in SK-N-MC cells (Lovenberg et al., 1999, 2000) show a high level of constitutive activity, resulting in the identification of several standard H₃ antagonists (thioperamide and clobenpropit) as inverse agonists in this cell system. Moreover, burimamide and impentamine, previously identified as H₄ antagonists (Ar-rang et al., 1983; Vollinga et al., 1995a, 1995b), behave as H₃ agonists at the recombinant H₃ receptors. The agonistic effects of impentamine could also be demonstrated on the hypothalamic histamine release in the rat brain using in vivo microdialysis. Moreover, in a series of impentamine analogs we were able to manipulate the intrinsic activity. VUF4904, an impentamine analog with an isopropyl group at the amino group of the side chain, bound with a relatively high affinity (12 nM) and acted as a neutral antagonist in the transfected SK-N-MC cells. These data indicate that ligands, previously identified as H₃ antagonists, can cover the whole spectrum of pharmacological activities, ranging from full inverse agonism to agonism, at the recombinant H₃ receptors heterologously expressed in SK-N-MC cells.

**Experimental Procedures**

**Materials.** (R)-α-Methylhistamine dihydrobromide was obtained from Sigma Research Biochemicals Inc. ( Zwijndrecht, The Netherlands). Burimamide was a kind gift of GlaxoSmithKline (Welwyn Garden City, Hertfordshire, UK). All other H₃ ligands were taken from laboratory stock or (re-)synthesized at the Vrije Universiteit Amsterdam (details will be published elsewhere). Forskolin, 3-isobutyl-1-methylxanthine, cyclic 3',5'-AMP, pertussis toxin, and bovine serum albumin were obtained from Sigma (Saint Louis, MO). Thioperamide, thiopenicillin, and streptomycin were from Sigma Research Biochemicals Inc. (Zwijndrecht, The Netherlands). 

**Cell Culture.** SK-N-MC cells, a human neuroblastoma cell line stably expressing the human histamine H₃ receptor (the 445-amino acid isoform) or the rat histamine H₂₃ receptor (Lovenberg et al., 1999, 2000), were grown in 10-cm² dishes at 37 °C in a humidified atmosphere with 5% CO₂ in Eagle’s minimal essential medium, supplemented with 10% fetal calf serum, 50 IU/ml penicillin, and 50 µg/ml streptomycin. After 2.5 h at 4 °C, the reaction was terminated by rapid dilution with 3 ml of ice-cold buffer, containing 140 mM NaCl, 3 mM KCl, 2.5 mM CaCl₂, 1 mM MgCl₂, and 5 mM glucose, pH 7.4, and filtration over 0.3% polyethylenimine-pretreated Whatman GF/C filters with two subsequent washes with 3 ml of buffer. Retained radioactivity was determined by liquid scintillation counting. Nonspecific binding was defined with 100 µM thioperamide as competing ligand.

In the present study, we describe that the human and rat histamine H₃ receptors stably expressed in SK-N-MC cells show a high level of constitutive activity, resulting in the identification of several standard H₃ antagonists (thioperamide and clobenpropit) as inverse agonists in this cell system. Moreover, burimamide and impentamine, previously identified as H₄ antagonists (Arrang et al., 1983; Vollinga et al., 1995a, 1995b), behave as H₃ agonists at the recombinant H₃ receptors. The agonistic effects of impentamine could also be demonstrated on the hypothalamic histamine release in the rat brain using in vivo microdialysis. Moreover, in a series of impentamine analogs we were able to manipulate the intrinsic activity. VUF4904, an impentamine analog with an isopropyl group at the amino group of the side chain, bound with a relatively high affinity (12 nM) and acted as a neutral antagonist in the transfected SK-N-MC cells. These data indicate that ligands, previously identified as H₃ antagonists, can cover the whole spectrum of pharmacological activities, ranging from full inverse agonism to agonism, at the recombinant H₃ receptors heterologously expressed in SK-N-MC cells.

**In Vivo Microdialysis.** Male Wistar rats weighing about 250 g were anesthetized with urethane (1.2 g/kg, i.p.) and placed in a stereotaxic apparatus. A dialysis probe (CMA/10; membrane length, 2 mm; CMA/Microdialysis AB, Stockholm, Sweden) was inserted into the anterior hypothalamic area with coordinates of AP, 1.5; L, 0.5; and V, 9.2 mm relative to the bregma, according to the atlas of Paxinos and Watson (1986). The anterior hypothalamic area was perfused with artificial cerebrospinal fluid containing 140 mM NaCl, 3 mM KCl, 2.5 mM CaCl₂, 1 mM MgCl₂, and 5 mM glucose, pH 7.4, through a dialysis probe at 1 µl/min using a microinfusion pump (CMA100, CMA/Microdialysis AB). Two hours after the insertion of a probe, samples were collected every 20 min with a minifraction collector (CMA140, CMA/Microdialysis AB) and frozen immediately at −40 °C until analysis. Impentamine and clobenpropit were added to cerebrospinal fluid at the concentration of 10 µM and administered through the dialysis membrane. After the experiment, the brains were removed for histological verification of sites of infusion. The concentration of histamine in the perfusate was assayed by HPLC (Yamatodani et al., 1985; Mochizuki et al., 1991) The recovery of histamine through the microdialysis membrane is about 40% (Mochizuki et al., 1991). In each microdialysis experiment, the average of the first three fractions was defined as basal release, and the subsequent fractions were expressed as a percentage of this. The statistical differences between groups were analyzed using one-way analysis of variance for repeated measurements. If significant effects versus the basal release were found, data were further analyzed by post hoc Newman-Keuls test.

**Data Analysis.** For the binding studies pIC₅₀ (negative logarithm of the ligand concentration that displaces the radioligand half-maximally) and pKᵢ values (negative logarithm of the equilibrium dissociation constant of the radioligand, i.e., the concentration, that occupies 50% of the available receptors at equilibrium) were calculated using nonlinear regression analysis using GraphPad Prism (GraphPad Software, San Diego, CA) and converted to pKᵢ (negative logarithm of the equilibrium dissociation constant for binding of the unlabeled drug) using the Cheng-Prusoff equation (Cheng and Pru-
soff, 1973). From the cAMP data pEC$_{50}$ (negative logarithm of the ligand concentration, that activates the receptor half-maximally) and pIC$_{50}$ values (negative logarithm of the ligand concentration, that inhibits the receptor half-maximally) were obtained by fitting these data to a sigmoidal relationship using GraphPad Prism. The intrinsic activities were calculated in comparison with the effects of the full agonist (R)-α-methylhistamine (1 μM) or the full inverse agonist iodophenpropit (10 μM).

All data are presented as mean ± S.E.M.; statistical comparisons were performed using the Student’s t test.

**Results**

The generation of the SK-N-MC cells stably expressing either the rat or human histamine H$_3$ receptor was described previously (Lovenberg et al., 1999, 2000). In the present study, we used SK-N-MC cell lines, expressing 516 nmol/mg of protein (n = 3) of the human histamine H$_3$ receptor or 627 ± 87 nmol/mg of protein (n = 3) of the rat histamine H$_3$ receptor, as assessed by [3H]α-methylhistamine binding.

As described previously, the H$_3$ agonists (R)-α-methylhistamine (pEC$_{50}$ = 9.26 ± 0.08) and imetit (pEC$_{50}$ = 9.28 ± 0.04) potently inhibited the 10 μM forskolin-stimulated production of cAMP in human H$_3$ receptor expressing cells (Fig. 1A; Table 1). In contrast, (R)-α-methylhistamine had no effect in the parental SK-N-MC cell line (Fig. 1A). As expected for a G$_1$-coupled receptor, the 1 μM (R)-α-methylhistamine effects at the human H$_3$ receptor (reduction to 11 ± 3% of the forskolin-induced cAMP levels) were abolished completely by an overnight pretreatment with 100 ng/ml pertussis toxin (99 ± 10% of forskolin-induced cAMP level, not shown).

Interestingly, we noticed an important difference between the forskolin-induced cAMP levels of the parental SK-N-MC cell line and the H$_3$ receptor expressing cells (Fig. 1B). As this could be an indication of constitutive H$_3$ receptor activation, we tested a variety of previously identified H$_3$ antagonists on the SK-N-MC cell line expressing the human H$_3$ receptor. Standard H$_3$ antagonists as thioperamide, clobenpropit, and iodophenpropit concentration-dependently increased the forskolin-induced cAMP levels in the transfected SK-N-MC cells (Fig. 1B; Table 1), whereas clobenpropit had no effect on the forskolin response in the parental cell line (Fig. 1B). In our experiments, iodophenpropit and clobenpropit acted as full inverse agonists (α = −1.0−0.9), whereas thioperamide acted as a partial inverse agonist (Table 1). The obtained pEC$_{50}$ values for the various inverse agonists correspond well with the respective affinities, as obtained in [3H]α-methylhistamine competition experiments (Table 1).

In search of neutral antagonists, we tested a variety of other H$_3$ antagonists. Surprisingly, the presumed H$_3$ antagonists burimamide, impentamine, and the imetit homolog VUF 8328 (Van der Goot et al., 1992) all acted as potent H$_3$ agonists at the human H$_3$ receptor with intrinsic activities between 0.8 and 0.9 (Fig. 2; Table 1). Comparing the pK$_i$ values of various agonists with their pEC$_{50}$ values revealed that in general the potencies of the agonists nicely parallel their affinities (Table 1). Only for the full agonists (R)-α-methylhistamine and perhaps imetit may some sort of receptor reserve be noticed (Table 1).

Based on the identification of agonism of impentamine, we studied several impentamine analogs to identify a neutral H$_3$ receptor antagonist. In this series of H$_3$ ligands, the amine function of impentamine was substituted or incorporated in a...
piperidine ring. Modification of the amine group results in a series of compounds with a wide spectrum of pharmacological activity, including the neutral antagonist VUF4904 (Fig. 3; Table 2).

Constitutive activity is not restricted to the human H<sub>3</sub> receptor. Whereas the rat and human H<sub>3</sub> receptor were expressed at similar levels (627 fmol/mg of protein versus 516 fmol/mg of protein), the forskolin-induced cAMP levels were always lower in the SK-N-MC cells expressing the human H<sub>3</sub> receptor (Fig. 4). These data indicate that in our experimental model the level of constitutive activity of the human H<sub>3</sub> receptor is more pronounced than that of the rat H<sub>3</sub> receptor. At the recombinant rat receptor, the H<sub>3</sub> ligands burimamide and impentamine also behave as H<sub>3</sub> agonists. In contrast to the human H<sub>3</sub> receptor, both ligands behave as full agonists at the rat H<sub>3</sub> receptor (Table 3). The constitutive activity, displayed by the rat H<sub>3</sub> receptor, can also be inhibited by compounds such as clobenpropit (Fig. 4; Table 3). Especially for the inverse agonists thioperamide and iodophenpropit, we confirmed the reported species differences (Lovenberg et al., 1999, 2000) with respect to their potencies in both receptor binding and functional assays (Table 3).

To investigate the predictive value of the data obtained with the recombinant receptors, impentamine and clobenpropit were tested in vivo. Previously, we showed by microdialysis the H<sub>3</sub> receptor-mediated effect on in vivo histamine release in the rat hypothalamus (Jansen et al., 1998). Using the same experimental set-up, we first evaluated the effects of impentamine. The mean values ± S.E.M. of the basal histamine release in the experiments in Fig. 5 were 0.078 ± 0.008 (n = 4) pmol/20 min. This value remained constant throughout the experimental period of 5 h under anesthesia (data not shown). After infusion of impentamine the histamine levels in the hypothalamus rapidly decreased to approximately 40% of the basal levels. Concomitant infusion of clobenpropit reversed the effect of impentamine and even caused an increase (±40%) in the histamine release above basal levels (Fig. 5).

**Discussion**

The recent cloning of the rat and human H<sub>3</sub> receptor cDNAs by Lovenberg et al. (1999, 2000) has had a great impact in the field of histamine research. The new information has been instrumental in identifying H<sub>3</sub> receptor isoforms (Drutel et al., 2001) and the H<sub>4</sub> receptor (Nakamura et al., 2000; Oda et al., 2000; Liu et al., 2001; Morse et al., 2001; Nguyen et al., 2001; Zhu et al., 2001) and has also been essential in deriving important information about the signaling properties of the H<sub>3</sub> receptor. Whereas for many years the actual signaling pathways for the H<sub>3</sub> receptor had been unknown, the use of cell lines expressing the H<sub>3</sub> receptor has led to the identification of at least three signal transduction pathways for the H<sub>3</sub> receptor: a G<sub>i</sub>-mediated inhibition of adenylate cyclase (Lovenberg et al., 1999, 2000; Drutel et al., 2001), the activation of the MAP kinase pathway (Drutel et al., 2001), and the stimulation of Na<sup>+</sup>/H<sup>+</sup> exchange (Silver et al., 2001).

Using SK-N-MC cell lines, stably expressing either the human and rat H<sub>3</sub> receptors at physiological receptor densi-
forskolin response in SK-N-MC cells, expressing the human H3 receptor. All data shown are the mean values were determined by the inhibition of the forskolin-stimulated (10 μM) cAMP production, whereas the inverse agonistic activity was determined by the increase of the forskolin response in SK-N-MC cells, expressing the human H3 receptor. All data shown are the mean ± S.E. of at least three experiments.

TABLE 2

Affinities and functional activities of several impentamine analogs at the human H3 receptor

<table>
<thead>
<tr>
<th>Ligand</th>
<th>pKᵢ</th>
<th>pEC₅₀</th>
<th>α</th>
</tr>
</thead>
<tbody>
<tr>
<td>Impentamine</td>
<td>8.29 ± 0.14</td>
<td>8.63 ± 0.23</td>
<td>0.9 ± 0.08</td>
</tr>
<tr>
<td>VUF5300</td>
<td>8.05 ± 0.09</td>
<td>8.67 ± 0.18</td>
<td>1.0 ± 0.02</td>
</tr>
<tr>
<td>VUF5207</td>
<td>7.81 ± 0.18</td>
<td>7.89 ± 0.1</td>
<td>0.7 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>VUF4904</td>
<td>7.89 ± 0.05</td>
<td>-0.1 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>VUF4903</td>
<td>7.97 ± 0.03</td>
<td>8.10 ± 0.17</td>
<td>-0.6 ± 0.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>VUF5202</td>
<td>8.63 ± 0.04</td>
<td>8.66 ± 0.11</td>
<td>-0.9 ± 0.1</td>
</tr>
</tbody>
</table>

<sup>a</sup> Indicates a significant difference compared with (R)-α-methylhistamine.
<sup>b</sup> Indicates a significant difference compared with iodophenpropit.

ties (500–600 fmol/mg of protein) (Yanai et al., 1994; Brown et al., 1996), we now show that both the rat and human H3 receptor display a high degree of constitutive activity. The forskolin-mediated cAMP production in SK-N-MC cells is inhibited strongly upon expression of the G<sub>i</sub>-coupled H3 receptor. The cAMP production can be further inhibited upon agonist stimulation of the H3 receptor and can be enhanced by a variety of H3 antagonists acting as inverse agonists at the H3 receptor. Thioperamide, clobenpropit, and iodophenpropit raise the cAMP levels in SK-N-MC cells expressing either the human or rat H3 receptor with potencies that match their receptor binding affinities. As reported previously (Lovenberg et al., 1999, 2000), an important species difference is noticed for thioperamide in both the binding and cAMP assay.

Burimamide was one of the key compounds used by Arrang et al. (1983) to demonstrate pharmacologically the existence of the H3 receptor in rat cerebral cortex slices. Remarkably, at recombinant H3 receptors, the presumed H3 antagonist burimamide acts as a H3 agonist. Whereas at the human H3 receptor burimamide acts as a partial agonist (α = 0.8), full agonism is observed at the rat receptor. It is interesting to note that via the use of heterologous expression systems, burimamide is reclassified for the second time. Previously, we showed that at the human H2 receptor burimamide acts as a weak partial agonist (Alewijnse et al., 1998). Because burimamide was developed originally as an H2 antagonist using histamine as a starting point (Black et al., 1972), the discovery of residual agonistic activity at histamine H2 and H3 receptors is perhaps not too surprising.

The use of transfected cell lines also suggests a reclassification for impentamine, the histamine homolog previously suggested to differentiate between H3 receptors in the guinea pig intestine and rat or guinea pig brain (Leurs et al., 1996; Harper et al., 1999). Impentamine is a potent H3 antagonist in the guinea pig intestine (p<sub>A₂</sub> = 8.4), but a partial agonist in the rat brain (p<sub>D₃</sub> = 8.2, α = 0.6) (Leurs et al., 1996). Moreover, radioligand binding studies at the H3 receptor in the guinea pig brain and intestine indicated that impentamine can discriminate between the receptors in the two prep-

TABLE 3

Affinities and functional activities of several H3 receptor ligands at the rat and human H3 receptor

<table>
<thead>
<tr>
<th>Ligand</th>
<th>pKᵢ</th>
<th>pEC₅₀</th>
<th>α</th>
<th>pKᵢ</th>
<th>pEC₅₀</th>
<th>α</th>
</tr>
</thead>
<tbody>
<tr>
<td>(R)-α-Methylhistamine</td>
<td>8.36 ± 0.07</td>
<td>9.26 ± 0.08</td>
<td>1.0 ± 0.05</td>
<td>8.04 ± 0.05</td>
<td>9.22 ± 0.16</td>
<td>1.0 ± 0.10</td>
</tr>
<tr>
<td>Impentamine</td>
<td>8.29 ± 0.14</td>
<td>8.63 ± 0.23</td>
<td>0.9 ± 0.08</td>
<td>8.30 ± 0.3</td>
<td>9.03 ± 0.13</td>
<td>1.1 ± 0.1</td>
</tr>
<tr>
<td>Burimamide</td>
<td>7.11 ± 0.1</td>
<td>6.67 ± 0.14</td>
<td>0.8 ± 0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.32 ± 0.09</td>
<td>7.16 ± 0.01</td>
<td>1.1 ± 0.02</td>
</tr>
<tr>
<td>Iodophenpropit</td>
<td>8.15 ± 0.05</td>
<td>7.6 ± 0.08</td>
<td>-1.0 ± 0.07</td>
<td>8.83 ± 0.07</td>
<td>8.03 ± 0.19</td>
<td>-1.0 ± 0.05</td>
</tr>
<tr>
<td>Clobenpropit</td>
<td>8.42 ± 0.02</td>
<td>8.44 ± 0.15</td>
<td>-0.9 ± 0.1</td>
<td>9.06 ± 0.08</td>
<td>8.54 ± 0.12</td>
<td>-1.0 ± 0.1</td>
</tr>
<tr>
<td>Thioperamide</td>
<td>7.18 ± 0.03</td>
<td>6.73 ± 0.2</td>
<td>-0.7 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.19 ± 0.02</td>
<td>8.99 ± 0.4</td>
<td>-1.0 ± 0.07</td>
</tr>
</tbody>
</table>

<sup>a</sup> Indicates a significant difference compared with (R)-α-methylhistamine.
<sup>b</sup> Indicates a significant difference compared with iodophenpropit.
Constitutive Activity of the Human H3 Receptor

After microdialysis, drugs (10 μM) were infused in the hypothalamus via the microdialysis probe. Fractions were collected every 20 min and the amount of histamine was determined by HPLC, as described under Experimental Procedures. *, a significant (P < 0.05) difference compared with basal levels of histamine release.

Fig. 5. Effect of impentamine and clobenpropit on the in vivo histamine release in the rat hypothalamus as measured by microdialysis. Drugs (10 μM) were infused in the hypothalamus via the microdialysis probe. Fractions were collected every 20 min and the amount of histamine was determined by HPLC, as described under Experimental Procedures. *, a significant (P < 0.05) difference compared with basal levels of histamine release.

arations (Harper et al., 1999). At both the recombinant rat and human H3 receptors, impentamine behaves as an effective agonist. Moreover, in vivo microdialysis shows that impentamine also acts as an H3 agonist in the rat hypothal- mus, inhibiting the basal release of histamine. Previously we showed that potent H3 agonists, like immeppip (Jansen et al., 1998), inhibit the hypothalamic histamine release to approximately the same extent as observed in this study for impen- tamine.

Although constitutive GPCR activity is now a widely ac- cepted pharmacological concept, effects due to the presence of the natural agonist cannot be ignored completely. The iden- tification of neutral antagonists has resolved this issue for the histamine H3 receptor and led to the recognition that the therapeutically important H3 antagonists are in fact inverse agonists (Smit et al., 1996). To identify a neutral H3 ag- onist we tested a variety of impentamine analogs at the human H3 receptor. The amine function of impentamine probably interacts with the aspartate residue Asp114 in transmembrane domain 3, which is highly conserved in the family of biogenic amines (De Esch et al., 2000). We hypothe- sized that modification of the amine function potentially could affect the agonistic properties of impentamine. Indeed, modification of the amine group dramatically affected the pharmacological activity of the ligand. Receptor affinity was reduced slightly for most analogs, unless a p-chloro-benzyl group was used (VUF5205, pKᵢ = 8.63). Remarkably, introduction of small alkyl groups resulted in reduced agonistic activity (di-methyl substitution, VUF5207, α = 0.7) or neutral antagonism (isopropyl substitution, VUF4904). Substitution of the amine group with a cyclohexyl ring or a p- chlorobenzyl group resulted in (partial) inverse agonists. Our data show that only subtle changes at the amine function alter the pharmacological activity of the ligands. At present, we do not have an explanation for this phenomenon, but this series of ligands may be of great help to understand the mechanism of receptor (in)activation. Detailed studies with receptor mutants, the development of similar, rigid analogs and the generation of a three-dimensional computer model to rationalize receptor-ligand interaction may also be useful in this respect.

In conclusion, in this study we show that both the rat and human H3 receptors show a considerable level of constitutive activity when expressed at physiological expression levels in SK-N-MC cells. This observation has important conse- quences for the classification of H3 receptor ligands, which can now be classified as inverse agonists, neutral antagon- ists, and agonists.

Constitutive activity of the rat H3 receptor was also reported very recently by Morisset et al. (2000). Interestingly, the constitutive activity of the rat H3 receptor was suggested to regulate brain histamine release in both rat and mouse (Morisset et al., 2000). The H3 receptor is, therefore, one of the few GPCRs for which it is known that they modulate important physiological processes by means of its constitutive activity. In light of the foreseen therapeutic application of H3 antagonists (Leurs et al., 1998), it remains to be established whether inverse agonists or neutral antagonists will be favored for clinical application.

References


Cheng Y and Prusoff W (1973) Relationship between the inhibition constant (Kᵢ) and the concentration of inhibitor which causes 50% inhibition (IC₅₀) of the enzymatic reaction. Biochem Pharmacol 22:3099–3108.


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