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Evaluation of the receptor selectivity of the H_3 receptor antagonists, iodophenpropit and thioperamide: an interaction with the 5-HT_3 receptor revealed

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1 In the present study we evaluated the receptor selectivity of the potent histamine H_3 receptor antagonist, iodophenpropit (IPP) in comparison with the prototype antagonist, thioperamide.

2 IPP proved to be a potent competitive H_3 receptor antagonist as measured against (R)-α-methylhistamine-induced inhibition of electrically-evoked contractions of the guinea-pig jejunum ($pA_2 = 9.12 \pm 0.06$, Schild slope: 1.0 ± 0.1, $n = 8$). In the same assay, thioperamide was slightly less potent ($pA_2 = 8.9 \pm 0.2$).

3 In radioligand binding studies, IPP showed a high affinity for the H_3 receptor. Displacement of [^{125}I]-IPP binding to rat cortex membranes by unlabelled IPP resulted in a K_i value of 0.97 ± 0.06 nM ($n = 3$). In contrast, IPP showed only a weak affinity for the histamine H_1- and H_2 receptor. Displacement of [^{3}H]-mepyramine and [^{125}I]-iodoaminopotentidine binding to respectively guinea-pig H_1- and human H_2 receptors by IPP resulted in K_i values of 1.71 ± 0.32 μM ($n = 3$) and 2.28 ± 0.81 μM ($n = 3$). For thioperamide the affinities for the H_1-, H_2- and H_3 receptor were respectively > 10 μM, > 10 μM and 4.3 ± 1.6 nM ($n = 7$).

4 Testing IPP and thioperamide in 39 different receptor binding assays revealed that IPP showed relatively high affinity for the 5-hydroxytryptamine 5-HT_3 receptor ($K_i = 11 \pm 1$ nM, $n = 3$), the α_2-adrenoceptor ($K_i = 120 \pm 5$ nM, $n = 3$) and the sigma receptor ($K_i = 170 \pm 70$ nM, $n = 3$). Thioperamide showed relatively high affinity for the 5-HT_3 receptor ($K_i = 120 \pm 30$ nM, $n = 3$) and the sigma receptor ($K_i = 180 \pm 90$ nM, $n = 3$).

5 Due to the low density of histamine H_3 receptors in the brain, the interaction of IPP with the 5-HT_3-, the α_2- and the sigma receptor might interfere with [^{125}I]-IPP binding to rat cortex membranes. Yet, in this preparation [^{125}I]-IPP binding was not influenced by ondansetron, yohimbine or haloperidol.

6 The interaction with the 5-HT_3 receptor was not restricted to IPP or thioperamide, but was also found with other H_3 receptor antagonists. The potent H_3 receptor agonist imetit, a compound belonging to the same chemical class of IPP, also interacted with the 5-HT_3 receptor ($K_i = 240 \pm 40$ nM). In contrast, histamine or the H_3 receptor agonist, (R)-α-methylhistamine showed no affinity for the 5-HT_3 receptor.

7 In the guinea-pig isolated ileum, imetit evoked concentration-dependent contractions, resulting in a pD_2 value of 4.72 ± 0.03 ($n = 9$). The contractions were antagonized by ondansetron, yielding a pA_2 value of 7.1 ± 0.1 ($n = 9$). Similarly ondansetron antagonized the contractions evoked by the 5-HT_3 receptor agonist, 2-methyl-5-HT with a pA_2 value of 7.3 ± 0.1 ($n = 4$). IPP and thioperamide did not mimic 2-methyl-5-HT but non-competitively inhibited the 2-methyl-5-HT-induced contractions of this preparation.

8 In an *in vivo* model for 5-HT_3 activity, the Von Bezold Jarisch reflex, thioperamide showed antagonism in low dosages, which correlated well with the affinity for the 5-HT_3 receptor site. Yet, at higher dosages no further 5-HT_3 receptor antagonism was observed. For IPP no 5-HT_3 receptor activity could be observed *in vivo*.

9 In the present study we showed that many H_3 receptor compounds, that are regarded as highly selective (including the prototype drug, thioperamide), also interact with the 5-HT_3 receptor, albeit at higher drug concentrations.

Keywords: Histamine H_3-receptor; iodophenpropit; thioperamide; receptor selectivity; 5-hydroxytryptamine 5-HT_3 receptor; guinea-pig intestine; rat brain; Von Bezold Jarisch reflex

Introduction

Concomitant with the increased knowledge of the role of H_3 receptors in (patho)physiological processes (Timmerman, 1990; Schwartz *et al.*, 1991) medicinal chemists have been developing highly selective ligands for the H_3 receptor (see reviews by Timmerman, 1990; Leurs *et al.*, 1991; Leurs & Timmerman, 1992). In 1987 Arrang *et al.* introduced (R)-α-methylhistamine and thioperamide as a highly selective H_3 receptor agonist and antagonist, respectively (Arrang *et al.*, 1987). These agents proved to be very useful for further pharmacological definition of the H_3 receptor. In order to obtain detailed insight into the interaction of ligands with the H_3 receptor, the search for potent agonists and antagonists continued. It was observed by various laboratories that for potent H_3 receptor agonism the protonated amine function of histamine could be replaced by other protonated groups (Garbarg *et al.*, 1992; Howson *et al.*, 1992; Van der Goot *et al.*,

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1992; Vollinga *et al.*, 1994). This observation resulted in the development of the potent and highly selective H_3 receptor agonists, imetit (Garbarg *et al.*, 1992; Howson *et al.*, 1992; Van der Goot *et al.*, 1992) and immepip (Vollinga *et al.*, 1994). In a search for new H_3 receptor antagonists, we noticed that methylation of the isothiourea moiety or lengthening the alkyl sidechain of imetit gave rise to moderate H_3 receptor antagonism (Van der Goot *et al.*, 1992). After a detailed exploration of these findings, clobenpropit was introduced as the most potent H_3 receptor antagonist known at this time ($pA_2 = 9.9$) (Van der Goot *et al.*, 1992).

The potency of this new class of H_3 receptor antagonists prompted us to develop an iodonated analogue of clobenpropit, iodophenpropit (IPP, VUF 4586) as a potential candidate for a radioligand for the H_3 receptor (Menge *et al.*, 1992). IPP appeared to be a suitable ligand for labelling of the H_3 receptor (Jansen *et al.*, 1992; 1994). This ligand binds with high affinity to a pharmacologically defined H_3 receptor binding site (Jansen *et al.*, 1992; 1994) and can be used to study receptor-G protein interaction or for autoradiographic receptor localization in the CNS (Jansen *et al.*, 1994).

We now describe a detailed survey of the receptor selectivity of the H_3 receptor radioligand, IPP in comparison with thioperamide, the prototype H_3 receptor antagonist which is usually applied in pharmacological studies. Both IPP and thioperamide appear to combine potent H_3 receptor antagonism with potent to moderate 5-hydroxytryptamine (5-HT) 5-HT_3 receptor antagonism. Moreover, we also show that the 5-HT_3 receptor activity does not affect the radioligand binding characteristics of IPP under our experimental conditions. Finally, we show that the chemically-related H_3 receptor agonist, imetit, possesses 5-HT_3 receptor agonist activity.

Methods

In vitro *H_3 receptor activity at the guinea-pig jejunum*

The *in vitro* histamine H_3 receptor activity was determined on the guinea-pig jejunum as described by Vollinga *et al.* (1992). Male Dunkin-Hartley guinea-pigs (350–400 g, Harlan CPB, Zeist, the Netherlands) were killed by cervical dislocation and the intestine was rapidly removed and kept in oxygenated (95% O_2/5% CO_2) Krebs buffer (mM: NaCl 118, KCl 5.6, $MgSO_4$ 1.18, NaH_2PO_4 1.28, $NaHCO_3$ 25 and glucose 5.5). Whole jejunum segments (*ca* 2 cm) were mounted between two platinum electrodes (4 mm apart) in warm (37°C) Krebs buffer under a load of 1 g. After 60 min equilibration the muscle was stimulated maximally (*ca* 15 V) with a frequency of 0.1 Hz and a duration of 0.5 ms with rectangular wave electrical pulses (Grass Stimulator S-88, Grass Instruments Co., Quincy, U.S.A.). Contractions were recorded isotonically (Hugo Sachs TL-2/HF-modem, Hugo Sachs Elektronik, Hugstetten, Germany). After 30 min of stimulation cumulative concentration-response curves for the H_3 receptor agonist (**R**)-α-methylhistamine were recorded. The contact time for each agonist concentration was 45–60 s. After a concentration-response curve had been obtained the tissue was washed for 30 min before a subsequent concentration-response curve was recorded. Antagonists were preincubated for 15 min during the stimulation before the preparations were challenged again with (**R**)-α-methylhistamine.

Receptor binding assays

All binding assays were performed according to well-documented methods (Van Wijngaarden *et al.*, 1993; Jansen *et al.*, 1994; Leurs *et al.*, 1994; Traiffort *et al.*, 1994). All drug solutions in the displacement studies were diluted and pipetted automatically (Tecan automatic dilution robot, type 5032. Tecan AG, Switzerland); the radioligand and tissues suspension were also added automatically by Filterprep 101 (Ismatec, Zürich, Switzerland), which further performed the assays up to filter (Whatman GF/B) collection and the addition of Scintillation Emulsifier-299 (Packard). All incubations were performed in triplicate. Overnight equilibration was followed by tritium counting.

In vitro *5-HT_3 receptor activity at the guinea-pig ileum*

The *in vitro* 5-HT_3 receptor activity was determined on the guinea-pig ileum essentially as described by Butler *et al.* (1988). Male Dunkin-Hartley guinea-pigs (200–250 g, Harlan CPB, Zeist, the Netherlands) were killed by cervical dislocation and the intense was rapidly removed and kept in oxygenated Krebs buffer. In contrast to Butler *et al.* (1988), in this study whole ileum segments (*ca* 2 cm) were used. The segments were equilibrated for 60 min at 37°C in oxygenated (95% O_2/5% CO_2) Krebs buffer under a load of 0.5 g. Drug-induced contractions were measured cumulatively and recorded isotonically. The cumulative additions were made very rapidly to avoid desensitization; a complete concentration-response curve was recorded in *ca* 50 s. Thereafter the tissue was washed for 30 min before a subsequent concentration-response curve was recorded. Antagonists were preincubated for 15 min before the preparations were challenged again with the agonists. Under these experimental conditions a pD_2 value of 5.54 ± 0.04 (mean ± s.e.mean, $n = 4$) was obtained for the 5-HT_3 receptor agonist 2-methyl-5-HT. The 2-methyl-5-HT-induced effects were competitively antagonized by the selective 5-HT_3 receptor antagonist ondansetron, yielding a pA_2 value of 7.31 ± 0.03 (Schild slope: 1.3 ± 0.1, mean ± s.e.mean, $n = 4$). These values are essentially the same as those reported by Butler *et al.* (1988).

In vivo *5-HT_3 receptor activity (Von-Bezold Jarisch reflex)*

Male Wistar rats (200–250 g, Harlan CPB, Zeist, the Netherlands) were anaesthetized with urethane (25% v/v, 5 ml kg^{-1}, i.p.) and 0.1 ml Hypnorm (i.m.). The arteria carotis, vena jugularis and the trachea were cannulated. The animals were ventilated during the experiment (80 strokes min^{-1}, 1 ml 100 g^{-1}). Blood pressure and heart rate were measured via the carotid cannula, whereas drugs were administered via the jugular vein. After stabilization of the animal a dose-response curve for 2-methyl-5-HT was recorded. Animals were pretreated for 5 min with the antagonists; four different antagonist doses were tested on each animal. For each antagonist 4 different animals were used.

Statistical analysis

In the functional experiments pD_2 values were obtained from the concentration-response curves by fitting the data points according to a logistic function. Antagonist potency was determined by the construction of a Schild-plot, using three different concentrations of the antagonist. Values shown are the mean ± s.e.mean of at least four different independent experiments.

In the radioligand binding studies the concentrations of unlabelled drug causing 50% of displacement of the specific binding (IC_{50} value) were obtained by computerized log-probit linear regression analysis of data obtained in experiments in which four to six different concentrations of the test compound were used. Inhibition constants (K_i) values were calculated using the Cheng-Prusoff equation. Mean ± s.e.mean values were calculated from at least three independent experiments.

Drugs

Histamine dihydrochloride (Brocacef), (**R**)-α-methylhistamine dihydrochloride (Research Biochemical International), amthamine dihydrobromide (synthezised according to Eriks *et al.*, 1992), IPP dihydrobromide (synthezised according to Menge *et al.*, 1992), clobenpropit dihydrobromide (synthesized according to Van der Goot *et al.*, 1992), impromidine trihy-

drochloride (gift from SmithKline Beecham), imetit dihydrobromide (synthesized according to Van der Goot *et al.*, 1992), 2-methyl-5-HT maleate (gift from Solvay Duphar) and zacopride fumarate (gift from Wyeth-Ayerst) were all dissolved in distilled water. Thioperamide (gift from Solvay Duphar) was dissolved in distilled water with 1 equivalent HCl. Granisetron (gift from Solvay Duphar) was dissolved in 10% ethanol with 1 equivalent HCl. Ondansetron hydrochloride (gift from Solvay Duphar) was dissolved in 65% ethanol with 1 equivalent HCl. The various stock solutions were diluted in the appropriate buffers for the radioligand binding studies and the *in vitro* experiments and in a 0.9% NaCl solution for the *in vivo* measurements.

Results

Selectivity of IPP at the three histamine receptor subtypes

The H_3 receptor antagonistic activity of IPP was tested on electrically stimulated segments of the guinea-pig jejunum. The inhibition of the neurogenic muscle contraction by (R)-α-methylhistamine was potently antagonized by IPP (Figure 1) with a pA_2 value of 9.12 ± 0.06 and a Schild slope of 1.0 ± 0.1 ($n = 17$ preparations of 8 different animals).

The potent H_3 receptor antagonism was confirmed in radioligand binding studies (Jansen *et al.*, 1992; 1994; this study). Displacement of [^{125}I]-IPP binding to rat brain cortex membranes with unlabelled IPP resulted in monophasic displacement curves (n_H value not significantly different from unity) (Figure 2). The affinity of IPP for the H_3 receptor obtained from these experiments (0.97 ± 0.06 nM, $n = 3$) correlates well with the pA_2 value obtained in the functional experiments (see above). For thioperamide a value of 4.3 ± 1.6 nM ($n = 7$) was obtained under similar conditions.

As can be seen from Figure 2 IPP is highly selective for the H_3 receptor, compared with the H_1- and H_2 receptors. The displacement of [^{3}H]-mepyramine and [^{125}I]-iodoaminopotentidine binding to the guinea-pig H_1- and the human H_2 receptor was monophasic (n_H values not significantly different from unity) and resulted in K_i values of 1.71 ± 0.32 μM ($n = 3$) and 2.28 ± 0.81 μM ($n = 3$) respectively, for the H_1- and the H_2 receptor. Also thioperamide hardly interacts with the H_1- and H_2 receptors. K_i values greater than 10 μM were observed (Table 1).

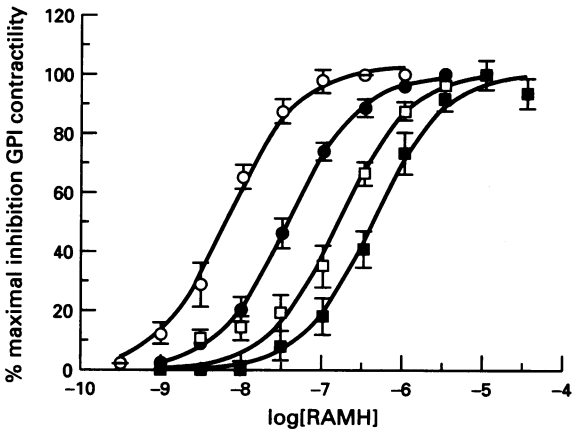

Figure 1 H_3 receptor antagonism by iodophenpropit (IPP) in the guinea-pig jejunum. Dose-response curves for the inhibition of electrically-induced twitches of the guinea-pig jejunal smooth muscle by the H_3 receptor agonist, (R)-α-methylhistamine (RAMH) were determined in the absence (○) and presence of three different concentrations of IPP (3 nM (●), 10 nM (□) and 100 nM (■)) after pretreatment for 15 min. Data shown are mean ± s.e.mean of eight independent experiments.

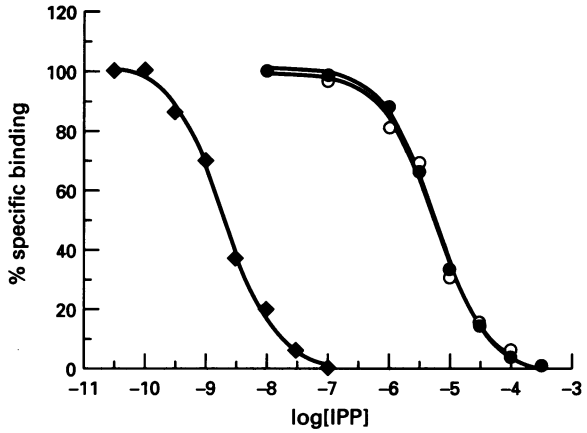

Figure 2 Displacement by iodophenpropit (IPP) of the specific [^{3}H]-mepyramine binding to membranes of CHO cells, expressing the guinea-pig H_1 receptor (○) (Traiffort *et al.*, 1994), specific [^{125}I]-iodoaminopotentidine binding to membranes of CHO cells, expressing the human H_2 receptor (●) (Leurs *et al.*, 1994) and specific [^{125}I]-iodophenpropit binding (◆) (Jansen *et al.*, 1994) to rat cortex membranes. Data from representative experiments out of three independent experiments performed in triplicate are shown. The respective radioligands were used at a concentration of 0.3–0.5 nM.

Receptor profile of IPP and thioperamide

IPP and thiopermide were tested in a variety of radioligand binding assays (Table 1). IPP showed a high affinity for the 5-HT_3 receptor (11 ± 1 nM), the α_2-adrenoceptor (120 ± 5 nM) and the sigma receptor (170 ± 70 nM). Moderate activity was found for the imidazoline I_2 receptor (530 ± 60 nM), the 5-HT_4 receptor (530 ± 110 nM) and the verapamil-sensitive Ca^{2+} channels (400–560 nM). Weak interactions of IPP were observed with the dopamine D_2 and D_3 receptor, the 5-HT_{1A} receptor, the 5-HT transporter, the muscarinic receptors and the sodium channel.

The pharmacological profile of thioperamide was distinct from IPP (Table 1). Thioperamide also showed a relatively high affinity for the 5-HT_3 receptor (120 ± 30 nM) and the sigma receptor (180 ± 90 nM). Weak interactions of thioperamide were observed with the α_2-adrenoceptor, the 5-HT_4 receptor, the tryptamine receptor and the muscarinic M_1 receptor.

[^{125}I]-IPP binding to rat brain cortex membranes

The 5-HT_3 receptor antagonist, ondansetron, the α_2-adrenoceptor antagonist, yohimbine, or the high affinity sigma receptor antagonist, haloperidol, were unable to displace the binding of 0.3 nM [^{125}I]-IPP to rat brain cortex membranes up to micromolar concentrations (Figure 3). These *in vitro* data were confirmed in autoradiographic studies, where neither ondansetron (1 μM) nor yohimbine (1 μM) prevented labelling of rat brain structures by [^{125}I]-IPP (data not shown).

5-HT_3 receptor interaction of various histaminergic drugs

The high 5-HT_3 receptor affinity of IPP and thioperamide was also displayed by other H_3 receptor ligands (Table 2), namely the antagonists, clobenpropit, impromidine, burimamide and the agonist imetit (Table 2). In contrast, the H_3 receptor agonists histamine and (R)-α-methylhistamine did not show any displacement in the 5-HT_3 receptor binding assay in concentrations up to 10 μM. Various 5-HT_3 receptor antagonists of different chemical classes did not interact with the histamine H_3 receptor at the tested concentrations.

In vitro *5-HT_3 receptor activity*

To determine the functional effects of the interaction of IPP, thioperamide and imetit with the 5-HT_3 receptor, these com-

Table 1 Pharmacological profile of IPP and thioperamide

Receptor site	*IPP* K_i (nM)	*Thioperamide* K_i (nM)
Histamine H_1	1,710 ± 320	> 10,000
Histamine H_2	2,280 ± 810	> 10,000
Histamine H_3	0.97 ± 0.06	4.3 ± 1.6
α_1-Adrenoceptor	1,500 ± 300	> 10,000
α_2-Adrenoceptor	120 ± 5	4,000 ± 800
β-Adrenoceptor	> 10,000	> 10,000
Imidazoline-I_2	530 ± 60	> 10,000
Dopamine D_1	> 10,000	> 10,000
Dopamine D_2	4,500 ± 800	> 10,000
Dopamine D_3	1,800 ± 400	> 10,000
5-HT_{1A}	2,300 ± 500	> 10,000
5-HT_{1B}	> 10,000	> 10,000
5-HT_{1D}	> 10,000	> 10,000
5-HT_{2A}	1,900 ± 300	> 10,000
5-HT_{2C}	> 10,000	> 10,000
5-HT_3	11 ± 1	120 ± 30
5-HT_4	530 ± 110	2,800
5-HT transporter	1,250 ± 120	> 10,000
Muscarinic M_1	1,500 ± 200	4,600 ± 600
Muscarinic M_2	1,980 ± 40	> 10,000
Muscarinic M_3	3,700 ± 100	> 10,000
Tryptamine	> 10,000	6,400 ± 1500
δ-Opiate	21,000 ± 2400	> 10,000
κ-Opiate	14,000 ± 1000	> 10,000
μ-Opiate	8,400 ± 2000	> 10,000
σ-Sigma	170 ± 70	180 ± 90
Benzodiazepine	> 10,000	> 10,000
CCK_A	> 10,000	> 10,000
CCK_B	> 10,000	> 10,000
Substance P	> 10,000	> 10,000
Melatonin	11,000 ± 3600	> 10,000
NMDA	> 10,000	> 10,000
$GABA_A$	> 10,000	> 10,000
Glycine	> 10,000	> 10,000
Glycine (strychnine insensitive)	> 10,000	> 10,000
Na^+ channel (BTX sensitive)$_{brain}$	1,000 ± 100	> 10,000
Ca^{2+} channel (DHP sensitive)$_{brain}$	> 10,000	> 10,000
Ca^{2+} channel (verapamil sensitive)$_{brain}$	400 ± 20	> 10,000
Ca^{2+} channel (verapamil sensitive)$_{heart}$	560 ± 180	> 10,000

K_i values for the various receptor sites were determined by radioligand binding studies as described in the Methods section. Data shown are the mean ± s.e.mean of at least 2 to 3 independent experiments each performed in triplicate.

pounds were tested on the guinea-pig isolated ileum. Neither IPP not thioperamide caused contractions of the guinea-pig ileum (data not shown). Yet, imetit was only *ca* 7 fold less active than the 5-HT_3 receptor agonist 2-methyl-5-HT ($pD_2 = 5.54 \pm 0.04$, $n = 4$), leading to a pD_2 value of 4.72 ± 0.03 (mean ± s.e.mean, $n = 9$). The imetit-induced contractions of the guinea-pig ileum were competitively antagonized by ondansetron (Figure 4). Schild analysis of the observed antag-

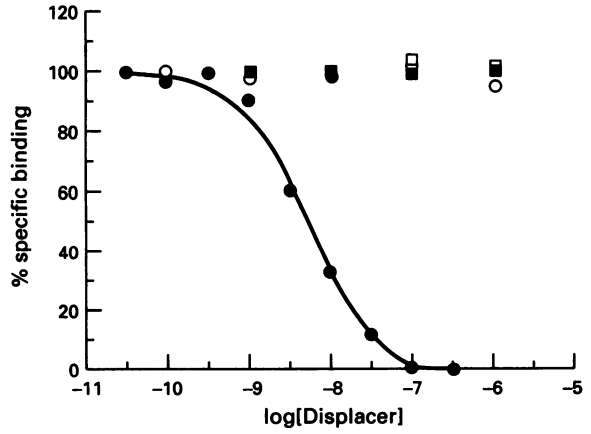

Figure 3 Displacement of total [^{125}I]-iodophenpropit ([^{125}I]-IPP) binding (0.2 nM) to rat cortex membranes by thioperamide (●), ondansetron (□), yohimbine (■) and haloperidol (○). Data shown are mean values of a representative experiment, performed in triplicate. Similar data were obtained in one or two other independent experiments.

Table 2 Affinities of various H_3- and 5-HT_3 receptor ligands for the H_3- and 5-HT_3 receptor

Compound	*H_3 receptor* K_i (nM)	*5-HT_3 receptor* K_i (nM)
Iodophenpropit	0.97 ± 0.06	11 ± 1
Clobenpropit	0.93 ± 0.06	38 ± 11
Thioperamide	4.3 ± 1.6	120 ± 30
Impromidine	51 ± 9	260 ± 30
Amthamine	> 10,000	> 10,000
Histamine	3.8 ± 9.6*	> 10,000
(R)-α-methylhistamine	3.5 ± 1.2*	> 10,000
Imetit	2.7 ± 0.8*	240 ± 40
Ondansetron	> 3,000	1.6 ± 0.2
Granisetron	> 10,000	0.78 ± 0.10
Mianserin	> 10,000	25 ± 9
Zacopride	> 10,000	0.083 ± 0.004

K_i values were determined by *in vitro* radioligand binding studies using [^{125}I]-IPP and [^{3}H]-GR65630 respectively. Data shown are the mean ± s.e.mean of at least 2 to 4 independent experiments each performed in triplicate. *K_i value for high affinity site. (Jansen *et al.*, 1994).

Table 3 *In vivo* activity of H_3- and 5-HT_3 receptor ligands on the 5-HT_3 receptor

Compound	5-HT_3 receptor activity *In vivo* K_i (nmol kg^{-1})	*In vitro* K_i (nM)
Iodophenpropit	NA	11 ± 1.1
Thioperamide	106 ± 25	120 ± 30
Ondansetron	7.3 ± 1.5	1.6 ± 0.2
Granisetron	1.0 ± 0.1	0.78 ± 0.10

K_i values were determined by measuring the antagonism of the 2-methyl-5-HT-induced bradycardia in anaesthetized rats after i.v. application of the drugs. Data shown are the mean ± s.e.mean of 4 different animals. For comparison the K_i values obtained from *in vitro* binding studies, using [^{3}H]-GR65630 are shown. NA not active.

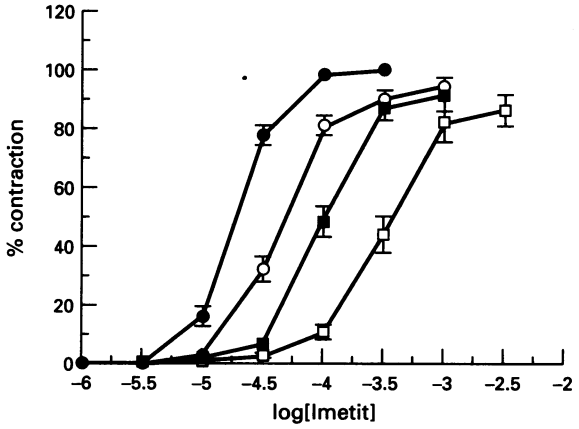

Figure 4 Imetit-induced contractions of guinea-pig ileum segments in the absence (●) or presence of 0.1 μM (○), 0.3 μM (■) and 1 μM (□) ondansetron. Data shown are mean ± s.e.mean from at least four independent experiments.

onism resulted in a pA_2 value of 7.11 ± 0.03 and a Schild slope of 1.1 ± 0.1 (mean ± s.e.mean, $n = 4$).

IPP and thioperamide affect the 2-methyl-5-HT-induced contractions in a non-competitive manner (Figure 5). At micromolar concentrations both IPP and thioperamide depressed the maximal 2-methyl-5-HT-induced contractions; in this respect IPP is *ca* 10 fold more active than thioperamide (Figure 5).

In vivo *5-HT_3 receptor activity*

In urethane-anaesthetized rats, intravenously applied thioperamide (Figure 6) antagonized the 2-methyl-5-HT-induced bradycardia (Von Bezold-Janisch reflex) at low dosages (10 and 30 μg kg^{-1}). At 100 μg kg^{-1} no additional antagonism was observed (Figure 6). The *in vivo* 5-HT_3 receptor activity calculated from the displacement of the 2-methyl-5-HT dose response curve at the two lowest thioperamide dosages correlates with the *in vitro* K_i values for the 5-HT_3 receptor (Table 3). For IPP no consistent antagonism was observed *in vivo* (data not shown).

Discussion

Several highly potent ligands for the H_3 receptor have in recent years been developed by various laboratories (Leurs *et al.*, 1991; Garbarg *et al.*, 1992; Howson *et al.*, 1992; Van der Goot *et al.*, 1992; Vollinga *et al.*,1994). This has resulted in the availability of potent H_3 receptor antagonist like thioperamide (Arrang *et al.*, 1987) and clobenpropit (VUF 9153) (Van der

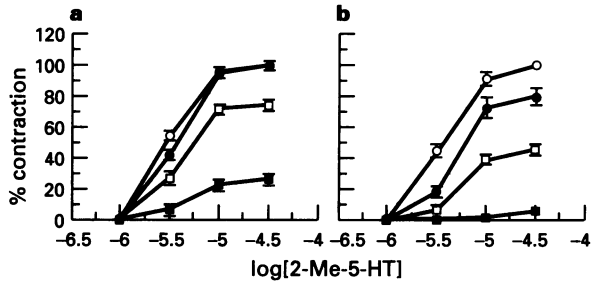

Figure 5 5-HT_3-induced contractions of guinea-pig ileum segments: (a) 2-methyl-5-HT-induced contractions in the absence (○) or presence of 1 μM (●), 3 μM (□) and 10 μM (■) iodophenpropit (IPP); (b) 2-methyl-5-HT-induced contractions in the absence (○) or presence of 10 μM (●), 30 μM (□) and 100 μM (■) thioperamide. Data shown are mean ± s.e.mean from at least four independent experiments.

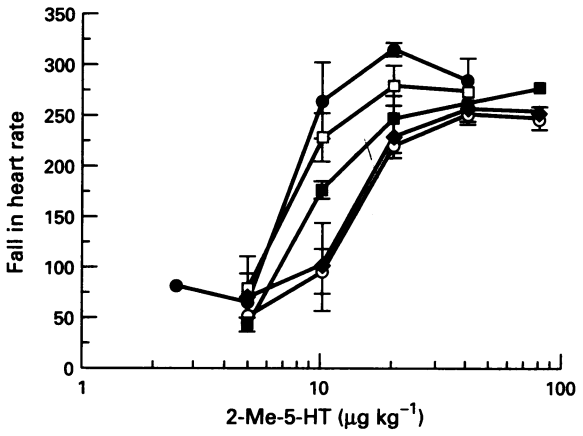

Figure 6 *In vivo* 5-HT_3 receptor activity of thioperamide. Urethane anaesthetized rats received i.v. vehicle (●), 3 μg kg^{-1} (□), 10 μg kg^{-1} (■), 30 μg kg^{-1} (○) and 100 μg kg^{-1} (◆) thioperamide and the 5-HT_3 receptor agonist, 2-methyl-5-HT. The 2-methyl-5-HT induced reduction in heart rate is the mean ± s.e.m of 4 different animals.

Goot *et al.*, 1992). Moreover, it appeared to be possible to incorporate [^{125}I]-iodine in the aromatic nucleus of the clobenpropit class of compounds (Menge *et al.*, 1992), resulting in [^{125}I]-IPP, a highly sensitive radiolabel for the H_3 receptor (Jansen *et al.*, 1992; 1994). In this study we show that unlabelled IPP is a highly potent antagonist at the H_3 receptor of the guinea-pig jejunum. Against the selective agonist (**R**)-α-methylhistamine, IPP shows competitive antagonism with a linear Schild plot with a slope of 1.0 and a pA_2 value of 9.12. This value closely corresponds to the reported affinity of [^{125}I]-IPP ($pK_D = 9.2$) for the H_3 receptor in rat brain tissue (Jansen *et al.*, 1992; 1994) and the pK_i value (9.0) for unlabelled IPP (this study). The affinity of IPP for the H_3 receptor is found to be more than thousand fold higher than for the other histamine receptor subtypes. This compound can therefore be regarded as a potent and selective H_3 receptor antagonist when histaminergic transmission is considered.

For possible therapeutic application of this class of compounds, receptor specificity data are of great importance. The potencies of IPP and thioperamide were therefore evaluated in 39 different receptor binding studies. From these studies it appears that IPP and the prototype H_3 antagonist, thioperamide (Arrang *et al.*, 1987) both show a reasonable affinity for the 5-HT_3 receptor in rat brain. Moreover, both compounds also interact with the sigma receptors in submicromolar concentrations, whereas IPP also shows a moderate affinity for the α_2-adrenoceptor.

The relatively high affinity of IPP for the 5-HT_3-receptor, the α_2-adrenoceptor and the sigma receptor promoted us to study the possible interaction of [^{125}I]-IPP with these receptor sites. It was previously noticed that [^{125}I]-IPP also binds to unidentified, non-H_3 receptor binding sites (Jansen *et al.*, 1992; 1994). Usually, this non-specific [^{125}I]-IPP binding does not give rise to major problems for the H_3 receptor assay in rat cortex membranes (Jansen *et al.*, 1992; 1994). Yet, in order to improve the [^{125}I]-IPP assay conditions and to understand better the molecular interactions of [^{125}I]-IPP, the contribution of labelling of the 5-HT_3-, and α_2-adrenoceptor and the sigma receptor was investigated, using high affinity antagonists for the three receptor sites. Ondansetron (5-HT_3 receptor antagonist), yohimbine (α_2-adrenoceptor antagonist) or haloperidol (sigma-antagonist) all failed to affect the [^{125}I]-IPP binding to rat cortex membranes. The relatively high level of non-specific binding of [^{125}I]-IPP therefore remains obscure from a molecular point of view.

The interaction of IPP and thioperamide with the 5-HT_3 receptor is shared by other H_3 receptor ligands. The antagonist, clobenpropit and impromidine, show affinities in the same concentration-range, while the agonist, imetit, also shows a reasonable affinity for the 5-HT_3 receptor. In contrast, histamine, amthamine or the H_3 receptor agonist (**R**)-α-methylhistamine were completely inactive in the concentration-range tested. These data indicate that the 5-HT_3 receptor activity can be separated from the H_3 receptor activity, as is also shown by the lack of H_3 receptor affinity of the potent 5-HT_3 receptor antagonist, ondansetron, granisetron, mianserin and zacopride (for review on 5-HT_3 receptor drugs see Kilpatrick *et al.*, 1990). The difference in selectivity of the H_3 receptor agonists histamine, (**R**)-α-methylhistamine and imetit is especially striking. Apparently the 5-HT_3 receptor affinity of the H_3 receptor ligands is due to the presence of a basic isothiourea (IPP, imetit, clobenpropit), thiourea (thioperamide) or guanidine moiety (impromidine) instead of a protonated amine function (histamine, (**R**)-α-methylhistamine). Interestingly, Bermudez *et al.* (1993) described a small series of arylureas derived from histamine. These compounds also showed a reasonably high affinity for the 5-HT_3 receptor, whereas no histamine receptor activity was investigated. On the basis of these data we conclude that the combination of an imidazole moiety and a basic sidechain, that can form hydrogen bond interactions, is sufficient for a high affinity interaction with the 5-HT_3 receptor. The substitution of the basic function with substituted aromatic residues can further modulate the 5-HT_3 receptor affinity (compare e.g. imetit vs IPP).

The arylurea analogues of histamine are reported to be quite potent 5-HT_3 receptor agonists (Bermudez *et al.*, 1993). In our study we identified imetit as a selective 5-HT_3 receptor agonist. In the guinea-pig ileum, imetit caused transient contractions, that were effectively antagonized by ondansetron. The pA_2 value for ondansetron against imetit does not differ from the value obtained against the selective 5-HT_3 receptor agonist, 2-methyl-5-HT (data this study, Butler *et al.*, 1988).

For IPP and thioperamide no 5-HT_3 receptor agonist effects are observed, but both drugs antagonized non-competitively the 2-methyl-5-HT-induced contractions of the guinea-pig ileum. The non-competitive nature of the antagonism precludes firm conclusions on the functional interaction of both drugs with the 5-HT_3 receptor. Yet, if one considers the known approximately 100 fold lower sensitivity of the guinea-pig 5-HT_3 receptor compared to its rat counterpart (Kilpatrick *et al.*, 1990) the active concentrations of imetit, IPP and thioperamide at the guinea-pig ileum may correspond to their affinity in rat brain. The ten fold higher potency at the ileum of IPP compared to thioperamide is striking and suggests that both drugs act at the 5-HT_3 receptor.

For thioperamide we were able to show 5-HT_3 antagonism *in vivo*. In the Von Bezold Jarisch reflex model, thioperamide dose-dependently antagonized the 2-methyl-5-HT-induced bradycardia. Suprisingly, at higher dose of thioperamide no further antagonism was observed. For IPP no inhibitory effects could be measured in this model. At present we do not have an explanation for these findings, but we suggest that due to the promiscuous nature of these drugs, various counteracting receptor systems in both the peripheral and central nervous systems can be modulated by these drugs *in vivo*.

In conclusion, our data indicate that various H_3 receptor ligands show reasonable affinity for the 5-HT_3 receptor. This activity should be considered in future when these drugs are used. Especially under *in vivo* conditions this activity may become apparent, since under these conditions it has already been shown, for example that a weak interaction of the H_3 receptor agonist (**R**)-α-methylhistamine with the H_1-receptor (Hey *et al.*, 1992) and α_2-adrenoceptor must be considered (Malinowska & Schlicker, 1993; Schlicker *et al.*, 1994; Timmerman *et al.*, 1995). Moreover, in a preliminary study on the cardiovascular effects of several H_3 receptor agonists in the rat, 5-HT_3 receptor effects for imetit were noticed *in vivo* (Timmerman *et al.*, 1995).

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