Paradoxical Stimulatory Effects of the “Standard” Histamine H₄-Receptor Antagonist JNJ7777120: the H₄ Receptor Joins the Club of 7 Transmembrane Domain Receptors Exhibiting Functional Selectivity

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ABSTRACT
The histamine H₄ receptor (H₄R) is expressed in several cell types of the immune system and is assumed to play an important pro-inflammatory role in various diseases, including bronchial asthma, atopic dermatitis, and pruritus. Accordingly, H₄R antagonists have been suggested to provide valuable drugs for the treatment of these diseases. Over the past decade, the indole derivative 1-[(5-chloro-1H-indol-2-yl)carbonyl]-4-methylpiperazine (JNJ7777120) has become the “standard” H₄R antagonist and has been extensively used to assess the pathophysiological role of the H₄R. However, the situation has now become more complicated by recent data (p. 749 and Naunyn Schmiedebergs Arch Pharmacol doi: 10.1007/s00210-011-0612-3) showing that JNJ7777120 can also activate /H9252/H9253 arrests in a supposedly Gi-protein-independent (pertussis toxin-insensitive) manner and that at certain H₄R species orthologs, JNJ7777120 exhibits partial agonist efficacy with respect to Gi-protein activation (steady-state high-affinity GTPase activity). These novel findings can be explained within the concept of functional selectivity or biased signaling, assuming unique ligand-specific receptor conformations with distinct signal transduction capabilities. Thus, great caution must be exerted when interpreting in vivo effects of JNJ7777120 as H₄R antagonism. We discuss future directions to get out of the current dilemma in which there is no “standard” H₄R antagonist available to the scientific community.

Introduction
Histamine (Fig. 1) is an important neurotransmitter and local mediator (Hill et al., 1997). A decade ago, several groups each independently identified a novel member of the HₓR family with unique pharmacological properties, the H₄R (for review, see Hough, 2001; Thurmond et al., 2008; Leurs et al., 2009). The H₄R is a Gα-protein-coupled receptor, causing inhibition of adenylyl cyclase and, in cells of the immune system, activation of phospholipase C via release of Gβγ-complexes (Fig. 2A). In cell membranes, activation of G- proteins by the H₄R can be monitored by histamine-stimulated [³²S]GTPγS binding to, or [γ-³²P]GTP hydrolysis by, G-proteins (Schneider et al., 2009). The discovery of the H₄R was highlighted in a Perspective article in Molecular Pharmacology (Hough, 2001). In this very first review-type pub-
lication on this topic, it was already noted that the pharma-
cological properties of the H4R, although clearly distinct from
those of other HxRs, differed from each other in various
studies. Most notably, one group reported on relatively high
affinity of H4R for H1R antagonists but other groups found no
interaction of the H4R with HxR antagonists (Hough, 2001;
Nguyen et al., 2001). Despite considerable efforts, these early
discrepancies have not been satisfactorily explained (Deml et
al., 2009). Now, again, the H4R causes headache concerning
its pharmacological properties.

The H4R is expressed in several cell types of the immune
system, including mast cells, eosinophils, dendritic cells, and
T lymphocytes. On the basis of this localization of the receptor
and studies with the H4R knockout mouse, it has been
suggested that the H4R plays a proinflammatory role in
bronchial asthma, atopic dermatitis, and pruritus and that
H4R antagonists could be useful drugs for the treatment for
these conditions (see, e.g., Thurmond et al., 2008; Leurs et
al., 2009). This suggestion has been corroborated by the
finding that the indole derivative 1-[(5-chloro-1H-indol-2-yl)
carbonyl]-4-methylpiperazine (JNJ7777120) (Fig. 1), a po-
tent H4R antagonist (Jablonowski et al., 2003; Venable et,
2005), exhibits anti-inflammatory effects in a mouse asthma
model (Dunford et al., 2006). Moreover, JNJ7777120 inhibits
the effects of histamine in various cell systems expressing the
H4R (see, e.g., Thurmond et al., 2008; Leurs et al., 2009).

Although JNJ7777120 has a relatively short plasma half-
life and limited bioavailability, rendering animal experi-
ments requiring continuous exposure to the compound tech-
nically difficult (Thurmond et al., 2004), researchers in the
H4R field readily embraced JNJ7777120 because of its avail-
ability and high H4R selectivity (Jablonowski et al., 2003;
Venable et al., 2005). Table 1 summarizes pharmacological
data for JNJ7777120 in various in vitro test systems. The
implementation of JNJ7777120 as “standard” H4R antago-
nist was also facilitated by the fact that the first known
standard H4R antagonist, thioperamide (Fig. 1), is not selec-
tive for the H4R but is also a potent H3R antagonist, render-
ing it potentially difficult to discriminate H3R and H4R ef-
fects in vivo. More precisely, thioperamide is a dual H4R/H3R
inverse agonist; i.e., according to the two-state model of 7TM
receptor activation, the compound stabilizes the inactive R
state, resulting in a reduction of basal G-protein activation
promoted by the agonist-free H4R and H3R (Schneider et al.,
2009; Schnell et al., 2010). Numerous H3R ligands structur-
ally related to JNJ7777120 have been synthesized (Venable
et al., 2005); with few exceptions (Schneider et al., 2010),
these compounds have not yet been characterized in depth
pharmacologically.

**Activation of β-Arrestin by JNJ7777120: A Twisted Story**

On this basis, Rosethorne and Charlton (2011), in this
issue of *Molecular Pharmacology*, provide important insights
into the pharmacological properties of the hH4R in general
and into the properties of JNJ7777120 in particular. Using
the U2OS osteosarcoma cell expression system, the authors
show that, as expected (Schneider et al., 2009), the endoge-
nous and full agonist histamine stimulates [35S]GTP binding
in cell membranes, whereas the inverse agonist thi-
peramide and JNJ7777120 do not affect GTP binding.

**Fig. 1.** Structures of selected H4R ligands analyzed in the present study.

**Fig. 2.** Functional selectivity of JNJ7777120 and thioperamide. A, at
hH4R, histamine is a full agonist with respect to G1- and β-arrestin
activation. JNJ7777120 is a partial inverse agonist with respect to G1-
protein activation and a partial agonist with respect to β-arrestin acti-
vation. B, at mH4R, rH4R, and cH4R, Histamine is a full agonist with
respect to G1 activation in Sf9 insect cell membranes, whereas
JNJ7777120 is a partial agonist at these H4R orthologs. The effects on
β-arrestin activation have not yet been studied. C, considering the effects
of thioperamide on G1-protein activation catalyzed by various H4R species
orthologs, effects may range from “full” inverse agonism [even thiopera-
amide is actually not a full inverse hH4R agonist (Schneider et al., 2009)]
over neutral antagonism to partial agonism.
ligand. To facilitate comparison of the data from various studies, inverse agonist or neutral antagonist properties at hH4R binding, JNJ 777120 exhibits, to a variable degree, partial

### TABLE 1

Characterization of JNJ777120 in different in vitro test systems

<table>
<thead>
<tr>
<th>Expression System</th>
<th>Assays</th>
<th>Important Findings Concerning JNJ777120</th>
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<tr>
<td>Human SK-N-MC cells</td>
<td>Radioligand binding cAMP-CRE gene reporter assay</td>
<td>Human H4R, [3H]histamine competition binding: Kᵢ = 4.1 nM, Kᵢ = 4 nM and pKᵢ = 7.9 nM cAMP assay with human, mouse, rat H4R; equipotent antagonistic potency; 1000-fold selectivity relative to H3R, H2R, and H3R no cross-reactivity with 50 other targets</td>
<td>Jablonowski et al., 2003</td>
</tr>
<tr>
<td>Mouse bone marrow-derived mast cells</td>
<td>In vitro mast cell chemotaxis assay</td>
<td>Antagonist: inhibition of chemotaxis in mast cells, induced by 10 μM of histamine: IC₅₀ = 40 nM</td>
<td>Thurmond et al., 2004</td>
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<tr>
<td>Endogenous (human eosinophils)</td>
<td>Flow cytometry, in vitro chemotaxis assays</td>
<td>Antagonist: inhibition of eosinophil shape change, induced by 1 μM of histamine: IC₅₀ = 300 nM; inhibition of chemotaxis, induced by 1 μM of histamine: IC₅₀ = 86 nM</td>
<td>Ling et al., 2004</td>
</tr>
<tr>
<td>Sf9 insect cells</td>
<td>Radioligand binding, steady-state GTPase assay</td>
<td>Fluorescence imaging Antagonist: inhibition of actin polymerization, induced by 300 nM of histamine: IC₅₀ = 6 nM</td>
<td>Barnard et al., 2008</td>
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<td>Human HEK 293/HEK 293T cells</td>
<td>Radioligand binding ([3H]histamine competition)</td>
<td>Substantial affinity differences of JNJ777120 at various species isoforms: human (Kᵢ = 5 nM), monkey (Kᵢ = 32 nM), pig (Kᵢ = 501 nM), dog (Kᵢ = 79 nM), mouse (Kᵢ = 4 nM), rat (Kᵢ = 4 nM) and guinea pig H4R (Kᵢ = 1 μM)</td>
<td>Schneider et al., 2009</td>
</tr>
<tr>
<td>Human U2OS cells</td>
<td>GTP-γS binding assay, β-arrestin recruitment assay, ERK phosphorylation</td>
<td>SRE-luciferase reporter gene assay (cH4R + Goα) Antagonist at canine H4R: rightward shift of the histamine concentration-response-curve, no pAᵢ reported</td>
<td>Jiang et al., 2008</td>
</tr>
<tr>
<td>COS-7 cells</td>
<td>Radioligand binding</td>
<td>Human HEK 293/HEK 293T cells</td>
<td>Lim et al., 2010</td>
</tr>
<tr>
<td>Mouse pituitary tumor AtT-20 cells</td>
<td>Adrenocorticotropic release (ELISA)</td>
<td>Antagonist: inhibition of ACTH secretion, induced by histamine (10 nM) or R-α- methylhistamine (100 nM): IC₅₀ = 360 or 230 nM, respectively</td>
<td>Meng et al., 2008</td>
</tr>
</tbody>
</table>

HEK, human embryonic kidney; ELISA, enzyme-linked immunosorbent assay.
2010; Rajagopal et al., 2010). For this reason, GPCRs should actually be more correctly referred to as 7TM receptors, giving credit to the universal heptahelical structure of these proteins instead of their signal transduction pathways, which are not necessarily G-protein-mediated.

So far, the data of Rosethorne and Charlton (2011) fit into established paradigms, but the headache starts with their finding that JNJ7777120 behaves as a partial agonist with respect to β-arrestin binding to hH4R (Fig. 2A; Table 1). This effect was observed at various H4R expression levels, ruling out the possibility that excess H4R molecules, referred to as receptor reserve, could account for the unexpected effects. Moreover, the effect of JNJ7777120 seems to be G-protein-independent, as suggested by the lack of influence of the ADP-ribosyltransferase PTX on β-arrestin binding. The authors provide evidence that the effect of JNJ7777120 on β-arrestin recruitment is mediated by the H4R and not through another receptor. Specifically, thioperamide, which is without stimulatory effect in this assay by itself, blocks the effects of JNJ7777120 on β-arrestin recruitment competitively, and the pA2 values of thioperamide for blockade of the JNJ7777120 response and the response of the H4R agonist (Schnell et al., 2011). Thus, JNJ7777120 may lead to a similar equilibrium in a particular 7TM receptor that is then capable of activating a unique pattern of G-protein-dependent and -independent signal transduction pathways (Galandrin et al., 2007; Rajagopal et al., 2010). β-Adrenergic receptor antagonists are a very prominent and well studied class of ligands for which functional selectivity has been documented (Galandrin et al., 2007). Indeed, the data from Rosethorne and Charlton (2011) may also act as agonist. It is noteworthy that, with respect to β-arrestin recruitment, JNJ7777120 acts only as partial agonist, but with respect to ERK activation, JNJ7777120 is actually a full agonist. However, the stimulatory effects of JNJ7777120 on ERK activation was only reported for an exceedingly high ligand concentration (100 μM), a concentration that is almost 10,000-fold higher than the EC50 for β-arrestin recruitment (Table 1). Thus, one cannot exclude the possibility that in addition to β-arrestin, other signal transduction pathways are involved in JNJ7777120-induced ERK activation. It will now be very important to study in great detail the effect of JNJ7777120 on ERK activation in more commonly used and well characterized expression systems such as HEK293 cells and in cells endogenously expressing hH4R. Eosinophils are a well established native cell system for studying H4R functions (Table 1). It is likely that the effects of JNJ7777120 strongly depend on the endogenous complement of signal transduction proteins in the cells haraboring hH4R.

Additional evidence that JNJ7777120 and structurally related compounds are more than just H4R “antagonists” comes from a recent analysis of a series of 25 indole, benzimidazole, and thienopyrrole compounds at the recombinant hH4R expressed in SF9 insect cells (Schneider et al., 2010). In this system, for a subset of compounds including JNJ7777120, we observed quite substantial differences between K values in [3H]histamine competition binding studies and EC50 values for inverse agonistic activity in the GTPase assays (Schneider et al., 2010) (Table 1). Although the data on JNJ7777120 compiled in Table 1 have to be compared with caution because they were obtained in different cell types and because different parameters were determined, it is evident that the apparent affinities/potencies of JNJ7777120 can vary considerably among the various studies, a property that is not commonly observed for classic receptor antagonists. For example, in the studies of Ling et al. (2004) and Barnard et al. (2008), the IC50 values for JNJ7777120 on various functional parameters in human eosinophils endogenously expressing hH4R differ by up to 50-fold (Table 1). These differences cannot be explained by the relatively small differences in the stimulatory histamine concentration used. Such data on divergent ligand affinities/potencies regarding various parameters support the notion that JNJ7777120 and related compounds stabilize functionally distinct H4R conformations.

Although the data from Rosethorne and Charlton (2011) is certainly unexpected for the H4R community and raise many questions of how JNJ7777120 effects in vitro and particularly in vivo should be interpreted, in a broader conceptual context, the findings are actually not that surprising. It is just that the H4R now joins the growing family of 7TM receptors showing functional selectivity or biased signaling. This concept states that any given ligand stabilizes a unique conformation in a particular 7TM receptor that is then capable of activating a unique pattern of G-protein-dependent and -independent signal transduction pathways (Galandrin et al., 2007; Rajagopal et al., 2010). β-Adrenergic receptor antagonists are a very prominent and well studied class of ligands for which functional selectivity has been documented (Galandrin et al., 2007).

**Even More Twists with JNJ7777120, Extending to Thioperamide**

But the JNJ7777120 story has some additional unexpected twists. First, we have recently studied the effects of JNJ7777120 at recombinant hH4R, mH4R, and cH4R expressed in SF9 cells, using the steady-state high-affinity GTPase assay as parameter (Schnell et al., 2011). It is important to characterize the effects of JNJ7777120 at those H4R species orthologs because mouse, rat, and dog are important laboratory animal species for assessing the pathophysiologial role of the H4R (Liu et al., 2001; Dunford et al., 2006). Most strikingly, at mH4R, rH4R, and cH4R expressed in S9 cells, JNJ7777120 exhibits strong partial agonism with respect to activation of G-proteins (Fig. 2B). However, a comparison of the efficacies must consider the constitutive activity, which is very high in the case of the hH4R and very low for the mH4R, rH4R and cH4R (Schnell et al., 2011). Thus, JNJ7777120 may lead to a similar equilibrium of active and inactive states for all H4R orthologs, which appears as inverse agonism at hH4R because of the shift of the basal equilibrium toward the active state (R*), and as partial agonism at mH4R, rH4R, and cH4R because of the different basal equilibria (presence or absence, respectively, of the R* activity).
state). The effect of JNJ7777120 on β-arrestin activation has not yet been studied with these H₄R species orthologs.

And then there is still another twist extending to thioperamide. To this end, in all studies including the study of Rosethorne and Charlton (2011), there has been consensus that thioperamide acts as H₄R inverse agonist (Leurs et al., 2009; Schneider et al., 2009). However, at cH₄R, thioperamide clearly exhibits partial agonistic efficacy, and at mH₄R and rH₄R, thioperamide is a neutral antagonist with respect to Gₛ protein activation (Schnell et al., 2011). Thus, depending on the species studied, thioperamide can stabilize either only inactive or both inactive and active H₄R conformations, and the functional behavior of this compound is clearly different from the behavior of JNJ7777120 (Fig. 2, B and C). It is also possible that the controversial effects of H₄R antagonists observed at the H₄R (Hough, 2001; Nguyen et al., 2001; Deml et al., 2009) are due to functional selectivity. At least, the H₄R antagonist data are reminiscent of what has been observed for JNJ7777120 (Hough, 2001; Deml et al., 2009) (Table 1), and a careful analysis of this problem is warranted.

What Is the Molecular Basis for the Divergent Effects of JNJ7777120 on Various Signaling Pathways Promoted by hH₄R? Also a Twisted Story

We have developed a model of the interaction of JNJ7777120 and related compounds with hH₄R (Schneider et al., 2010). We suggested that JNJ7777120 prevents the indole ring of Trp⁶.₄₈, the key element of the proposed 7TM receptor-activating toggle switch, from changing the inactive vertical position into the horizontal position (Schneider et al., 2010). However, JNJ7777120 stabilizes both inactive and active hH₄R states (Fig. 2). Therefore, the compound was docked into models of the inactive hH₄R (Fig. 3A) and of the active hH₄R (Fig. 3B). The positively charged amine moiety of JNJ7777120 interacts electrostatically with the highly conserved Asp³.₃₂ in both states. In the inactive hH₄R, the indole moiety of JNJ7777120 adopts a nearly vertical position with respect to the longitudinal axis of the receptor, placed between transmembrane domains III and VI. This conformation stabilizes the indole ring of Trp⁶.₄₈ in a vertical position, too (Fig. 3A). These assumptions correspond to published data of related compounds (Schneider et al., 2010). For the active state of the hH₄R, the docking studies suggest an alternative binding mode of JNJ7777120 in which the indole moiety is stacked between the aromatic side chains of Trp⁶.₄₈ and Tyr⁶.₅₁. This interaction stabilizes the indole ring of Trp⁶.₄₈, discussed as being involved in the rotamer toggle switch during receptor activation, in a more horizontal position (Fig. 3B). Based on the present models, no differences in amino acids directly interacting with JNJ7777120 in the binding pocket of inactive and active hH₄R are obvious. Unfortunately, no conclusions can be drawn from the models about the molecular mechanism by which JNJ7777120 induces and stabilizes a β-arrestin-binding conformation of the hH₄R, but in the following, we will propose a mechanism based on the available literature.

Nonvisual arrestins 2 and 3 preferentially bind to active phosphorylated 7TM receptors but, e.g., in the case of β₂ adrenergic and M₂ muscarinic receptors, also to phosphorylated inactive states (~2-fold lower binding; Gurevich and Gurevich, 2006). However, unphosphorylated receptor states must be active to bind arrestins with sufficient affinity. Thus, recruitment of β-arrestin to an inactive (not G-protein coupled) receptor state requires phosphorylation of multiple serine and threonine residues in the intracellular loops and the C-terminal tail, 7TM receptor regions that all have been shown to contain phosphorylation sites relevant for arrestin binding (Gurevich and Gurevich, 2006). 7TM receptors are mainly phosphorylated by GRKs. Among them, GRKs 5 and 6 are independent of translocation by Gβγ-subunits and exclusively responsible for ERK1/2 activation by arrestins (Kim et al., 2005; Ren et al., 2005).

Because the JNJ7777120-mediated recruitment of β-arrestin supposedly does not depend on G-proteins (Rosethorne and Charlton, 2011), JNJ7777120 may stabilize a specific hH₄R state that does not activate Gₛ-proteins but in the first place facilitates phosphorylation of newly exposed serine and threonine residues by Gβγ-independent GRKs. Some of the phosphorylated serine/threonine sites or clusters may then interact with the lysine- and arginine-rich polar core of β-arrestin. Specific phosphorylation patterns differentially orient β-arrestin on the receptor and stabilize different active arrestin conformations, leading to structurally and functionally distinct arrestin-receptor complexes (Gurevich and Gurevich, 2006). To enable high-affinity hH₄R-binding and activation of such a functionally distinct β-arrestin state resulting in prolonged ERK activation (Rosethorne and Charlton, 2011), JNJ7777120 should therefore expose the proper pattern of hH₄R phosphorylation sites by conformational changes in the intracellular loops and the C-terminal tail. These conformational changes may also enable interactions with additional arrestins.

Fig. 3. Model of the interaction of JNJ7777120 with inactive and active hH₄R. Homology models of the inactive and active hH₄R were generated as described previously (Schneider et al., 2010). For modeling of the inactive hH₄R, the crystal structure of the β₂ adrenergic receptor (Protein Data Bank 2RH1) was used as template, whereas for modeling of the active hH₄R, the crystal structure of active opsin (Protein Data Bank 3DQB) was applied. JNJ7777120 was docked into both models, using SYBYL 7.3 (Tripos, St. Louis, MO). A, JNJ7777120 docked into the inactive hH₄R. B, JNJ7777120 docked into the active hH₄R.
arrestin regions, contributing to the activation of the complex via induced fit.

And Even More Twists: Are the Effects of JNJ7777120 on β-Arrestin Recruitment Really G-Protein-Independent?

The conclusion that JNJ7777120 induces β-arrestin recruitment in a G-protein-independent manner is based on the finding that the ligand does not stimulate [35S]GTPγS binding and on the lack of effect of PTX on β-arrestin recruitment (Rosethorne and Charlton, 2011). PTX, via ADP-ribosylation of G- protein α-subunits, uncouples 7TM receptors from G-proteins and is a most valuable tool for unmasking G-protein-dependent pathways (Ui and Katada, 1990). Although inhibitory effects of PTX on receptor-mediated signaling can be readily interpreted [specifically whether the proper controls with the B-oligomer (and not only the carrier solvent) are performed], the interpretation of negative PTX data is more complicated. In particular, it is difficult to completely ADP-ribosylate all G- protein α-subunits in cells because G- proteins are so abundant (Ui and Katada, 1990). For this reason, PTX effects are often only incomplete. Accordingly, one cannot exclude the possibility that a fraction of the available G-proteins in the osteosarcoma cell expression system used by Rosethorne and Charlton (2011) was still functional and, evidently, those non–ADP-ribosylated G-proteins could participate in signaling, including β-arrestin recruitment and ERK activation (Walters et al., 2009). At the membrane level, activation of G-protein may be too small to be detected, but the β-arrestin assay may be sufficiently sensitive to detect G-protein activation.

Rosethorne and Charlton (2011) incubated the cells for 20 h with 200 ng/ml PTX, but even a 24-h incubation with PTX at a concentration of 1 μg/ml may be insufficient to functionally eliminate all G-proteins from receptor-coupling (Ui and Katada, 1990). It is possible to assess the effectiveness of PTX-catalyzed ADP-ribosylation by treating membranes from PTX-treated cells with preactivated PTX and [32P]NAD and then performing SDS polyacrylamide electrophoresis with subsequent quantitative autoradiography. Effective ADP-ribosylation of G-proteins in intact cells results in poor incorporation of [32P]ADP-ribose in the subsequent membrane incubation with activated PTX. Unfortunately, this important control experiment was not performed in the study of Rosethorne and Charlton (2011).

Moreover, the specific activity of PTX from various commercial suppliers can be quite different and vary considerably from batch to batch, so that it is always essential to include a positive control experiment to document functionality of PTX. Unfortunately, the source of PTX is not mentioned in the study by Rosethorne and Charlton (2011), and the authors also did not present a positive control experiment showing that PTX actually functioned properly. Such a positive control could have been provided by demonstrating a lack of stimulatory effect of histamine on [35S]GTPγS binding in membranes from PTX-treated cells and a decrease in basal [35S]GTPγS binding, reflecting uncoupling of constitutively active receptors from G-proteins (Seifert and Wenzel-Seifert, 2003).

Considering the constitutive activity of the hH4R, the function of JNJ7777120 as weak partial inverse agonist or even neutral antagonist on [35S]GTPγS binding in the study of Rosethorne and Charlton (2011) implies that at least a small part of JNJ7777120-bound hH4R molecules stays in an active state, further activating G- proteins. A fraction of remaining functional G-proteins would not necessarily change β-arrestin recruitment after PTX incubation and might also lead to the congruent concentration-response curves of JNJ7777120-mediated β-arrestin binding with and without PTX (Rosethorne and Charlton, 2011).

Alternatively, Rosethorne and Charlton (2011) could have studied the effects of PTX on histamine- and JNJ7777120-induced ERK activation. Based on the time course, the effects of histamine would be expected to be PTX-sensitive, whereas the effects of JNJ7777120 would be predicted to be PTX-insensitive (Luttrell and Gesty-Palmer, 2010). However, no such control experiments were performed. PTX-sensitive arrestin recruitment has been reported for G- coupled receptors (Walters et al., 2009). Intriguingly, the effect of histamine on β-arrestin recruitment in the osteosarcoma cell line is apparently PTX-insensitive, too (Rosethorne and Charlton, 2011). This result is not necessarily in contrast with the possibility that, in the presence of PTX, histamine recruits β-arrestin in a G-protein-independent manner differently from a Giγ-dependent manner without PTX, because β-arrestin binding curves may be similar in both cases. But this possibility would imply that the time course of ERK activation by histamine changes after PTX treatment (Luttrell and Gesty-Palmer, 2010; Rosethorne and Charlton, 2011), a consequence that must be checked to exclude insufficient PTX function in the study of Rosethorne and Charlton (2011).

Moreover, one cannot dismiss the possibility that JNJ7777120 stabilizes an hH4R conformation that enables the receptor to interact even with ADP-ribosylated G- protein α-subunits or to preferentially interact with PTX-insensitive G-proteins. Because of slow guanine nucleotide exchange, it can be very difficult or impossible to detect activation of PTX-insensitive G-proteins in the [35S]GTPγS binding assay (Wenzel-Seifert and Seifert, 2000), but activation of PTX-insensitive G-proteins can be assessed more readily with the G-protein photoaffinity labeling/immunoprecipitation technique (Laugwitz et al., 1996). However, such experiments were not conducted in the study of Rosethorne and Charlton (2011). These limitations regarding the G-protein aspect of the study of Rosethorne and Charlton (2011) should be addressed in future studies but do not question the fundamental issue of paradoxical effects of JNJ7777120 at the hH4R level. We simply have to be cautious with the conclusion that JNJ7777120 recruits β-arrestin without any involvement of active G proteins.

Are H4R Agonists an Alternative to JNJ7777120 and Thioperaamide?

So, if pharmacological effects of JNJ7777120 and thiopera-mide can no longer be taken for granted as antagonist actions, what about the use of agonists as experimental tools for assessing the pathophysiological role of hH4R? We have recently reviewed the hH4R agonist literature (Igel et al., 2010). Unfortunately, the situation with agonists is not easier than with hH4R “agonists.” For example, 5(4)-methylhistamine, originally described as an hH4R agonist (Black et al., 1972), displays selectivity for recombinant hH4R relative
to the other H₄Rs (Lim et al., 2005). However, when applied in vivo, the actual concentration of the ligand in a particular organ is unknown, so effects on H₄Rs other than the H₄R, specifically the H₄R, cannot be excluded. The anti-inflammatory effects of 5(4)-methylhistamine and JNJ7777120 in a mouse asthma model could be interpreted as agonistic effects of the ligands on the H₄R (Morgan et al., 2007; Neumann et al., 2010), but the effects of 5(4)-methylhistamine could also be mediated via activation of H₄R, exhibiting an established anti-inflammatory role (Hill et al., 1997).

We have described the cyanoguanidine 2-cyano-1-[4-(1H-imidazol-4-yl)butyl]-3-[2-phenylthio)ethyl]guanidine (UR-PI376) as a potent and selective H₄R agonist (Igel et al., 2009a), but for studies in mouse, rat, and dog, the compound is not useful because of low potency and efficacy (Schnell et al., 2011). The N²-acetylated imidazolopyrrolguanidine H₄R agonist N³-[3-(1H-imidazol-4-yl)propyl]-N²-propionylguanidine (UR-PI294) (Igel et al., 2009b) should also only be used with caution, considering the mechanistically still unexplained and very unusual “superagonism” at recombinant H₄R, measuring G-protein-catalyzed GTP hydrolysis as parameter (Schnell et al., 2011).

Conclusions and Future Studies. Even at the very beginning, the H₄R pharmacology caused headache, as exemplified by contradictory and still unresolved H₄R antagonist effects (Hough, 2001). This initial phase was followed by a relatively headache-free phase in which these intriguing H₄R antagonist effects were put aside and JNJ7777120 rapidly advanced to the status of “standard” H₄R antagonist. And now we have headache, again, because multiple ligands encompassing classic H₄R “antagonists” such as JNJ7777120 and thioiperamide, and even agonists show paradoxical, unexpected, unexplained, and complex effects in various systems (Table 1; Figs. 2 and 3).

The recent data from Rosethorne and Charlton (2011) and other groups have important implications for future research in the H₄R field. First, considering the lack of a standard H₄R antagonist, it is necessary to study multiple structurally diverse compounds at multiple H₄R orthologs, assessing multiple G-protein-dependent and -independent parameters, in agonist, antagonist, and inverse agonist modes. There is no way to avoid these painful experimental approaches because even among compounds that are structurally very similar, unexpected pharmacological differences may be uncovered (Schneider et al., 2010). Second, the above-mentioned pharmacological studies have to be accompanied by mechanistic studies aiming at the elucidation of the structural basis for the functional diversity, both with respect to receptors and ligands. This is also not an easy task because the H₄R species orthologs at multiple H₄R orthologs. Third, crystal structures of the H₄R would be most useful; again, however, structures with multiple ligands and multiple interacting proteins such as G-proteins and arrestins would be required. From all these considerations, it becomes evident that the challenges in the H₄R field are formidable, and presently, it is not clear whether a “standard” H₄R antagonist will ever be identified. But perhaps another strategy will help us out of the dilemma in a relatively short period of time: It is possible that other groups have observed paradoxical effects of H₄R ligands in general and JNJ7777120 in particular as well but have elected not to publish the data so far because they “don’t fit” to current, or more correctly, as is outlined in this article, past paradigms. Open and unbiased documentation of H₄R ligand effects in recombinant and native systems will help us understand biased H₄R signaling and the still poorly understood pathophysiological function of the H₄R.

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Authorship Contributions

Participated in research design: Seifert, Dove, and Buschauer. Contributed new reagents or analytic tools: Dove and Strasser. Performed data analysis: Seifert, Schneider, Dove, Brunskole, Neumann, Strasser, and Buschauer. Wrote or contributed to the writing of the manuscript: Seifert, Schneider, Dove, Brunskole, Neumann, Strasser, and Buschauer.

References


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