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 showing that JNJ7777120 can also activate /H9252-arrestin 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 mediators (Hill et al., 1997). A decade ago, several groups each independently identified a novel member of the H4R family with unique pharmacological properties, the H4R (for review, see Hough, 2001; Thurmond et al., 2008; Leurs et al., 2009). The H4R is a G 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 Gα-proteins by the H4R can be monitored by histamine-stimulated [35S]GTPγS binding to, or [γ-32P]GTP hydrolysis by, Gα-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 pharmacological 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 H1R 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 potent H4R antagonist (Jabloňowski et al., 2003; Venable et al., 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 experiments requiring continuous exposure to the compound technically difficult (Thurmond et al., 2004), researchers in the H4R field readily embraced JNJ7777120 because of its availability and high H4R selectivity (Jabloňowski 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 antagonist was also facilitated by the fact that the first known standard H4R antagonist, thioperamide (Fig. 1), is not selective for the H4R but is also a potent H1R antagonist, rendering it potentially difficult to discriminate H4R and H1R effects in vivo. More precisely, thioperamide is a dual H4R/H1R 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 H1R (Schneider et al., 2009; Schnell et al., 2010). Numerous H4R ligands structurally 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 endogenous and full agonist histamine stimulates [35S]GTP binding in cell membranes, whereas the inverse agonist thioperamide (Schneider et al., 2009) is a dual H4R/H1R 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 H1R (Schneider et al., 2009; Schnell et al., 2010). Numerous H4R ligands structurally 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.

![Fig. 1. Structures of selected H4R ligands analyzed in the present study.](image)

According to conventional definition, histamine, 5(4)-methylhistamine, UR-PI376, and UR-PI376 are H4R agonists, whereas thioperamide and JNJ7777120 are H4R antagonists. The classification of the compounds depends substantially on the specific system analyzed, and most strikingly, even the “standard” antagonists JNJ7777120 and thioperamide can exhibit agonistic effects at H4R, reflecting functional selectivity or biased signaling.

![Fig. 2. Functional selectivity of JNJ7777120 and thioperamide. A, at hH4R, histamine is a full agonist with respect to Gj- and β-arrestin activation. JNJ7777120 is a partial inverse agonist with respect to Gj-activation and a partial agonist with respect to β-arrestin activation. B, at mH4R, rH4R, and cH4R, Histamine is a full agonist with respect to Gj 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 Gj-protein activation catalyzed by various H4R species orthologs, effects may range from “full” inverse agonism (even thioperamide is actually not a full inverse hH4R agonist (Schneider et al., 2009)) over neutral antagonism to partial agonism.](image)
operate effectively reduces $[^{35}S]$GTP$\gamma$S binding, reflecting constitutive activity of hH$_4$R. With respect to $[^{35}S]$GTP$\gamma$S binding, JNJ 777120 exhibits, to a variable degree, partial inverse agonist or neutral antagonist properties at hH$_4$R (Schneider et al., 2009, 2010; Rosethorne and Charlton, 2011) (Fig. 2A; Table 1). In addition to stimulation of $[^{35}S]$GTP$\gamma$S binding, histamine also stimulates binding of $\beta$-arrestin to hH$_4$R. $\beta$-Arrestin recruitment to 7TM receptors has traditionally been linked to receptor uncoupling from G-proteins and desensitization (Luttrell and Gesty-Palmer, 2010; Rajagopal et al., 2010). More recently, however, it has become clear that $\beta$-arrestin can also serve as signal-transducing protein, stimulating G-protein-independent signal transduction pathways such as ERK (Luttrell and Gesty-Palmer, 2010). It is interesting to note that thioperamide effectively reduces $[^{35}S]$GTP$\gamma$S binding, reflecting constitutive activity of hH$_4$R (Jablonski et al., 2003). More recently, however, it has become clear that thioperamide also serves as signal-transducing protein, stimulating G-protein-independent signal transduction pathways such as ERK (Luttrell and Gesty-Palmer, 2010).

**TABLE 1**

Characterization of JNJ777120 in different in vitro test systems

<table>
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<tr>
<th>Expression System</th>
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<th>Important Findings Concerning JNJ777120</th>
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<td>Human SK-N-MC cells</td>
<td>Radioligand binding cAMP-CRE gene reporter assay</td>
<td>Human H$_4$R; $[^{3}H]$histamine competition binding: $K_i = 4.1$ nM, $K_i = 4$ nM and $K_i = 7.9$ nM cAMP assay with human, mouse, rat H$_4$R; equipotent agonist potency; 1000-fold selectivity relative to H$_3$R, H$_2$R, and H$_3$R no cross-reactivity with 50 other targets</td>
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<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 $\mu$M of histamine: IC$_{50} = 40$ nM</td>
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<td>Endogenous (human eosinophils)</td>
<td>Flow cytometry, in vitro chemotaxis assays</td>
<td>Antagonist: inhibition of eosinophil shape change, induced by 1 $\mu$M of histamine: IC$<em>{50} = 300$ nM; inhibition of chemotaxis, induced by 1 $\mu$M of histamine: IC$</em>{50} = 86$ nM</td>
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<tr>
<td>Sf9 insect cells</td>
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<td>Human HEK 293/HEK 293T cells</td>
<td>Radioligand binding ($[^{3}H]$histamine competition)</td>
<td>Human HEK 293/HEK 293T cells; $[^{3}H]$histamine competition binding assay: $K_i = 50$ nM</td>
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<tr>
<td>COS-7 cells</td>
<td>Radioligand binding</td>
<td>Substantial affinity differences of JNJ777120 at various species isoforms: human ($K_i = 5$ nM), monkey ($K_i = 32$ nM), pig ($K_i = 501$ nM), dog ($K_i = 79$ nM), mouse ($K_i = 4$ nM), rat ($K_i = 4$ nM) and guinea pig H$_4$R ($K_i = 1$ $\mu$M)</td>
</tr>
<tr>
<td>Human U2OS cells</td>
<td>GTP$\gamma$S binding assay, $\beta$-arrestin recruitment assay, ERK phosphorylation</td>
<td>Antagonist at canine H$_4$R; rightward shift of the histamine concentration-response curve, no $\alpha_2$A reported</td>
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<td>Mouse pituitary tumor AtT-20 cells</td>
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<td>$[^{3}H]$histamine competition binding assay: $K_i = 50$ nM</td>
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**HEK**, human embryonic kidney; **ELISA**, enzyme-linked immunosorbent assay.
Moreover, the effect of JNJ7777120 seems to be Gi-protein-receptor reserve, could account for the unexpected effects. The authors provide evidence that the effect of JNJ7777120 on β-arrestin recruitment is mediated by the H4R and not through another receptor. Specifically, thiopepiramide, 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 thiopepiramide for blockade of the JNJ7777120 response and the response of the H4R agonist clobenpropit are very similar. Unfortunately, the pA2 for the endogenous H4R ligand histamine was not reported. This is not trivial because the apparent affinity of thiopepiramide may be ligand-dependent.

Most striking is the finding that β-arrestin binding to hH4R is not a dead-end. In particular, JNJ7777120 induces very effective and prolonged ERK activation, whereas histamine induces only transient ERK activation. These time courses are typical for arrestin- and G-protein-dependent signal transduction, respectively (Luttrell and Gesty-Palmer, 2010), but the two pathways were not dissected with PTX by Rosethorne and Charlton (2011). In any case, these data show that JNJ7777120 is capable of stabilizing a conformation in hH4R that induces β-arrestin recruitment and stimulates an important downstream signaling pathway, at least in an osteosarcoma cell line. Thus, JNJ7777120 cannot be considered the “standard” H4R antagonist anymore, but depending on the parameter assessed, JNJ7777120 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 hH4R functions (Table 1). It is likely that the effects of JNJ7777120 strongly depend on the endogenous complement of signal transduction proteins in the cells harboring 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 hH4R conformations.

Although the data from Rosethorne and Charlton (2011) are 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 Thiopepiramide**

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 pathophysiological 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*
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 thiopera-

amide. To this end, in all studies including the study of
Rosethorne and Charlton (2011), there has been consensus
that thioperaamide acts as H4R inverse agonist (Leurs et al.,
2009; Schneider et al., 2009). However, at cH4R, thioper-

amide clearly exhibits partial agonistic efficacy, and at mH4R
and rH4R, thioperaamide is a neutral antagonist with respect
to Gβγ-protein activation (Schnell et al., 2011). Thus, depend-
ing on the species studied, thioperaamide can stabilize either
only inactive or both inactive and active H4R conformations,
and the functional behavior of this compound is clearly dif-
derent from the behavior of JNJ7777120 (Fig. 2, B and C). It
is also possible that the controversial effects of H4R antago-

nists 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 hor-
izontal position (Schneider et al., 2010). However, JNJ7777120
stabilizes both inactive and active hH4R states (Fig. 2). There-
fore, 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 con-
formation stabilizes the indole ring of Trp6.48 in a vertical posi-
tion, 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 alter-
native 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 con-
clusions 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 fol-
lowing, 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 phosphory-
lated 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 ser-
ine 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 ex-
clusively 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 Gβγ-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-ar-
restin. Specific phosphorylation patterns differentially orient
β-arrestin on the receptor and stabilize different active ar-
restin 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 pro-
longed 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 conforma-
tional changes may also enable interactions with additional

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 Gαi-protein α-subunits, uncouples 7TM receptors from G-proteins and is a most valuable tool for unmasking Gαi-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 Gαi-proteins in cells because Gαi-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 Gαi-proteins in the osteosarcoma cell expression system used by Rosethorne and Charlton (2011) was still functional and, evidently, those non–ADP-ribosylated Gαi-proteins could participate in signaling, including β-arrestin recruitment and ERK activation (Walters et al., 2009). At the membrane level, activation of Gαi-protein may be too small to be detected, but the β-arrestin assay may be sufficiently sensitive to detect Gαi-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 Gαi-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 [γ-32P]ADP-NAD and then performing SDS polyacrylamide electrophoresis with subsequent quantitative autoradiography. Effective ADP-ribosylation of Gαi-proteins in intact cells results in poor incorporation of [γ-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 Gαi-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 Gαi-proteins. A fraction of remaining functional Gαi-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 Gαi-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 Gαi-protein-independent manner differently from a Gβγ-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 Gαi-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 Thioperamide?

So, if pharmacological effects of JNJ7777120 and thioperamide 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 “antagonists.” For example, 5(4)-methylhistamine, originally described as an hH4R agonist (Black et al., 1972), displays selectivity for recombinant hH4R relative
to the other HRs (Lim et al., 2005). However, when applied in vivo, the actual concentration of the ligand in a particular organ is unknown, so effects on HRs other than the H4R, specifically the H2R, 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 H2R, 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-P1376) as a potent and selective hH4R 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 N\(^\text{\text{\textdagger}}\)-acylated imidazolylpropylguanidine H2R agonist N\(^\text{3}\)-[3-(1H-imidazol-4-yl)propyl]-N\(^\text{2}\)-propionylguanidine (UR-P1294) (Igel et al., 2009b) should also only be used with caution, considering the mechanistically still unexplained and very unusual "superagonism" at recombinant H\(_2\)R, 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 thioperamide, 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, unexplained, and complex effects 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 are structurally very different from each other (Lim et al., 2008, 2010; Schnell et al., 2011). In fact, combinations of amino acids and entire receptor regions may account for the different pharmacological properties of 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.

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Performed data analysis: Seifert, Schneider, Dove, Brunskole, Neumann, Strasser, and Buschauer.

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