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REVIEW

The histamine H3 receptor: from discovery to clinical trials with pitolisant

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The third histamine receptor was discovered in 1983 by a traditional pharmacological approach, consisting of assessing the inhibitory effect of histamine on its own release from depolarized rat brain slices. The same *in vitro* test was used to design, in 1987, the first highly selective and potent H3-autoreceptor ligands, the antagonist thioperamide and the agonist (R)alphamethylhistamine which enhances and inhibits, respectively, the activity of histaminergic neurons in brain. The use of these research tools was instrumental in establishing the main functions of cerebral histaminergic neurons, namely their role in maintenance of wakefulness, attention, learning and other cognitive processes. In 1990, the cloning of the gene of the H3-receptor, a member of the superfamily of heptahelical receptors coupled to G proteins, paved the way to the demonstration of the high constitutive activity of the receptor, including its native form, and its participation in the tonic control of histamine release; it also facilitated the development of H3-receptor inverse agonist programs in many drug companies. Pitolisant (BF2.649, 1-{3-[3-(4-chlorophenyl)propoxy]propyl}piperidine, hydrochloride) is the first inverse agonist to be introduced in the clinics. Its wake-promotion activity was evidenced in excessive diurnal sleepiness of patients with narcolepsy, Parkinson's disease or Obstructive Sleep Apnea/Hypopnea, in which this activity is characterized by a mean decrease of the Epworth Sleepiness Scale by about five units. The procognitive activity of this novel class of drugs may also find therapeutic applications in dementias, schizophrenia or attention deficit hyperactivity disorder.

LINKED ARTICLES

This review is dedicated to the memory of Sir James Black who recently passed away and whose achievements in classical pharmacology and drug design have been examples to the author throughout his career. *BJP* published an issue in 2010 on Analytical Receptor Pharmacology in Drug Discovery, which was dedicated to the memory of Sir James Black. To view this issue visit http://dx.doi.org/10.1111/bph.2010.161.issue-6 In 2009 *BJP* published a Histamine themed issue. To view this issue visit http://dx.doi.org/10.1111/bph.2009.157.issue-1

Abbreviations

H3R, histamine H3 receptor; HA, histamine; OSA, obstructive sleep apneoa/hypopneoa.

Introduction

The existence of a third histamine receptor was established by the beginning of the 1980s, rather than earlier, because it is only at this time that both conceptual and experimental tools had been made available during the few preceding years. Indeed it was only by the mid-1970s, that histamine was shown to be added to the growing list of novel cerebral neurotransmitters (Schwartz, 1975; 1977; Haas *et al.*, 2008), at a time when most neuroscientists admitted that only acetylcholine and catecholamines were playing such a role. In agreement, electrolytic lesions of the medial forebrain bundle had been shown to elicit dramatic decreases in the telencephalic level of L-histidine decarboxylase (Garbarg *et al.*, 1974). This histamine-synthesizing enzyme, characterized in rat brain a few years earlier (Schwartz *et al.*, 1970), had previously shown to be present in isolated nerve-endings. Hence, the lesion studies were interpreted as reflecting the anterograde degeneration of a major histaminergic neuronal bundle emanating from an area posterior to the lateral hypothalamic area, and projecting in a diffuse manner to the whole telencephalon, a disposition largely confirmed, several years later, with the development of histochemical methods (Watanabe *et al.* 1983; Panula *et al.*, 1984). In addition, depolarization-induced and calcium-dependent release of histamine (Verdiere *et al.*, 1975), metabolic inactivation

pathways (Schwartz *et al.*, 1971) and presence of the two classes of receptors, H1 and H2, mediating its effects in peripheral tissues had all been recently uncovered in the brain (Baudry *et al.*, 1975; Haas and Bucher, 1975).

In other words, at the beginning of the 1980s, after nearly a decade of efforts, almost the whole machinery of histaminergic neuronal transmission had been uncovered, and, conceptually, the time had come to assess whether – like shown shortly before for catecholaminergic neurons – histaminergic neurons were endowed with autoreceptors.

Discovery of the histamine H3 receptor

Autoreceptors, that is, receptors expressed on neuronal cell bodies or terminals and inhibiting their own transmitter release or synthesis, had become, at the beginning of the 1980s, a field of intense research. Such receptors were often and conveniently studied by labelling the endogenous neurotransmitter stores of noradrenaline or dopamine neurons via radioactive amine uptake in brain slices or synaptosomes through their high-affinity transport systems. When the analogy between catecholaminergic and histaminergic neurons had become apparent, it was tempting to assess whether autoreceptors were also present on the latter.

There had been attempts in several laboratories to perform release experiments following incubation of brain preparations with radiolabelled histamine, but resulting data were hardly interpretable. On our side, we avoided this approach, knowing from previous studies that high-affinity transport systems for histamine could not be evidenced. However, we had put a lot of efforts earlier to develop a method to label endogenous stores of histamine selectively through the incubation of the cerebral preparations by the 3H-aminoacid precursor (Verdiere *et al.*, 1975). To succeed, we had painstakingly developed ion-exchange chromatography methods on small columns, first to purify 3H-histidine (by eliminating a preformed 3H-histamine contamination present in the commercial samples and thereby, reducing a background noise), then to isolate small amounts of synthesized 3H-histamine (present only in L-histidine decarboxylase- expressing cells) from huge excess of 3H-histidine (actively uptaken in all cells).

In 1982, with Jean-Michel Arrang and Monique Garbarg (Arrang *et al.*, 1983), we applied this process to slices from rat cerebral cortex, a brain area already known from deafferentation studies (Barbin *et al.*, 1975), to contain exclusively terminals from histaminergic neurons, and which represents an abundant source of tissue. In order to conduct conveniently large numbers of parallel incubations (necessary to construct dose–response curves, the golden rule in traditional pharmacology), a very simple device was deliberately selected. It consisted of common test tubes in which slice batches were distributed, oxygenated superficially at the beginning and depolarized by the addition of potassium ions.

Quite rapidly, this simple process worked reliably and reproducibly, and it was found that addition of nonradioactive histamine in the medium reduced the amount of 3H-histamine released by the depolarizing stimulus. This histamine-induced braking effect on the amine's own release was result from a receptor-mediated effect, rather than a kind of isotopic dilution effect: it could be blocked competitively by known histamine antagonists and mimicked by histamine analogues. Clearly this implied the involvement of a presynaptic auto-inhibitory receptor. Was this receptor one of the two already defined at this time?

As a consequence of the beautiful work of traditional pharmacology performed a few years earlier by James Black, Robin Ganellin and their colleagues (Black *et al.*, 1972), we had on hand the necessary tools to answer this question, that is, a series of compounds acting selectively at either the H1 receptor (H1R) or the H2R, each of these tools being characterized by its apparent affinity constant. From their dose–response curves, we derived apparent dissociation constants of antagonists (using the Schild plot analysis) and the relative potencies of agonists. Using these classical pharmacology indexes carefully, it became clear that we were dealing with a non-HIR, non-H2R: for instance, the effect of histamine was hardly affected by mepyramine at micromolar concentrations (whereas the drug displays nanomolar affinity at the H1R) and impromidine, a potent H2R agonist, behaved as a rather potent antagonist in our model, two examples among many others that fortified our conviction that we had discovered a novel histamine receptor.

In the letter to *Nature* describing these findings (Arrang *et al.*, 1983), we proposed to call H3 receptor this novel entity, and this was one of the last receptors discovered by 'traditional' pharmacological approaches, that is, without the contribution of molecular biology.

H3-receptor ligands: from research tools to drug candidates

At the beginning of the eighties, the use of selective ligands, like agonists or antagonists, was almost the only way to understand the function of a novel receptor. Obviously, such research tools were lacking in the case of the H3 receptor. A program to design such tools was started through a collaboration between the groups of Jeanne-Marie Lecomte at Bioprojet (a young research company founded a few years earlier), Jean-Charles Schwartz at Institut National de la Santé et de la Recherche Médicale (INSERM) in Paris and the medicinal chemistry groups of Max Robba, at the University of Caen, and of Walter Schunack, at the University of Berlin. The starting point was the structure of histamine itself, namely because the natural agonist was, presumably, tightly binding to the receptor, as revealed by its EC50, lower by several orders of magnitude than at the H1 or H2 receptors. Hence, the imidazole ring was retained while the ethylamine side-chain of histamine was either substituted or rigidified in a piperidine ring. The synthesized compounds were tested on the original system upon which the H3 receptor had been discovered, that is, depolarized rat brain slices labelled with 3H-histidine.

In a few years, two main tools were derived from this program: thioperamide, a potent antagonist (Ki = 4 nM) and (R)alphamethylhistamine, a potent agonist (15-fold more potent than histamine itself), both being highly selective versus the H1 and H2 receptors (Arrang *et al.*, 1987).

One of the first applications of these tools was the identification of H3Rs on other classes of cerebral neurons, for example, catecholamine or serotonine neurons (Schlicker *et al.*, 1994).

In addition the agonist, when tritiated, constituted a useful radioprobe, first to map the receptor distribution autoradiographically in the brain, (revealing for instance, its high density in striatum or some hypothalamic areas), then to show the modulation of H3R binding by guanylnucleotides, thereby indicating the belonging of the novel receptor to the superfamily of G-protein coupled receptors (Arrang *et al.*, 1990).

The antagonist was shown to potently enhance histamine turnover in rat brain, a property which proved to be extremely useful to uncover the functional role of histaminergic neurons as it became, for the first time, feasible to activate experimentally histaminergic transmissions. Thereafter, numerous studies using thioperamide, could establish or confirm the role of histaminergic pathways in, for example, arousal, cognition, hormonal controls or various behaviours (reviewed in Schwartz *et al.*, 1991; Haas *et al.*, 2008).

Could these research tools be developed as drugs?

It became rapidly clear that this was not the case: the agonist was rapidly inactivated by first-pass hepatic metabolism, whereas thioperamide displayed hepatotoxicity, presumably attributable to its thioamide group. Hence, the program to design an H3R antagonist as a drug useful to improve wakefulness and cognitive deficits in the clinics was continued with Robin Ganellin being embarked in this European enterprise after he had become Professor at University College London. Hundreds of compounds were prepared and tested in our University laboratories, a number of which like ciproxifan (Ligneau *et al.*, 1998) were extremely potent *in vivo*, but had to be abandoned after toxicity tests performed by Bioprojet. The program also produced [^{125}I] iodoproxyfan, a highly sensitive radioprobe, and potent H3R agonists, for example, Imetit or BP2.94. Meanwhile, whereas drug companies remained essentially uninterested in this research, several other university groups succeeded in designing H3R ligands with high potency such as clobenpropit or GT-2331 (recently reviewed in Sander *et al.* 2008; Stocking and Letavic, 2008; Raddatz *et al.*, 2010). Only the latter compound was apparently submitted to a clinical trial, in adult attention deficit hyperactivity disorder (ADHD), but the result was never disclosed and, instead of being an antagonist, its partial agonistic properties were thereafter discovered.

Two important discoveries modified this landscape. The first one was the demonstration that the imidazole residue, so far considered as a 'must' in H3R ligands, could be replaced by nitrogen-containing heterocycles, thereby leading to potent derivatives devoid of some of the drawbacks of imidazole-containing compounds, such as interaction with CYP450s or poor brain penetration. The second one was the 'deorphanisation' of the human H3R, that is, the identification of an 'orphan' heptahelical receptor after its expression and using the H3R ligands that had been designed during the preceding years (Lovenberg *et al.*, 1999).

This last discovery had several important applications. It provided a human recombinant protein facilitating the screening of molecules and, thereby, triggering the involvement of several large multinational companies, such as Johnson & Johnson, Abbott, Pfizer, GSK, Pfizer or Hoffman-La Roche in H3R antagonist research programs (reviewed in Sander *et al.*, 2008; Raddatz *et al.*, 2010), the results of which are described in a very large number of patent applications. In addition, homology cloning of the H3R in several animal species disclosed marked pharmacological differences associated with limited amino acid sequence differences such as in the rat, a species in which site-directed mutagenesis experiments identified only two amino acids in trans-membrane domains as responsible for such a difference that, indeed, has to be taken into consideration for drug selection and development (Ligneau *et al.*, 2000).

In addition, the existence of various isoforms produced by alternative mRNA splicing was demonstrated (Schwartz *et al.*, 2001), although the functional significance of the process has remained essentially unclear, as is still the case for the dopamine D2R, the first heptahelical receptor for which the process was disclosed (Giros *et al.*, 1989).

More importantly for drug selection and development was the discovery of a marked constitutive activity in the H3R, attributable to a short amino acid sequence in the sixth trans-membrane domain (Schwartz *et al.*, 2001). Constitutive activity in heptahelical receptors over-expressed in artificial cell systems is a rather trivial observation but, in the case of the H3R, it can even be detected in the native receptor in rat brain; furthermore, endogenous histamine release *in vitro*, or *in vivo*, can only be triggered by inverse agonists reversing this constitutive activity, suggesting that potency of various antagonist/inverse agonists may depend on their intrinsic activity as inverse agonists (Morisset *et al.*, 2000).

Based upon these considerations, BF2.649, now known under its international nonproprietary name denomination of Pitolisant (also formerly designated tiprolisant by the World Health Organization), a N-piperidyl derivative (Figure 1) was selected for development.

Pitolisant, the first inverse agonist/antagonist to proceed to the clinics

Pitolisant was progressively found to gather a number of biological properties required to be administered in humans and, by this way, to verify the validity of hypothesis regarding the clinical utility of H3R antagonist/inverse agonists (summarized in Table 1; see also Ligneau *et al.*, 2007a,b).

It displays a low nanomolar apparent affinity as competitive antagonist at the human recombinant or native receptor from post-mortem brain samples. It is, also at low nanomolar concentrations and with high intrinsic activity, an inverse agonist which not only blocks the braking effect of histamine or H3R agonists on endogenous histamine release from depolarized synaptosomes, but also enhances this release over the basal level. Its high selectivity was shown by the absence of

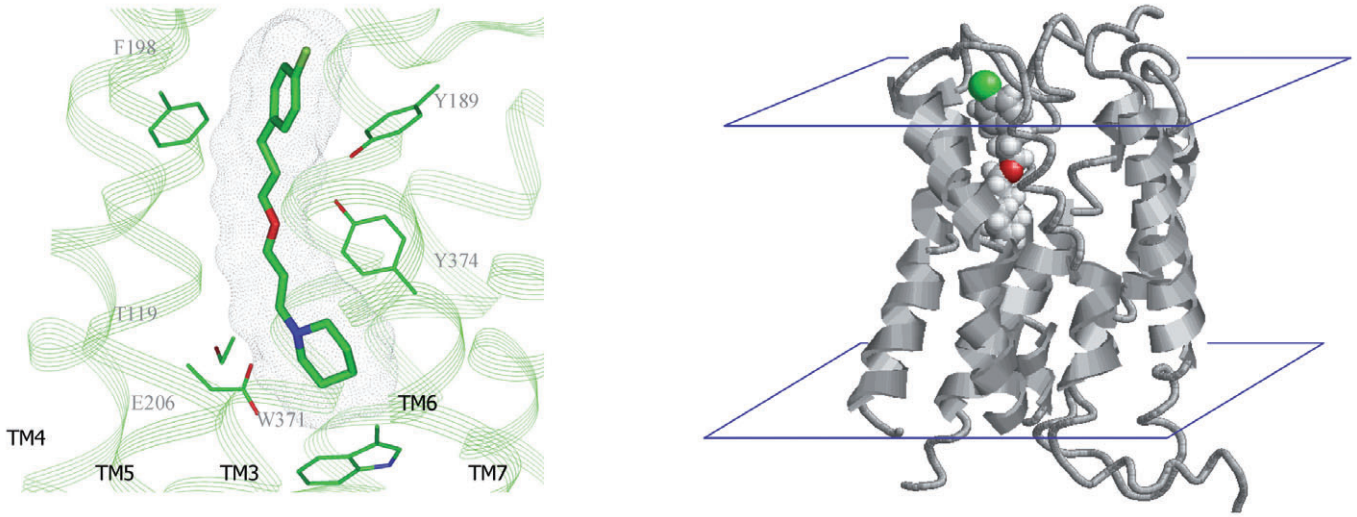

Figure 1

Pitolisant (BF2.649, 1-{3-[3-(4-chlorophenyl)propoxy]propyl}piperidine, hydrochloride) in a model of the histamine H3 receptor. Topology of the H3 receptor model is shown in a membrane bilayer (parallel blue planes). The antagonist binding site, within the trans-membrane core and just below the extracellular loops, is oriented perpendicularly to the membrane, according to the refined binding site of H3 receptor (Levoin *et al.*, 2008). Crucial residues and membrane spanning segments (TM 3-7) are shown in the right inset. Glu206 forms a salt bridge with the piperidine, the hydroxyl of Tyr374 is H-bonded with the central oxygen of the ligand, and Phe198 and/or Tyr189 make π-stacking with the para-chlorophenyl.

Table 1

Summary of biological properties of Pitolisant (BF2.649)

Dissociation constant (Ki) at the hH3R	0.3–1.0 nM
Dissociation constant (Ki) at the rH3R	17 nM
Inverse agonism (EC50) at the hH3R	1.5 nM
Activation of HA release in mouse brain (ED50, p.o.)	1.6 $mg \cdot kg^{-1}$
Mouse brain/plasma ratio of AUCs	~11
Recombinant hHERG channel inhibition (IC50)	1.3 µM (Safety Ratio = ~10^3)
Rabbit Purkinje fiber action potential change	~10 µM (Safety Ratio = ~10^4)
Cardiovascular changes (BP) dog telemetry (ED50, i.v.)	〉1.5 $mg \cdot kg^{-1}$ (Safety Ratio 〉 5)
Cardiovascular changes (QTc) dog telemetry (ED50, i.v.)	〉4.5 $mg \cdot kg^{-1}$ (Safety Ratio 〉 15)
NOAEL dose in 6-month rat toxicity	30 $mg \cdot kg^{-1} \cdot day^{-1}$ (Safety Ratio = 37.3)
NOAEL dose in 9-month monkey toxicity	12 $mg \cdot kg^{-1} \cdot day^{-1}$ (Safety Ratio = 9.6)
Cytochrome P450 isozyme inhibition (IC50)	2.6 µM (2D6, dextromethorphan), 〉10 µM (other isozymes)
Cytochrome P450 isozyme induction (EC50)	10 µM (2A6, 2B6), >100 µM (other isozymes)
Pharmacokinetics in healthy volunteers (20 mg, p.o.)	Cmax = ~30 $ng \cdot mL^{-1}$, Tmax = 3 h, T1/2 = 11 h

Safety Ratios are based upon the plasma level at human therapeutic dosage (20 $mg \cdot d^{-1}$, p.o.).

significant interaction with nearly a hundred of various human receptors or channels, at a 100 nM concentration.

At the rodent H3R it was found to be about 20 times less potent than at the human H3R. In spite of this limited *in vitro* potency, Pitolisant administered orally to mice enhanced brain histaminergic activity at a rather low dosage, as assessed using the level of *tele*-methyl histamine as a reliable index. This apparent discrepancy could be accounted for by the high oral bioavailability (84% when comparing areas under the curves determined by radioreceptor assay after oral and intravenous administrations) and very high brain penetration of the compound and/or pharmacologically active metabolites in this species (Ligneau *et al.*, 2007b).

Pitolisant also enhanced acetylcholine in microdialysates of rat prefrontal cortex and hippocampus, and dopamine in prefrontal cortex. In contrast, dopamine turnover was not enhanced in the striatal complex comprising the nucleus accumbens, an observation which suggests that this differen-

tiated monoamine-releasing effect of the H3R inverse agonist reflects an indirect and selective action of endogenous histamine on subpopulations of monoaminergic neurons rather than an effect via H3-heteroreceptors which are present on striatal dopamine neurons (Schlicker *et al.*, 1994). In addition, this observation also accounts for the lack of psychomotor activation and dependence liability of the drug, the latter assessed in three different models in rodents and monkey (P. Beardsley, M. Uguen, X. Ligneau and J-C. Schwartz, in preparation); both features oppose H3R inverse agonists to other classes of wake-promoting drugs like amphetamines.

The preclinical safety of the drug, allowing it to be administered in humans, was demonstrated in a series of regulatory tests in which the safety ratio, that is, the ratio of highest non-effective concentrations (or plasma concentrations at the no observable adverse effect level doses) to plasma concentrations at human therapeutic dose were found acceptable. Notably, 6-month rat and 9-month monkey toxicity studies did not show any significant histopathological or biochemical alteration, and the only toxic manifestation at very high doses, by several orders of magnitude higher than those necessary to saturate cerebral H3Rs, were behavioural manifestations consisting mainly in convulsive episodes. Also, the cardiovascular safety, assessed by the ratio of human ether-à-go-go related gene channel IC50 to hH3R Kb or free (20% of the total) plasma concentration at therapeutic dose, were found acceptable, as confirmed by telemetry in dogs and, now, even more importantly, by thorough electrocardiographic assessment in a large number of healthy volunteers and patients.

In healthy human volunteers, single oral doses up to 120 mg (representing six times the therapeutic dose) were well tolerated without any adverse, physiological, namely cardiovascular, or biological manifestation; at 120 mg, however, there were some minor signs of irritability. Although behavioural manifestations in these young male volunteers were quite mild, signs of increased vigilance, particularly at the end of the day, could be detected by monitoring their quantitative electroencephalogram (EEG) (with a shift towards high frequency waves which are known to accompany states of increased vigilance) or in a test like the Critical Flicker Fusion Test, which revealed an improved attention.

The pharmacokinetic parameters were found consistent with a once-a-day administration in the morning, and a plasma level already well reduced at the end of the day to ensure a lack of waking effect during night. The decay in plasma level was associated with the appearance of hydoxylated metabolites produced by both cytochromes 3A4 and 2D6, followed by opening of the piperidine ring, leading to a highly hydrosoluble major metabolite, totally inactive at the H3R, which constitutes, in humans, the main form of elimination of the drug in the urines. CYP 2D6 activity towards 25 μM dextromethorphan, a recommended conventional substrate, was inhibited by Pitolisant with an IC50 value, ~10 times higher than its plasma levels at therapeutic dosage. Together with the fact that Pitolisant metabolism involves two distinct CYP 450 isoforms, this tends to avoid any major metabolic drug–drug interaction with compounds interacting with CYP 2D6. This was shown when Pitolisant (40 mg) was co-administered with olanzapine (10 mg) to a group of healthy volunteers: no significant change in each drug plasma levels was detected as compared with their individual administration (P. Robert, pers. comm.).

Taken together, these data indicated that Pitolisant was definitely a 'druggable' compound that could be used to explore, for the first time, the H3R inverse agonist utility in therapeutics.

Preclinical data obtained with a variety of H3R inverse agonists of diverse chemical structure strongly suggest that this class of drugs may find therapeutic applications in two large categories of indications: wakefulness impairments and cognitive disorders. Although the two types of disorders might largely overlap, it is convenient to consider them separately.

Therapeutic applications of histamine H3-receptor inverse agonists in excessive daytime sleepiness

The critical role of histaminergic neurons in the maintenance of wakefulness, suggested for the first time in the mid-seventies (Schwartz, 1977) has been, thereafter, confirmed by a large series of experimental approaches (reviewed by Lin, 2000), including the seminal observation that enhancement of histaminergic neuron activity by thioperamide, the first H3R inverse agonist, triggers a long-lasting period of arousal that is blocked by mepyramine in cats, that is, blockade of post-synaptic H1Rs (Lin *et al.*, 1990). This wake-promoting activity that is shared by a variety of H3R inverse agonists (Sander *et al.*, 2008; Stocking and Letavic, 2008; Raddatz *et al.*, 2010) can be observed in several animal species, for example, mice, and is associated with enhanced activity not only of histaminergic neurons but also of other ascending waking pathways, that is, noradrenergic, cholinergic or dopaminergic neurons as shown with Pitolisant (Ligneau *et al.*, 2007a,b).

Consequently, the use of H3R inverse agonists in excessive daytime sleepiness disorders was obviously a rational hypothesis that was explored in clinical trials with Pitolisant in three diseases; it was preceded, when feasible, by studies in available animal models.

Narcolepsy is a rare disabling disorder affecting about 25 over 100 000 persons and characterized by excessive daytime sleepiness and abnormal rapid eye movement (REM) sleep manifestations, including cataplexy (sudden loss of muscle tone triggered by strong emotions), direct transition from wakefulness to REM sleep (DREMs) periods, sleep paralysis and hypnagogic hallucinations (Dauvilliers *et al.*, 2007). Narcolepsy is caused by deficient neurotransmission by orexins (also known as hypocretins; Chemelli *et al.*, 1999), excitatory peptides which are released by neurons from the lateral hypothalamus with widespread projections, namely to aminergic neurons known to be involved in the control of wakefulness, for example, histaminergic or noradrenergic neurons. Histaminergic neurons seem even necessary to the waking action of orexins (Huang *et al.*, 2001), and reduced levels of histamine in the cerebrospinal fluid (CSF) of narcoleptic patients were recently reported (Nishino *et al.*, 2009), although this finding was thereafter challenged (Croyal *et al.*, 2011).

Table 2

Summary of the effects of Pitolisant on excessive diurnal sleepiness: changes in Epworth sleepiness scores in narcolepsy, Parkinson's disease and obstructive sleep apnoea

	Epworth sleepiness score (means ± SEM)	
	At inclusion	After treatment
Narcolepsy[a]	17.2 ± 0.7	12.4 ± 0.9*
Parkinson's disease[b]	16.6 ± 0.7	10.8 ± 1.1*
Obstructive sleep apnoea[c]	15.7 ± 0.9	9.8 ± 1.5*

[a]One-month treatment at individually adjusted dosage (10, 20 or 40 mg, o.d., $n = 26$).
[b]One-month treatment at 20 mg, o.d. ($n = 21$).
[c]Three-day treatment at 40 mg, o.d. ($n = 12$).
*$P < 0.05$.

We explored the hypothesis that the lack of orexins could be circumvented by activating histaminergic neurons pharmacologically, first in orexin (–/–) mice, a reliable model of narcolepsy. Quite unexpectedly, if one compares with some human CSF data (Nishino *et al.*, 2009), these mice were found to display normal indices of brain histaminergic and noradrenergic transmission, and Pitolisant enhanced the activity of these two major wake-promoting systems to the same extent as in their wild-type counterparts; this indicated that the two ascending waking systems can still be stimulated in this narcolepsy model (Lin *et al.*, 2008).

Pitolisant enhanced wakefulness during the lights-off (active) period of orexin (–/–) mice, as it does in the wild-type mice. In addition, it reduced in these 'narcoleptic' mice, the number of characteristic DREMs episodes, that is, direct transitions from wakefulness to REM sleep. Although this interpretation was challenged, Chemelli *et al.* (1999) proposed that these episodes correspond to cataplexy episodes in narcoleptic patients, and another H3R antagonist was described to decrease cataplectic episodes in the Doberman 'narcoleptic' dog (Bonaventure *et al.*, 2007).

Starting from these promising preclinical data, a single-blind pilot trial with a simple design was undertaken in 22 severely affected narcoleptic patients which received placebo on the first week and Pitolisant (40 mg, once a day), during the next week. The drug elicited a statistically significant and clinically meaningful reduction of their diurnal somnolence, assessed either by the Epworth Sleepiness Scale (a self-administered questionnaire for patients evaluating their chances of dozing in eight different situations often encountered in daily life) as well as by the number or duration of the diurnal sleep/somnolence episodes (Table 2 and Lin *et al.*, 2008). The drug was generally well tolerated with, however, a few patients experiencing nocturnal insomnia that could have been related to individual drug overdosage.

Hence, in a second 'proof-of-concept', open label study, 26 patients were individually titrated over 3 weeks, receiving 10, 20 or 40 mg of Pitolisant for 1 month and, optionally, for up to 9 months (Y. Dauvilliers and I. Arnulf, pers. comm.). In those patients that were maintained at 20 mg, as in those receiving 40 mg, the reduction in the Epworth score was of about five units; a similar reduction was also recorded after 3 and even 9 months among the fraction of patients extending their treatment. Interestingly also, there was a significant reduction in the frequency of cataplexy episodes in those patients which had remained clearly affected in spite of their continuing anticataplectic treatment, mainly by antidepressant drugs.

Taken together, these preliminary studies suggested that, in narcolepsy, H3R inverse agonists may have long-lasting anti-sleepiness and, possibly, anticataplectic efficacy and may be well tolerated. This has to be confirmed in double-blind, placebo-controlled studies that are now in progress with Pitolisant.

Parkinson's disease is a degenerative disorder in which 30–40% of patients suffer from excessive diurnal sleepiness, even when their motor symptomatology is well controlled by dopaminergic or other treatments, and eventually, culminates in sleep attacks without prodrome in 1–4% of these patients (Arnulf, 2005). Dr J.S. Lin has developed a reliable model of the condition in cats lesioned with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine which display both the motor signs and the excessive sleepiness of Parkinson's disease (Arnulf *et al.*, 2005). In these animals, treatment with either levodopa or dopaminergic direct agonists improves motor symptomatology, but Pitolisant had to be added to reduce sleepiness and, importantly, the H3R inverse agonist did not compromise the motor effect of the anti-parkinsonian drugs (Arnulf, 2009; I. Arnulf, H. Moller, P. Lehert, J.M. Lecomte, J.C. Schwartz and J.S. Lin in preparation).

In a first exploratory single-blind trial on 26 Parkinson's disease patients suffering from excessive diurnal sleepiness receiving placebo for one week, followed by 40 mg Pitolisant, once a day for 1 week, the drug-induced improvement of the Epworth score was significant (Table 2, Arnulf, 2009; Arnulf I, Moller H, Lehert P, Lecomte JM, Schwartz JC and Lin JS, in preparation). Furthermore, a majority of the patients (19) continued the treatment (in open form) for 3 months, at the end of which the score improvement was maintained. Meanwhile, the motor symptomatology, evaluated by the Unified Parkinson's Disease Rating Scale score, which was mild (the ongoing anti-parkinsonian treatment was maintained all over the trial), was not significantly affected.

These initial positive data were confirmed in a double-blind dose-ranging study in 107 patients receiving placebo, 5, 10, 20 or 40 mg Pitolisant for 1 month. Statistical analysis of the relationship between dose and effect showed it monotonic ($P = 0.017$) between placebo and the highest dose, that is, the higher the dose, the stronger the effect and the minimum efficacious dose was 20 mg ($P = 0.035$).

At this dose, the median decrease of the Epworth score was of five units (Arnulf, 2009; Arnulf, Moller, Lehert, Lecomte, Schwartz and Lin, in preparation).

The beneficial effect of a brain histamine-releasing drug in this pathology is consistent with the observations that histaminergic neurons do not degenerate (Garbarg *et al.*, 1983), and that cerebral H3 receptors maintain their responsiveness (Anichtchik *et al.*, 2001). In contrast, the poor benefit provided by modafinil (Arnulf, 2005) is attributable to its mode of action via dopaminergic neurons (Volkow *et al.*, 2009), degenerated in this disease.

Obstructive Sleep Apnea (OSA) is also often accompanied with excessive diurnal sleepiness, even, sometimes, when the patients are submitted to nasal Continuous Positive Airway Pressure.

A 'proof-of-concept', single-blind trial was conducted on 21 patients suffering from moderate to severe OSA with excessive daytime sleepiness (EDS) which received placebo for 1 week, followed by Pitolisant (40 mg/day) for another week. The main efficacy criterion was the Osler test (Oxford Sleep Resistance test), consisting in placing the patient in a dark room, instructing him to remain awake and measuring the delay for falling asleep. Pitolisant treatment significantly improved the Osler score by nearly seven points ($P < 0.01$) and the Epworth score was also significantly improved (Table 2) (P. Levy, Pepin and Gormand, pers. comm.).

In all these studies, Pitolisant treatment was generally well tolerated with only minor adverse events such as nausea (4.9% vs. 0.6% with placebo), headache (9.7% vs. 2.9%), gastralgies (2.7 vs. 0.6%) or moderate insomnia (9.7% vs. 5.2%), and a similar safety pattern was found in other studies, a total of over 500 patients or healthy volunteers having already taken the drug for variable durations.

Hence, the positive results of several single-blind trials and one double-blind dose-finding trial in three pathologies in which EDS is a common symptom have substantiated the idea that H3R inverse agonists constitute a valuable and safe therapeutic option.

Are H3R antagonist/inverse agonists therapeutically useful as cognitive enhancers?

The waking action of Pitolisant in cats, mice and healthy volunteers is associated with a shift of the power distribution pattern of the cortical EEG in favour of the highest frequency rhythms, which are known to accompany cognitive activities such as attention or learning. This is consistent with many observations (reviewed in Hancock and Fox, 2004; Passani *et al*. 2004; Stocking and Letavic, 2008) made in several laboratories and using various animal models, of enhanced attention (such as the effect of ciproxifan on the five-choice test in rats (Ligneau *et al*., 1998) or facilitated learning (such as the effect of thioperamide or Pitolisant on the two-trial object-recognition test in mice (Ligneau *et al*., 2007a).

In addition, the procognitive (namely, the pro-attentional) potential of H3-receptor inverse agonists, now evident not only in rodents but also in healthy human volunteers, is obviously encouraging for exploring their therapeutic utility in diseases in which cognitive deficits represent the major symptomatology.

Schizophrenia is one of such diseases in which various preclinical data have suggested the interest of H3R inverse agonists (Arrang, 2007), particularly Pitolisant (Ligneau *et al*., 2007a,b).

This hypothesis is currently explored by C. Tamminga and colleagues in an ongoing double-blind trial in which patients that are already on typical (haloperidol) or atypical antipsychotic drugs (risperidone, aripiprazole, paliperidone) receive, in addition, 20 mg Pitolisant. The potential changes are being assessed by a battery of cognitive scales as well as by typical psychosis scales.

Lewy's bodies dementia patients, could benefit from an association of H3R inverse agonists with their usual treatment with acetylcholinesterase inhibitors since the two classes of drugs enhance extracellular acetylcholine in brain by distinct mechanisms and their actions were found additive in microdialysis experiments performed in rat hippocampus (D. Perrin and X. Ligneau, pers. comm.). In addition patients with this type of dementia suffer from excessive daytime sleepiness for which the wake-promoting effect of H3R agents might be useful. The hypothesis is currently explored in a double-blind trial with Pitolisant performed by F. Pasquier and colleagues.

ADHD is a disorder namely characterized in children and adults by an impaired attention. The potential interest of H3R inverse agonists in this pathology is suggested by their pro-attentional activity in rodents which is not accompanied by psychomotor activation and abuse potential, as is the case for Ritaline or amphetamines. In agreement, Pitolisant enhanced dopamine release in the prefrontal cortex but not in ventral striatum (Ligneau *et al*. 2007a; Ligneau *et al*., 2007b).

Recently, a single-blind trial with this drug in 20 adult patients resulted in a progressive improvement in the Conners' adult ADHD rating scales as well as in the attention deficit hyperactivity disorder rating scale rating scales (J. Mendelewicz and P. Oswald, pers. comm.). Obviously, this preliminary result requires confirmation in a double-blind trial in adults and children.

In conclusion, whereas the beneficial effects of an H3R inverse agonist in Excessive Daytime Sleepiness seem already reasonably well substantiated with Pitolisant, those in cognitive disorders require additional testing. This question will also be settled when clinical data with other compounds apparently in ongoing trials (PF-03654746, GSK189254, GSK239512, MK-0249, MK-3134, JNJ-17216498 and ABT-288) will become available.

Conflicts of interest

The author is co-founder, shareholder and scientific director of Société civile de recherche Bioprojet.

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