Pitolisant, a wake-promoting agent devoid of psychostimulant properties: Preclinical comparison with amphetamine, modafinil, and solriamfetol

Stéphane Krief | Isabelle Berrebi-Bertrand | Isabelle Nagmar | Martin Giret | Simon Belliard | David Perrin | Marilyne Uguen | Philippe Robert | Jeanne-Marie Lecomte | Jean-Charles Schwartz | Olivier Finance | Xavier Ligneau

Bioprojet-Biotech, Saint-Grégoire, France

Correspondence
Xavier Ligneau and Stéphane Krief,
Bioprojet-Biotech, 4 rue du Chesnay-Beauregard, 35760 Saint-Grégoire, France.
Email: x.ligneau@bioprojet.com; s.krief@bioprojet.com

Funding information
Bioprojet biotech

Abstract
Several therapeutic options are currently available to treat excessive daytime sleepiness (EDS) in patients suffering from narcolepsy or obstructive sleep apnea. However, there are no comparisons between the various wake-promoting agents in terms of mechanism of action, efficacy, or safety. The goal of this study was to compare amphetamine, modafinil, solriamfetol, and pitolisant at their known primary pharmacological targets, histamine H3 receptors (H3R), dopamine, norepinephrine, and serotonin transporters, and in various in vivo preclinical models in relation to neurochemistry, locomotion, behavioral sensitization, and food intake. Results confirmed that the primary pharmacological effect of amphetamine, modafinil, and solriamfetol was to increase central dopamine neurotransmission, in part by inhibiting its transporter. Furthermore, solriamfetol increased levels of extracellular dopamine in the nucleus accumbens, and decreased the 3,4-dihydroxyphenylacetic acid (DOPAC)/DA ratio in the striatum, as reported for modafinil and amphetamine. All these compounds produced hyperlocomotion, behavioral sensitization, and hypophagia, which are common features of psychostimulants and of compounds with abuse potential. In contrast, pitolisant, a selective and potent H3R antagonist/inverse agonist that promotes wakefulness, had no effect on striatal dopamine, locomotion, or food intake. In addition, pitolisant, devoid of behavioral sensitization by itself, attenuated the hyperlocomotion induced by either modafinil or solriamfetol. Therefore, pitolisant presents biochemical, neurochemical, and behavioral profiles different from those of amphetamine and other psychostimulants such as modafinil or solriamfetol. In conclusion, pitolisant is a differentiated therapeutic option, when compared
1 | INTRODUCTION

Excessive daytime sleepiness (EDS) refers to difficulty maintaining desired wakefulness and alertness during the day with unintended lapses into drowsiness or sleep. It is prevalent in various sleep-wake disorders such as narcolepsy, obstructive sleep apnea (OSA), idiopathic hypersomnia, central sleep apnea, Parkinson’s disease, and restless legs syndrome.1,2

Among wake-promoting agents that are utilized for narcolepsy, modafinil/armodafinil, sodium oxybate, methylphenidate, solriamfetol, and pitolisant are approved by the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) (excepted armodafinil and methylphenidate in Europe). In the presence of both EDS and cataplexy, primary treatments would be sodium oxybate or pitolisant as these drugs address both symptoms, and accordingly are currently the two only approved drugs by the FDA and the EMA.3,4 Therapeutic guidelines for EDS and cataplexy have been recently updated.5-7 Stimulants such as methylphenidate and amphetamines are second-line drugs for EDS in narcolepsy, because of their sympathomimetic side effects, rebound hypersomnia, abuse potential and tolerance.2,8

Most of these drugs, such as sodium oxybate, solriamfetol, and modafinil, have a psychostimulant component that is related, at least in part, to their ability to promote dopamine (DA) and norepinephrine (NE) neurotransmission,9-11 either due to inhibition of the reuptake (and/or enhanced transporter internalization or transport reversal) of DA and NE and/or stimulation of their neuronal release.12,13 Modafinil has been shown to enhance DA in various brain regions including the striatum.14,15 Sodium oxybate has also been shown to facilitate indirectly the release of DA in the nucleus accumbens.16 Solriamfetol enhances DA (and NE) neurotransmission in both in vitro assays and in vivo in the rat striatum, probably as a result of inhibition of DA and NE transporters (dopamine transporter [DAT] and norepinephrine transporter [NET]).17

Besides these drugs, the novel therapeutic class of histamine H3 receptor (H3R) inverse agonists/antagonists has been developed. The first in this class, pitolisant, has been approved by the EMA and the FDA for the treatment of EDS in narcoleptic patients with or without cataplexy.4,18 A large body of experimental data documents the role of cerebral histaminergic neurons in the maintenance of wakefulness and the modulation of several other physiological functions such as attention, learning and memory processes.19,20 Central nervous effects of histamine are mediated by the H1, H2, and H3 receptor subtypes.20,21 Among these, the H3R, first described as an autoreceptor, regulates both the synthesis and release of histamine from histaminergic neurons.22 The H3Rs display a high constitutive activity in vivo23, and inverse agonists stimulate histaminergic neurotransmission. H3Rs also function as heteroreceptors that modulate the release of various neurotransmitters, including acetylcholine, glutamate, gamma-aminobutyric acid (GABA), serotonin, and DA.21,24

The present study explored the pharmacological profile of pitolisant compared with those of other wake-promoting drugs that belong to the psychostimulant class, namely modafinil and solriamfetol. These compounds have been already characterized on their own,24,17,26 but no studies have compared their pharmacology side by side. The current approach focused on the effect of these drugs in vitro on DAT, NET, and serotonin transporter (SERT) activities, as well as in vivo on DA turnover in selected brain areas, and particularly in several behavioral animal models that are generally used to reveal psychostimulant potential.

2 | MATERIALS AND METHODS

2.1 | In vitro [35S]-GTPγS binding on the H3 histamine receptor

Human embryonic kidney (HEK293) cells that expressed the human H3 histamine receptor (NM_007232) stably were grown until confluence (37°C in a 95:5 air:CO₂ atmosphere), collected, then centrifuged at 300 g for 15 min at 4°C. Pellets were resuspended in buffer I (Tris·HCl 50 mM, MgCl₂ 10 mM, NaCl 140 mM, pH 7.4) that was supplemented by phenyl methyl sulfonyl fluoride (1 mM). The suspension was stirred gently and submitted to mechanical pressure exerted through a syringe with a 25-26G needle. The cell lysate was then centrifuged at 300 g for 5 min at 4°C to eliminate nuclei and cell debris. The resulting supernatant was then centrifuged at 48 000 g for 30 min at 4°C. The final pellet was resuspended in buffer I. The aliquots were frozen in liquid nitrogen and stored at −80°C until required. Protein content was measured by the Bradford method.27 Membranes were thawed, diluted to a final concentration of 2.5 µg protein/180 µl/well in 96-well polystyrene microplates and incubated for 30 min at room temperature with test compounds in buffer I that was supplemented with guanosine diphosphate (10 µM). Labeled [35S]-guanosine 5′-O-[(gamma-thio)] triphosphate (GTPγS) (0.2-0.3 nM, 1250 Ci/mmol, Perkin Elmer) was added for an additional 30 min. The reaction was stopped after the transfer to a Millipore GF/C HTS microplate (MSFCN6B50) by filtration of the incubation mix followed by three 250 µl washes. The filter-bound radioactivity was measured in a Microbeta TRLUX® scintillation counter after 50 µl of scintillation liquid had been added (OptiPhase SuperMix, PerkinElmer). The binding of [35S]-GTPγS with psychostimulants, for the treatment of EDS, as this agent does not show any amphetamine-like properties within in vivo preclinical models.

KEYWORDS
behavior, modafinil, neurochemistry, pitolisant, psychostimulants, rodents, solriamfetol
was determined for the reference agonist (R)-α-methylhistamine (maximal stimulation over basal set as 100%), and for the test compounds in the agonist mode, to calculate their half-maximal effective concentrations (EC₅₀). Activity below basal, that is, negative percentages, indicated inverse agonism. In the antagonist mode, the compounds were solubilized as stock solution in dimethyl sulfoxide (10⁻² M) and tested against (R)-α-methylhistamine-induced binding at its 80% maximal effective concentration (EC₅₀) (obtained at 1 μM), to calculate their half-maximal inhibitory concentrations (IC₅₀). EC₅₀ and IC₅₀ were determined with GraphPad Prism software version 7 (GraphPad Software LLC). The IC₅₀ was used to determine functional affinity of each compound by calculating the Kᵽ(Kᵽ = IC₅₀/(1 + ([Histamine]/EC₅₀ of Histamine))) according to Cheng and Prusoff.²₈

2.2 | In vitro NET and DAT activity assays

The human transporters for NET (NET or SLC6A2, #NM_001172504) and DA (DAT or SLC6A3, #NM_001044) were stably expressed in Chinese hamster ovary (CHO) cells, and that for serotonin (SERT or SLC6A4 #NM_001045) in HEK293 cells. The neurotransmitter transport activity in each expressing cell line was measured with a homogeneous fluorescence-based neurotransmitter transporter uptake assay according to the manufacturer’s recommendation (#R8173, Molecular Devices). Initial experiments were aimed at determining V_max of substrate transport at 25°C as well as K_m for each cell line. The obtained K_m were 0.5x, 1.0x, and 0.15x (x being an arbitrary unit of substrate concentration) for the NET, DAT, and SERT cell expressing lines, respectively. Then, Kᵽ for the test compounds were determined. For these purposes, cells were seeded in 96-well clear-bottom microplates (Costar #3882) at density 200 000 per well in 40 μl of Hank’s balanced salt solution buffer (Gibco #14065-049) that was supplemented with 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) (Dutschler #L0180-100), pH 7.4. The test compounds (10 μl, 10x concentration) were added for 30 min, then a 50 μl aliquot of fluorescent substrate was added (0.25x, 1x and 1x, for NET, DAT and SERT, respectively). The transport reaction was held in the dark within the initial velocity period window, that is, 3 h for NET and DAT and 5 min for SERT. Fluorescence-associated transport was measured in a functional drug screening system FDS/μCELL plate reader (Hamamatsu Photonics) at 480 nm excitation and 540 nm emission wavelengths. The IC₅₀ were determined with GraphPad Prism software version 7. Each Ki was deduced from the IC₅₀ using the following equation: Ki = IC₅₀/(1+([Substrate]/Kₘ)), according to Cheng and Prusoff.²₈

2.3 | Animals

Animals were housed in groups under a 12:12 h light/dark cycle (lights on at 7:00 a.m.) at a controlled temperature of 21 ± 2°C and humidity of 45 ± 15% with free access to food and water, except when noted below. Experiments were conducted in accordance with European ethical standards (2013/118/EEC) and approved by the local ethical committee (CEA n° 79). Male Wistar rats (220–300 g) and male Swiss mice were obtained from Janvier (Le Genest Saint-Isle, France). Male C57BL/6J and male OF1 mice were obtained from Charles River (Saint-Germain-Nuelles, France).

2.4 | Pharmacokinetics of solriamfetol and modafinil in mice

Male Swiss mice received solriamfetol (10 mg/kg, p.o., per os) or modafinil (10 mg/kg, p.o.) under a 10 ml/kg administration volume with methylcellulose (1%) as vehicle. Plasma and brain concentrations of solriamfetol and modafinil were determined through the use of liquid chromatography with tandem mass spectrometry analytical method. A generic internal standard was added to plasma and brain homogenates, and samples were extracted by use of Oasis® HLB (Waters) solid-phase extraction plates. Eluted samples were separated on an Acquity® UPLC BEHCh18 column (Waters) at 50°C using water/acetonitrile as the mobile phase (run time 3.5 min). The mass spectrometry ion source electrospray ionization was used in positive multiple reaction monitoring mode. The lower limit of quantification was 1 ng/ml for both compounds.

2.5 | In vivo DA turnover in the rat striatum

Rats received vehicle (methylcellulose 1%, 5 ml/kg, p.o.), solriamfetol (3 or 30 mg/kg, p.o.) or d-amphetamine (2 mg/kg, by intraperitoneal injection (i.p.) in saline). Sixty minutes later, they were killed by decapitation and their striata were dissected out, weighed, frozen in liquid nitrogen and stored at −80°C. In a similar experiment, the effects of pitolisant (10 mg/kg, i.p. in saline) were explored 30 and 90 min after treatment in comparison with those of d-amphetamine (2.5 mg/kg, i.p. in saline). Tissues were homogenized in 2.5 ml of a 0.4 N perchloric acid/2.7 mM ethylenediaminetetraacetic acid solution. After centrifugation (8000 rpm, 20 min, 4°C), supernatants were analyzed by use of high-pressure liquid chromatography coupled to electrochemical detection, according to the method of Ligneau et al.²⁹ Tissue concentrations of DA, DOPAC, serotonin (5-HT), and 5-hydroxyindole-3-acetic acid (5-HIAA) were determined and the corresponding ratios (DOPAC/DA and 5-HIAA/5-HT) were calculated.

2.6 | In vivo microdialysis in the rat nucleus accumbens

Anesthetized rats (chloral hydrate 400 mg/kg, i.p.) were positioned in a Kopf stereotaxic frame. A guide cannula
(CMA/12 microdialysis probe, Phymep) was implanted into the nucleus accumbens (anterior-posterior, +1.2 mm from bregma; medial-lateral, +0.18 mm; dorsal-ventral, −5.8 mm from dura) according to the atlas of Paxinos and Watson. It was secured with dental cement and anchor screws into the skull. Rats were single-housed for postoperative recovery for at least 5 days. Then, microdialysis experiments were performed as described previously by use of a CMA/12 microdialysis probe (2-mm length) to measure the effects on extracellular DA of vehicle (methylcellulose 1% in water, 5 ml/kg, p.o.), or solriamfetol (30 and 100 mg/kg, p.o.).

2.6.1 Assay of tele-methylhistamine in the brain of mice

Male OF1 mice (22–25 g) were fasted for 16 h before p.o. administration of vehicle (methylcellulose 1% in water, 10 ml/kg, p.o.) or of solriamfetol (30 and 100 mg/kg, p.o.). Ninety minutes after the treatments, animals were killed. The brain was dissected out and homogenized in 10 volumes (w/v) of ice-cold 0.4 N perchloric acid. The clear supernatant that was obtained after centrifugation (2000 g for 30 min at 4°C) was stored at −20°C before the level of tele-methylhistamine was measured by enzyme immunoassay as described previously.

2.7 Spontaneous locomotor activity in the mouse

To evaluate the effects of the drugs on spontaneous locomotor activity, C57BL/6J mice (24–26 g) were introduced individually into individual boxes (20.5 × 11.0 × 20.0 cm height; 25 lux) of an infrared detection actimeter (Metronic, Pessac, France) between 8:30 a.m. and 10:00 a.m. to measure horizontal movements. Counts of locomotor activity were incremented each time the animal moved from one half of the cage to the other and were recorded continuously for 30 min (5-min intervals), before mice received vehicle (methylcellulose 1% in water, 10 ml/kg, p.o.), pitolisant (10 or 20 mg/kg, p.o.), solriamfetol (1, 3, 10, 30, or 100 mg/kg, p.o.), modafinil (128 mg/kg, p.o.), or d-amphetamine (3 mg/kg, i.p.). Mouse locomotion was further recorded over 5.5 h.

In another set of experiments, aimed at evaluating the effects of pitolisant on solriamfetol or modafinil-induced locomotor activity, C57BL/6J mice (24–26 g) received between 8:30 a.m. and 10:00 a.m. either vehicle (methylcellulose 1% in water, 10 ml/kg, p.o.) or pitolisant (10 or 20 mg/kg, p.o.) and were then placed individually into the infrared detection actimeter to measure horizontal movements as described above. Counts of locomotor activity were recorded continuously for 30 min before mice received vehicle (methylcellulose 1% in water, 10 ml/kg, p.o.), solriamfetol (30 or 100 mg/kg, p.o.) or modafinil (64 or 128 mg/kg, p.o.) and mouse locomotion was further recorded over 5.5 h.

2.8 Behavioral drug sensitization in the rat

2.8.1 Apparatus

Rats were tested in black, wooden open fields (76 × 76 × 45 cm height) located in a dimly lit room (5 lux). A video-tracking system (Ethovision XT4.1, Noldus) enabled behavioral analyses based on center-point detection.

2.8.2 Experimental procedure

After 1-hour of habituation in the test room before each daily session, male Wistar rats (240–270 g) received vehicle (methylcellulose 1% in water, 4 ml/kg, p.o.), pitolisant (10 mg/kg, i.p. in saline), solriamfetol (60 or 100 mg/kg, p.o.), or modafinil (64 mg/kg, i.p. in cyclodextrin 30%) immediately before they were placed in the open field. Each rat was allowed five exploration sessions in an open field (always the same for each rat) between 1:00 p.m. and 5:00 p.m. on 5 successive days. Locomotor activity was recorded continuously for 40 min.

2.9 Food intake in the mouse

C57BL/6J mice (24–26 g) were single-housed in reversed cycle (12 h/12 h, lights off at 9:00 a.m.). Fifteen minutes before the lights were turned off, the mice received vehicle (methylcellulose 1% in water, 10 ml/kg, p.o.), pitolisant (10 or 20 mg/kg, p.o.), solriamfetol (30 or 100 mg/kg, p.o.), modafinil (64, 96 or 128 mg/kg, p.o.) or d-amphetamine (3 mg/kg, i.p.). Food consumption of each mouse was recorded at 1:00 p.m., i.e., 4 h after lights-off.

2.9.1 Drugs

Pitolisant (BF2.649 hydrochloride salt), solriamfetol and modafinil were obtained from Bioprojet (Paris, France), d-amphetamine and cocaine-HCl were obtained from Sigma-Aldrich (Saint Quentin Fallavier, France). Except when indicated, drug doses were expressed as free bases.

Compounds were tested at their pharmacologically active doses. For pitolisant, the doses used were those eliciting a maximal effect mediated by the H3R. For the other drugs, waking, locomotor stimulating, and/or neurotransmitter modulating doses were selected based on data from the literature. In mice, the doses were as follows: pitolisant at 10 and 20 mg/kg, p.o.,25,32 solriamfetol at 30 and 100 mg/kg, p.o.,17 modafinil at 64 and 128 mg/kg, p.o.,32–34 and d-amphetamine at 3 mg/kg, i.p.34,35 In rats, pitolisant was used at 10 mg/kg, i.p.,36 solriamfetol at 30 and 100 mg/kg, p.o.,17 modafinil at 120 mg/kg, i.p.,36–39 and d-amphetamine at 2–2.5 mg/kg, i.p.40–43
2.9.2 | Statistical analyses

Statistics were calculated through the use of GraphPad Prism 7.0 (GraphPad Software). The statistical significance of differences between experimental groups was assessed by paired Student-Fisher t-tests or analysis of variance (ANOVA), and $p < .05$ was taken as the threshold of significance.

3 | RESULTS

3.1 | In vitro affinities on the H3R, and on DAT, NET, and SERT

In the $[^{35}\text{S}]-\text{GTP} \gamma \text{S}$ functional binding assay, performed on membranes from cells that stably expressed the human H3R, pitolisant reversed the effects of (R)-$\alpha$-methylhistamine (1 $\mu$M) with a $K_b$ value of 2.2 nM (Figure 1, Table 1). Modafinil, solriamfetol and D-amphetamine were all inactive at the H3R. In the DAT, NET and SERT functional transporter assays, indatraline, which was used as a reference inhibitor, had Ki values of 2.7, 1.2, and 7.0 nM, respectively, D-amphetamine was also a potent inhibitor with Ki values of 140 nM for DAT and 12 nM for NET, but had low affinity for SERT (8660 nM). The other compounds were either inactive or poor inhibitors, with Ki values in the micromolar range. Their order of inhibition potency was ranked as, for DAT: modafinil > solriamfetol > pitolisant, for NET: pitolisant > solriamfetol > modafinil (inactive); and for SERT: pitolisant > solriamfetol = modafinil (inactive).

3.2 | In vivo

3.2.1 | Pharmacokinetics of pitolisant, solriamfetol, and modafinil in mouse plasma and brain

Compounds were tested here at their pharmacologically active doses: pitolisant at 10 and 20 mg/kg, p.o., solriamfetol at 30 and 100 mg/kg, p.o., and modafinil at 64 and 128 mg/kg, p.o. Drug levels were measured in both plasma and brain to ascertain their main pharmacokinetics parameters, i.e., maximum concentration observed ($C_{\text{max}}$), the time at which the maximum concentration was observed ($T_{\text{max}}$), and the area under the concentration-time curve between 0 and 8 h after administration (AUC$_{0-8h}$). Both pitolisant and solriamfetol were found to be high brain-penetrating agents, with a brain/plasma AUC$_{0-8h}$ ratio of 23.5 and 9.6, respectively, whereas levels of modafinil were similar in plasma and brain. All the compounds reached a $T_{\text{max}}$ in the brain 30 min post-dose. The
corresponding \( C_{\text{max}} \) at their pharmacologically active doses (calculated from Table 2 values by linear extrapolation when needed) were between 10 000 and 20 000 ng/g (approximately 35–70 µM) for pitolisant, 12 000 and 40 000 ng/g (–60–200 µM) for solriamfetol, and 6000 and 12 000 ng/g (–22–45 µM) for modafinil.

3.2.2 Effects of drugs on DA and serotonin biomarkers in the rat striatum

\( \alpha \)-amphetamine administration produced a decrease in the DOPAC/DA ratio in the rat striatum that varied from 30%, measured 60 min after a 2 mg/kg, i.p. injection, to 51% 30 min after a 2.5 mg/kg, i.p. injection (Figure 2). This decrease resulted from a diminution of DOPAC (–15%) together with an increase in DA (±25%). Treatment with solriamfetol at 30 mg/kg, p.o. significantly decreased the DOPAC/DA ratio by 14% (but not at 3 mg/kg, p.o.) 60 min after the dose; this effect represented about 50% of that of \( \alpha \)-amphetamine (at 2 mg/kg, i.p.). This decrease mainly resulted from a diminution of DOPAC (–11%) with no significant changes in DA (±4%). By contrast, treatment with pitolisant (10 mg/kg, i.p.) caused no effect on this ratio, at either 30 or 90 min post-dosing. No effect was observed on the 5-HIAA/5-HT ratio in the rat striatum 60 min after dosage of \( \alpha \)-amphetamine.

### TABLE 1

| Affinities of pitolisant, solriamfetol, modafinil, \( \alpha \)-amphetamine and indatraline to the human histamine H3 receptor and to the human dopamine, norepinephrine and serotonin transporters (DAT, NET, and SERT) in in vitro assays (data from Figure 1) |
| --- |
| hH3 receptor \( K_b \) (nM) | hDAT \( K_i \) (nM) | hNET \( K_i \) (nM) | hSERT \( K_i \) (nM) |
| Pitolisant | 2.2 | 4100 | 2500 | 5790 |
| Solriamfetol | >10 000 | 2400 | 4800 | >10 000 |
| Modafinil | >10 000 | 1800 | >10 000 | >10 000 |
| \( \alpha \)-amphetamine | >10 000 | 140 | 12 | 8660 |

Indatraline was used as a reference compound, and \( K_i \) values obtained in our assays were 2.7, 1.2, and 7.0 nM, for DAT, NET, and SERT, respectively.

### TABLE 2

| Pharmacokinetic parameters of pitolisant, solriamfetol, and modafinil in male Swiss mice. Mean ± SEM of 3 mice |
| --- |
| Plasma | Brain | Brain/plasma AUC ratio |
| --- | --- | --- |
| | C\(_{\text{max}}\) (ng/ml) | \( T_{\text{max}} \) (h) | AUC\(_{0-8h}\) (ng/ml × h) | C\(_{\text{max}}\) (ng/g) | \( T_{\text{max}} \) (h) | AUC\(_{0-8h}\) (ng/g × h) | |
| Pitolisant | 441 ± 69 (1.5 µM) | 0.5 | 1475 | 10 346 ± 1.099 (35 µM) | 0.5 | 34 686 | 23.5 |
| 10 mg/kg, p.o. | Solriamfetol | 731 ± 104 (3.7 µM) | 0.5 | 1150 | 3935 ± 452 (20 µM) | 0.5 | 10 990 | 9.6 |
| 10 mg/kg, p.o. | Modafinil | 953 ± 144 (3.5 µM) | 1.5 | 2162 | 965 ± 156 (3.5 µM) | 0.5 | 2298 | 1.1 |
| 10 mg/kg, p.o. | *Data from Ligneau et al.* 29 |

\( * \) Data from Ligneau et al. 29

### FIGURE 2

DOPAC/DA ratio in the rat striatum. Effect of solriamfetol (3 or 30 mg/kg, p.o.) and \( \alpha \)-amphetamine (2 mg/kg, i.p.) 60 min after dosing (left), or of pitolisant (10 mg/kg, i.p.), and \( \alpha \)-amphetamine (2.5 mg/kg, i.p.) 30 and 90 min after dosing (right). Mean ± SEM of 11–16 rats. Statistics: ANOVA \( F(3,42) = 15.24, p < .001 \) (left); ANOVA \( F(2,33) = 68.51, p < .001 \) (right, 30 min); ANOVA \( F(2,45) = 21.02, p < .001 \) (right, 90 min), with Dunnett’s post-hoc multiple comparisons test: *\( p < .05 \); **\( p < .01 \) versus vehicle.
3.2.3 Effects of drugs on extracellular DA in the nucleus accumbens of freely moving rats

Extracellular levels of DA in the nucleus accumbens were increased after solriamfetol administration at 100 mg/kg, p.o. (+138% of basal levels 30 min after administration) while no effects were observed after administration of the lower dose (30 mg/kg, p.o.). Consequently, the area under the curve (calculated during the 90 min post-administration period) of the 100 mg/kg, p.o. solriamfetol group was significantly enhanced by 123% (p < .05) (Figure 3).

3.2.4 Effects of drugs on tele-methylhistamine level in the brains of mice

Solriamfetol (30 and 100 mg/kg, p.o.) caused no significant effect on tele-methylhistamine levels in the brain of mice (not shown).

3.2.5 Effects of drugs on locomotor activity in mice, and on behavioral drug sensitization induced by repeated drug administration in rats

Both α-ampetamine (3 mg/kg, i.p.) and modafinil (128 mg/kg, p.o.) stimulated the cumulative locomotor activity that was recorded over 5.5 h post-dosing by 9-fold as compared with control mice (Figure 4). Treatment with solriamfetol at a dose of 100 mg/kg, p.o. also significantly stimulated the locomotion by fivefold as compared with control mice, but the 30 mg/kg, p.o. dose had no effect. By contrast, administration of pitolisant (10 and 20 mg/kg, p.o.) caused no significant effect by itself. In these conditions, pitolisant (20 mg/kg, p.o.) reduced the activity that was induced by 30 and 100 mg/kg p.o. doses of solriamfetol; administration of pitolisant fully normalized the hyperlocomotion that was induced by the 30 mg/kg dose of solriamfetol and it significantly reduced (by 68%) that induced by the 100 mg/kg dose (Figure 5A). Likewise, pitolisant (20 mg/kg, p.o.) reduced significantly (by 58%) the modafinil-induced hyperlocomotion (64 mg/kg modafinil, p.o.) (Figure 5B). The highest dose of modafinil that was tested, 128 mg/kg, p.o., caused less hyperlocomotion than the 64 mg/kg dose did, and this was not further reduced by pitolisant.
In rats, pitolisant (10 mg/kg, i.p.) had no hyperlocomotion effects either upon acute or after 5-day-repeated administrations (Figure 6). In contrast, administration of modafinil (64 mg/kg, i.p.) significantly increased rat locomotor activity after acute administration (+145% versus controls, \( p < .05 \)) and this was further enhanced steadily from day to day, reaching a plateau at day 4; by the fifth day of repeated administration, locomotion was strongly stimulated by 419% compared with control rats \( (p < .001) \) and by 53% versus modafinil-treated rats at day 1 \( (p < .05) \). Administration of solriamfetol at the dose of 60 mg/kg, p.o., caused no significant locomotion

**Figure 4** Spontaneous locomotor activity in the mouse. Effect of pitolisant (10 or 20 mg/kg, p.o.), solriamfetol, 10, 30 or 100 mg/kg, p.o., modafinil (128 mg/kg, p.o.) or d-amphetamine (3 mg/kg, i.p.). Time-course of locomotor effects (left) and cumulated locomotor activity over the 30–360 min period (right). Mean ± SEM of 8–16 mice. ANOVA provides \( F(9, 125) = 18.14 \) and \( p < .001 \), followed by a Fisher’s LSD test: *** \( p < .001 \) versus vehicle

**Figure 5** Drug-induced locomotor activity in mice is mitigated by pitolisant. (A) Effect of pitolisant (20 mg/kg, p.o.) on solriamfetol (30 or 100 mg/kg, p.o.)-induced locomotor activity. Time-course of locomotor effects (left) and cumulative locomotor activity over the 30–360 min period post solriamfetol dosing (right). Mean ± SEM of 12–13 mice. ANOVA provides \( F(4, 59) = 7.738 \) and \( p < .001 \), followed by a Fisher’s LSD test: * \( p < .05 \); *** \( p < .001 \) versus vehicle; $ \( p < .05 \), and $$$ \( p < .001 \) versus corresponding solriamfetol groups. (B) Effect of pitolisant (20 mg/kg, p.o.) on modafinil (64 or 128 mg/kg, p.o.)-induced locomotor activity. Mean ± SEM of 16 mice. ANOVA provides \( F(4, 75) = 13.66 \) and \( p < .001 \), followed by a Fisher’s LSD test: *** \( p < .001 \) versus vehicle; $$$ \( p < .001 \) versus corresponding modafinil groups.
and was, by day 5, increased by 185% versus controls (p < .001), and by 106% when compared with corresponding solriamfetol-treated rats at day 1 (p < .001).

3.2.6 | Effects of drugs on food intake in mice

In mice, administration of pitolisant (10 and 20 mg/kg, p.o.) did not modify the food intake during the initial 4 h of the night phase (Figure 7). By contrast, solriamfetol at 30 and 100 mg/kg, p.o., inhibited food intake by 25% and 40%, respectively. Likewise, administration of modafinil caused a dose-dependent reduction in food intake by 40%, 76%, and 96% at the doses of 64, 96, and 128 mg/kg, p.o.

Hypophagic effects of α-amphetamine (3 mg/kg, i.p.) were shown, with a 56% decrease in food consumption compared with controls.

4 | DISCUSSION

Psychostimulants are a broad class of sympathomimetic drugs, the effects of which can include increased locomotion, arousal, vigilance, anorexia, vigor, wakefulness, and attention. They comprise drugs of abuse, such as cocaine and methamphetamine, and therapeutic drugs such as α-amphetamine, methylphenidate, modafinil, and solriamfetol. Currently, therapeutic options to treat EDS associated with narcolepsy or OSA include the wake-promoting agents pitolisant, modafinil and solriamfetol. However, there are a limited number of clinical studies comparing safety and efficacy of these drugs.

Therefore, the current study was aimed at evaluating these compounds preclinically. The present in vitro and in vivo comparisons show that α-amphetamine, modafinil, and solriamfetol cause DA extracellular levels in the nucleus accumbens, hyperlocomotion, behavioral sensitization and hypophagia, which are common features of psychostimulants and drug-abuse potential, whereas pitolisant was devoid of such effects.

Most of these drugs such as amphetamine-like drugs (α-amphetamine, methylphenidate), solriamfetol, and modafinil have a psychostimulant component related, at least in part, to their ability to promote the DA and NE neurotransmission.

All the psychostimulant-related compounds that were tested here enhanced DA neurotransmission in the striatum and more importantly in the nucleus accumbens, a key region for addiction. Modafinil, whose mode of action is not yet fully elucidated, modulates several transmitters in the central nervous system, including DA, NE, 5-HT, glutamate and GABA. In particular, modafinil has been reported to increase levels of extracellular DA in the monkey striatum, in the nucleus accumbens in rodents and in humans, which is a feature of drugs of abuse. The increase in the rat nucleus accumbens was modest as compared with that elicited by α-amphetamine. Similarly, solriamfetol significantly increased levels of extracellular DA in the rat nucleus accumbens (Figure 3) in agreement with effects reported in the rat striatum and its DAT inhibitory potency (Table 1). These data were consistent with the decrease in the DOPAC/DA ratio that was found in the striata of rats receiving solriamfetol or α-amphetamine and in the nucleus accumbens of rats treated with modafinil. For α-amphetamine, the decrease in the DOPAC/DA ratio in the striatum resulted from both an enhanced DA release and a decreased DA uptake due to the DAT
blockade, and/or its internalization or transport reversal. For solriamfetol and modafinil, the decrease in DOPAC/DA ratio resulted mainly from DAT inhibition. The decrease in the DOPAC/DA ratio in the striatum showed a gradation: d-amphetamine (2 mg/kg, i.p.) > modafinil (64 mg/kg, i.p.) > solriamfetol (3 and 30 mg/kg, p.o.) (Figure 2). In contrast, pitolisant had no effect on the DOPAC/DA ratio (Figure 2) or the levels of extracellular DA in the rat nucleus accumbens as reported for other H3R antagonists. The NE turnover in the cortex and/or prefrontal cortex of rodents is enhanced by d-amphetamine, solriamfetol, and modafinil in line with their in vitro affinities to NET (Table 1). For pitolisant, similar NE cortical effects were observed, presumably attributable to its nanomolar potency in blocking the H3 histamine heteroreceptor rather than to its micromolar affinity towards the NET (Table 1).

No major effects on serotonin turnover have been reported in the mouse prefrontal cortex for pitolisant in either the rat prefrontal cortex or the striatum for modafinil or here in the rat striatum for solriamfetol, d-amphetamine or pitolisant (data not shown). These results agree with the lack of affinities for SERT for these compounds (Table 1).

When considering its primary target (H3R), pitolisant showed a K<sub>ll</sub> value of 2.2 nM in the functional [35S]-GTPyS binding assay (Table 1), which agreed with affinities already reported. Modafinil, solriamfetol and d-amphetamine were inactive at the H3R while ciproxifan and ABT-239 were selective and potent H3R antagonist/inverse agonist, enhanced in vivo the brain tele-methylhistamine level (a reliable index of histaminergic neurotransmission) with an ED<sub>50</sub> value of 1.6 mg/kg, p.o., in mice, whereas modafinil was less potent and solriamfetol showed no effect (not shown).

By enhancing the histaminergic neurotransmission, pitolisant promotes wakefulness, vigilance and attention in rodents and cats and in orexin<sup>-/-</sup> mice, a unique model of narcolepsy. It did this without stimulating locomotion. This lack of locomotion effect has been reported for other H3R antagonists such as thioperamide, ciproxifan and ABT-239. In contrast, the wake-promoting agents modafinil, solriamfetol and d-amphetamine elicited hyperlocomotion in rodents (Figure 4), as already reported for modafinil in rats and mice, for solriamfetol in mice, and for d-amphetamine. Interestingly, pitolisant could prevent both solriamfetol-induced and modafinil-induced hyperlocomotion in mice (Figure 5), as already described for cocaine- and methamphetamine-induced hyperlocomotion. The mechanisms by which pitolisant reduced drug-induced hyperlocomotion in mice remains to be established, for example, by exploring a larger set of doses of compounds and their corresponding pharmacokinetics to evaluate any contribution of potential drug–drug interactions to pharmacodynamics. Behavioral locomotor sensitization due to repeated drug exposure is a known feature of drugs of abuse. This phenomenon, described for d-amphetamine and modafinil, occurred to some extent with solriamfetol (Figure 6), whereas it was absent for the two H3R antagonists/inverse agonists, pitolisant, and ABT-288.

Hypophagy is another well-known feature of psychostimulants such as d-amphetamine. Both modafinil and solriamfetol reduced food intake in mice (Figure 7) as reported with modafinil in rats and in humans. The potential role of central histamine and the H3R in the control of food intake and body weight is debated (reviewed in ref. 65). The H3R antagonist A331440 reduced long-term food consumption and had anti-obesity effects in mice fed a high-fat diet. Similar results were obtained with the H3R antagonist NNC 38-1049 in diet-induced obesity in rats. Here, in regular conditions, acute pitolisant showed no effects on standard food intake in normal mice, in contrast with psychostimulants. These preclinical data agree with the decreased appetite reported clinically and specified in product labels as a common adverse effect for modafinil and solriamfetol, and uncommon for pitolisant.

All these in vivo data were obtained in rodents at doses of the compounds eliciting brain concentrations above those of their respective target's Ki values, thus allowing a maximal pharmacodynamic effect. Corresponding plasma exposures in mice (calculated from C<sub>5000</sub> to 10,000 ng/ml at 200 mg/kg) hence, caution should be taken before any extrapolation of these data to humans as the behavioral results reported here in rodents were obtained at drug exposures above those reached in human at therapeutic doses for solriamfetol (~820 ng/ml at 150 mg/kg) pitolisant (~36 ng/ml at 40 mg/kg) and modafinil (~5000 to 10,000 ng/ml at 200 mg/kg) Hence, caution should be taken before any extrapolation of these data to humans as the behavioral results reported here in rodents were obtained at drug exposures above those reached in human at therapeutic doses for solriamfetol (ratio mouse/human C<sub>5000</sub> of 2.7 and 9), pitolisant (ratio mouse/human C<sub>5000</sub> of 25), and in the human range for modafinil (ratio mouse/human C<sub>5000</sub> of ~1). The comparison of drug exposure between species would also need to be refined based on affinities of the compounds at rodent receptors/transporters targets, brain-to-plasma drug ratios, as well as on the effective unbound free drug brain concentrations.

Altogether, these data demonstrate that pitolisant possesses a neurochemical profile and causes behavioral effects strongly different from those of modafinil, solriamfetol, and d-amphetamine which all share to various extents some common behavioral features of psychostimulants such as hyperlocomotor effects, locomotor sensitization and reduction in food intake. Furthermore, the potential lack of drug-abuse liability for the H3R antagonist class of drug is supported by preclinical in vitro and in vivo investigations performed with ABT-288 and with pitolisant in rodents and primates. This was confirmed in clinics, where pitolisant demonstrated a significantly lower potential for abuse compared with phentermine and had an overall profile similar to placebo in non-dependent recreational stimulant users. The EMA and FDA apply no restricted use recommendations to it. The two other wake-promoting drugs, modafinil and solriamfetol have different clinical profiles with regard to their drug abuse liability, solriamfetol has abuse potential similar to or lower than that of phentermine, and modafinil has a limited potential for large-scale abuse. The US Drug Enforcement Administration (DEA) classifies these as Schedule IV drugs.

In conclusion, the present study has evaluated and compared, for the first time in preclinical in vitro and in vivo models, wake-promoting agents currently used in therapeutic settings. Data confirmed their mode of action in vitro on the monoamine transporters (for modafinil, solriamfetol and d-amphetamine) or the H3R (for pitolisant). In vivo, modafinil, solriamfetol, and d-amphetamine...
behave as psychostimulants, inducing locomotion and/or behavioral sensitization, and increasing DA extracellular levels in the nucleus accumbens. These results are in line with preclinical and clinical abuse liability signals that have led the DEA to classify them as scheduled II (o-amphetamine) and IV (modafinil and solriamfetol) agents. Pitolisant, by contrast, is devoid of psychostimulant effects or drug abuse potential. Pitolisant, a wake-promoting medicine that reduces occurrence of EDS and cataplexy attacks in narcoleptic patients, is a first-in-class H3R antagonist/inverse agonist with pharmacological, biochemical and behavioral properties strongly differentiated from those of the psychostimulants currently available.

ACKNOWLEDGEMENT
The excellent technical assistance of Pauline Borne for pharmacokinetic experiments is acknowledged. This research was funded by Bioprojet-Biotech.

DISCLOSURE
SK, IBB, IN, MG, SB, DP, MU, PR, OF, and XL are paid employees of Bioprojet-Biotech. JML and JCS are shareholders of Bioprojet. The authors declare that they have no conflict of interest. The funding sources did not influence design and conduct of the study; collection, management, analysis and interpretation of the data and preparation, review or approval of the manuscript.

AUTHOR CONTRIBUTIONS
Krief, Berrebi-Bertrand, Ligneau, Schwartz, and Lecomte participated in research design. Krief, Nagmar, Giret, Belliard, Perrin, Uguen, and Ligneau conducted experiments. Krief, Berrebi-Bertrand, Robert, Perrin, Uguen, and Ligneau performed data analysis. Krief, Berrebi-Bertrand, Ligneau, Schwartz, and Finance wrote or contributed to the writing of the manuscript.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID
Stéphane Krief https://orcid.org/0000-0002-5543-731X
Xavier Ligneau https://orcid.org/0000-0003-3097-4482

REFERENCES
1. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5(TM)). American Psychiatric Publishing; 2013.
2. Takenoshita S, Nishino S. Pharmacologic management of excessive daytime sleepiness. Sleep Med Clin. 2020;15:177-194.
3. Syed YY. Pitolisant: first global approval. Drugs. 2016;76:1313-1318.
4. Thorpy MJ. Recently approved and upcoming treatments for narcolepsy. CNS Drugs. 2020;34:9-27.
5. Bassetti CLA, Callweit U, Vignatelli L, et al. European guideline and expert statements on the management of narcolepsy in adults and children. J Sleep Res. 2021. ahead of print. https://doi.org/10.1111/jsr.13387
6. Franceschini C, Pizza F, Cavalli F, Plazzi G. A practical guide to the pharmacological and behavioral therapy of narcolepsy. Neurotherapeutics. 2021;18:6-19.
7. Gandhi KD, Mansukhani MP, Silber MH, Kolla BP (2021) Excessive daytime sleepiness: a clinical review. Mayo Clin Proc 96:1288-1301.
8. Abad VC. Profile of solriamfetol in the management of excessive daytime sleepiness associated with narcolepsy or obstructive sleep apnea: focus on patient selection and perspectives. Nat Sci Sleep. 2021;13:75-91.
9. Koob GF, Volkow ND. Neurocircuitry of addiction. Neuropsychopharmacology. 2010;35:217-238.
10. Volkow ND, Wise RA, Balzer R. The dopamine motive system: implications for drug and food addiction. Nat Rev Neurosci. 2017;18:741-752.
11. Foster SL, Weinschenker D. (2019) Chapter 15 - the role of nor-epinephrine in drug addiction: past, present, and future. In: Torregrossa M (Ed.), Neural Mechanisms of Addiction. Academic Press. pp. 221-236.
12. Robertson SD, Matthies HJG, Galli A. A closer look at amphetamine-induced reverse transport and trafficking of the dopamine and nor-epinephrine transporters. Mol Neurobiol. 2009;39:73-80.
13. Schmitt KC, Rothman RB, Reith MEA. Nonclassical pharmacology of the dopamine transporter: atypical inhibitors, allosteric modulators, and partial substrates. J Pharmacol Exp Ther. 2013;346:2-10.
14. Dopheide MM, Morgan RE, Rodvelt KR, Schachtman TR, Miller DK. Modafinil evokes striatal [3H]dopamine release and alters the subjective properties of stimulants. Eur J Pharmacol. 2007;568:112-123.
15. Volkow ND, Fowler JS, Logan J, et al. Effects of modafinil on dopamine and dopamine transporters in the male human brain: clinical implications. JAMA. 2009;301:1148-1154.
16. Kamal RM, van Noorden MS, Franek E, Dijkstra BAG, Loonen AJM, De Jong CAJ. The neurobiological mechanisms of gamma-hydroxybutyrate dependence and withdrawal and their clinical relevance: a review. Neuropsychopharmacology. 2016;73:65-80.
17. Baladi MG, Forster MJ, Gatch MB, et al. Characterization of the neurochemical and behavioral effects of solriamfetol (JSP-110), a selective dopamine and norepinephrine reuptake inhibitor. J Pharmacol Exp Ther. 2018;366:367-376.
18. Kollb-Sielecka M, Demolis P, Emmerich J, Markey G, Salmonson T, Haas M. The European Medicines Agency review of pitolisant for treatment of narcolepsy: summary of the scientific assessment by the Committee for Medicinal Products for Human Use. Sleep Med. 2017;33:125-129.
19. Schwartz JC, Arrang JM, Garbarg M, Pollard H, Ruat M. Histaminergic transmission in the mammalian brain. Physiol Rev. 1991;71:1-51.
20. Haas HL, Sergeeva OA, Selbach O. Histamine in the nervous system. Physiol Rev. 2008;88:1183-1241.
21. Panula P, Chazot PL, Cowart M, et al. International union of basic and clinical pharmacology. XCVIII. Histamine receptors. Pharmacol Rev. 2015;67:601-655.
22. Arrang JM, Garbarg M, Schwartz JC. Auto-inhibition of brain histamine release mediated by a novel class (H3) of histamine receptor. Nature. 1983;302:832-837.
23. Morisset S, Rouleau A, Ligneau X, et al. High constitutive activity of native H3 receptors regulates histamine neurons in brain. Nature. 2000;408:860–864.
24. Schlücker E, Kathmann M. Role of the histamine H3 receptor in the central nervous system. Handb Exp Pharmacol. 241. Springer; 2016;277-299.
25. LigneauX,PerrinD,LandaIsL.etal.BF2.649[1-3-[3-[4-Chlorophenyl] propoxy]propyl]piperidine, hydrochloride, a nonimidazoline inverse agonist/antagonist at the human histamine H3 receptor: preclinical pharmacology. J Pharmacol Exp Ther. 2007;320:365-375.
26. Minzenberg MJ, Carter CS. Modafinil: a review of neurochemical actions and effects on cognition. Neuropsychopharmacology. 2008;33:1477-1502.
27. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 1976;72:248-254.
28. Cheng Y, Prusoff WH. Relationship between the inhibition constant (K1) and the concentration of inhibitor which causes 50 percent inhibition (IS0) of an enzymatic reaction. Biochem Pharmacol. 1973;22:3099-3108.
29. Ligneau X, Landais L, Perrin D, et al. Brain histamine and schizophrenia: potential therapeutic applications of H3-receptor inverse agonists studied with BF2.649. Biochem Pharmacol. 2007;73:1215-1224.
30. Paxinos G, Watson C. The Rat Brain in Stereotaxic Coordinates. 4th ed. Academic Press; 1998:1-237.
31. Ligneau X, Lin J, Vanni-Mercier G, et al. Neurochemical and behavioral effects of ciproxifan, a potent histamine H3 receptor antagonist. J Pharmacol Exp Ther. 1998;287:658–666.
32. Lin J-S, Dauvilliers Y, Arnulf I, et al. An inverse agonist of the histamine H(3) receptor improves wakefulness in narcolepsy: studies in orexin-/– mice and patients. Neurobiol Dis. 2008;30:74-83.
33. Willie JT, Renthal W, Chemelli RM, et al. Modafinil more effectively induces wakefulness in orexin-null mice than in wild-type littermates. Neuroscience. 2005;130:983-995.
34. Paterson NE, Fedolak A, Olivier B, Hanania T, Caldarone B. Psychostimulant-like discriminative stimulus and locomotor sensitization properties of the wake-promoting agent modafinil in rodents. Pharmacol Biochem Behav. 2010;95:449-456.
35. Chen R, Zhang M, Park S, Gnegy ME. C57BL/6J mice show greater amphetamine-induced locomotor activation and dopamine efflux in the striatum than 12952/SvHsd mice. Pharmacol Biochem Behav. 2007;87:158-163.
36. Uguen M, Perrin D, Bellard S, et al. Preclinical evaluation of the abuse potential of Pitolisant, a histamine H3 receptor inverse agonist/antagonist compared with Modafinil. Br J Pharmacol. 2013;169:632-644.
37. Touret M, Sallan-Moulin M, Jouvet M. Awakening properties of modafinil without paradoxical sleep rebound: comparative study with amphetamine in the rat. Neurosci Lett. 1995;189:43-46.
38. Mereu M, Chun LE, Prisinzano TE, Newman AH, Katz JL, Tanda G. The unique psychostimulant profile of (S)-modafinil: investigation of behavioral and neurochemical effects in mice. Eur J Neurosci. 2017;45:167-174.
39. Alam N, Chaudhary K. Repeated restraint stress potentiates methylenidate and modafinil-induced behavioral sensitization in rats. Naunyn Schmiedebergs Arch Pharmacol. 2020;393:785-795.
40. Robinson TE. Behavioral sensitization: characterization of enduring changes in rotational behavior produced by intermittent injections of amphetamine in male and female rats. Psychopharmacology. 1984;84:466-475.
41. Di Chiara G, Imperato A. Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. Proc Natl Acad Sci USA. 1988;85:5274-5278.
42. Ferraro L, Antonelli T, O’Connor WT, Tanganelli S, Rambert FA, Fuxe K. Modafinil: an antinarcotic drug with a different neurochemical profile to D-amphetamine and dopamine uptake blockers. Biol Psychiatry. 1997;42:1181-1183.
43. Vanderschuren LJ, Schoffelmeer ANM, Mulder AH, De Vries TJ. Dopaminergic mechanisms mediating the long-term expression of locomotor sensitization following pre-exposure to morphine or amphetamine. Psychopharmacology. 1999;143:244-253.
44. Hyttel J, Larsen JJ. Neurochemical profile of Lu 19–005, a potent inhibitor of uptake of dopamine, noradrenaline, and serotonin. J Neurochem. 1985;44:1615-1622.
45. Berridge CW. Neural substrates of psychostimulant-induced arousal. Neuropsychopharmacology. 2006;31(11):2332-2340.
46. Wood S, Sage JR, Shuman T, Anagnostaras SG. Psychostimulants and cognition: a continuum of behavioral and cognitive activation. Pharmacol Rev 2014; 66:193–221.
47. Dauvilliers Y, Bassetti C, Lammers GJ, et al. Pitolisant versus placebo or modafinil in patients with narcolepsy: a double-blind, randomised trial. Lancet Neurology. 2013;12:1068-1075.
48. Andersen ML, Kessler E, Murakane KS, McClung JC, Tufik S, Howell LL. Dopamine transporter-related effects of modafinil in rhesus monkeys. Psychopharmacology. 2010;210:439-448.
49. Zolowska D, Jain R, Rothman RB, et al. Evidence for the involvement of dopamine transporters in behavioral stimulant effects of modafinil. J Pharmacol Exp Ther. 2009;329:738-746.
50. Jones SR, Gaintinov RR, Wightman RM, Caron MG. Mechanisms of amphetamine action revealed in mice lacking the dopamine transporter. J Neurosci. 1998;18:1979-1986.
51. Fox GB, Ensheshade TA, Pan JB, et al. Pharmacological properties of ABT-239 [4-[(2R)-2-Methylpyrrolidinyl]ethyl-benzofuran-5-yl]benzonitrile]: II. Neuropharmacological characterization and broad preclinical efficacy in cognition and schizophrenia of a potent and selective histamine H3 receptor antagonist. J Pharmacol Exp Ther. 2005;313:176-190.
52. Ensheshade TA, Brownman KE, Miller TR, et al. Pharmacological properties and proognitive effects of ABT-288, a potent and selective histamine H3 receptor antagonist. J Pharmacol Exp Ther. 2012;343:233-245.
53. Tanda G, Pontieri FE, Frau R, Chiara GD. Contribution of blockade of the noradrenaline carrier to the increase of extracellular dopamine in the rat prefrontal cortex by amphetamine and cocaine. Eur J Neurosci. 1997;9:2077-2085.
54. Rowley HL, Kulkarni RS, Gosden J, Brammer RJ, Hackett D, Heal DJ. Differences in the neurochemical and behavioural profiles of lis-dexamfetamine methylenidate and modafinil revealed by simultaneous dual probe microdialysis and locomotor activity measurements in freely-moving rats. J Psychopharmacology. 2014;28(3):254-269.
55. Garbag M, Tuong MD, Gros C, Schwartz JC. Effects of histamine H3-receptor ligands on various biochemical indices of histaminergic nervous activity in rat brain. Eur J Pharmacol. 1989;164:1-11.
56. Clopham J, Kilpatrick JG, Thioperamide, the selective histamine H3 receptor antagonist, attenuates stimulant-induced locomotor activity in the mouse. Eur J Pharmacol. 1994;259:107-111.
57. Pillot C, Ortiz J, Héron A, Ridray S, Schwartz J-C, Arrang J-M. Ciproxifan, a histamine H3-receptor antagonist/inverse agonist, potentiates neurochemical and behavioral effects of haloperidol in the rat. J Neurosci Off J Soc Neurosci. 2002;22:7272-7280.
58. Simon P, Hémet C, Costentin J. Analysis of stimulant locomotor effects of modafinil in various strains of mice and rats. Fund Clin Pharmacol. 1996;10:431-435.
59. Giros B, Jaber M, Jones SR, Wightman RM, Caron MG. Hyperlocomotion and indifference to cocaine and amphetamine in mice lacking the dopamine transporter. Nature. 1996;379:606-612.
60. Kitanaka J, Kitanaka N, Hall FS, et al. In vivo evaluation of effects of histamine H3 receptor antagonists on methamphetamine-induced hyperlocomotion in mice. Brain Res. 2020;1740:146873.
61. Kalivas PW, Sorg BA, Hooks MS. The pharmacology and neural circuitry of sensitization to psychostimulants. Behav Pharmacol. 1993;4:315-334.
62. Stekete JD, Kalivas PW. Drug wanting: behavioral sensitization and relapse to drug-seeking behavior. Pharmacol Rev. 2011;63:348-365.
63. Wu-Oliva R, Fukushima DF, Borci AR, et al. Addictive potential of modafinil and cross-sensitization with cocaine: a pre-clinical study. Addict Biol. 2011;16:565-579.
64. Hudzik TJ, Basso A, Boyce-Rustay JM, et al. Assessment of the abuse liability of ABT-288, a novel histamine H3 receptor antagonist. Psychopharmacology. 2013;228(2):187-197.
65. Nicolaidis S, De Saint HZ. Nonamphetamine awakening agent modafinil induces feeding changes in the rat. Brain Res Bull. 1993;32:87-90.
66. Makris AP, Rush CR, Frederick RC, Kelly TH. Wake-promoting agents with different mechanisms of action: comparison of effects of modafinil and amphetamine on food intake and cardiovascular activity. Appetite. 2004;42:185-195.
67. Perez GA, Haney M, Foltin RW, Hart CL. Modafinil decreases food intake in humans subjected to simulated shift work. Pharmacol Biochem Behav. 2008;90:717-722.
68. Diaz NF, Flores-Herrera H, García-López G, Molina-Hernández A. Central histamine, the H3-receptor and obesity therapy. CNS Neurol Disord Drug Targets. 2019;18:516-522.
69. Hancock AA, Bennani YL, Bush EN, et al. Antiobesity effects of A-331440, a novel non-imidazole histamine H3 receptor antagonist. Eur J Pharmacol. 2004;487:183-197.
70. Malmöf K, Zaragoza F, Golozoubova V, et al. Influence of a selective histamine H3 receptor antagonist on hypothalamic neural activity, food intake and body weight. Int J Obes. 2005;29:1402-1412.
71. Zomorodi K, Kankam M, Lu Y. A Phase I, randomized, crossover, open-label study of the pharmacokinetics of solriamfetol (JZP-110) in healthy adult subjects with and without food. Clin Ther. 2019;41:196-204.
72. Lecendreux M, Plazzi G, Franco P, et al. Pharmacokinetics of pitolisant in children and adolescents with narcolepsy. Sleep Med. 2020;66:220-226.
73. Robertson P, Hellriegel ET. Clinical pharmacokinetic profile of modafinil. Clin Pharmacokinet. 2003;42(2):123-137.
74. Setnik B, McDonnell M, Mills C, et al. Evaluation of the abuse potential of pitolisant, a selective H3-receptor antagonist/inverse agonist, for the treatment of adult patients with narcolepsy with or without cataplexy. Sleep. 2020;43.
75. Carter LP, Henningfield JE, Wang YG, et al. A randomized, double-blind, placebo-controlled, crossover study to evaluate the human abuse liability of solriamfetol, a selective dopamine and norepinephrine reuptake inhibitor. J Psychopharmacol Oxf Engl. 2018;32:1351-1361.
76. Jasinski DR, Kovacević-Ristanović R. Evaluation of the abuse liability of modafinil and other drugs for excessive daytime sleepiness associated with narcolepsy. Clin Neuropharmacol. 2000;23:149-156.
77. Myrick H, Malcolm R, Taylor B, LaRowe S. Modafinil: preclinical, clinical, and post-marketing surveillance–a review of abuse liability issues. Ann Clin Psychiatry. 2004;16(2):101-109.