BIOCHEMICAL PHARMACOLOGY 73 (2007) 1157–1171

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The brain H_3-receptor as a novel therapeutic target for vigilance and sleep–wake disorders

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ARTICLE INFO

Article history:
Received 11 September 2006
Accepted 3 January 2007

Keywords:
Sleep–wake cycle
Histamine
H_3-receptor
Cortical EEG
Arousal
Vigilance
Sleep–wake disorders
Narcolepsy
Somnolence
Modafinil
Psychostimulant
Knockout mice

ABSTRACT

Brain histaminergic neurons play a prominent role in arousal and maintenance of wakefulness (W). H_3-receptors control the activity of histaminergic neurons through presynaptic autoinhibition. The role of H_3-receptor antagonists/inverse agonists (H_3R-antagonists) in the potential therapy of vigilance deficiency and sleep–wake disorders were studied by assessing their effects on the mouse cortical EEG and sleep–wake cycle in comparison to modafinil and classical psychostimulants. The H_3R-antagonists, thioperamide and ciproxifan increased W and cortical EEG fast rhythms and, like modafinil, but unlike amphetamine and caffeine, their waking effects were not accompanied by sleep rebound. Conversely, imetit (H_3R-agonist) enhanced slow wave sleep and dose-dependently attenuated ciproxifan-induced W, indicating that the effects of both ligands involve H_3-receptor mechanisms. Additional studies using knockout (KO) mice confirmed the essential role of H_3-receptors and histamine-mediated transmission in the wake properties of H_3R-antagonists. Thus ciproxifan produced no increase in W in either histidine-decarboxylase (HDC, histamine-synthesizing enzyme) or H_1- or H_3-receptor KO-mice whereas its waking effects persisted in H_2-receptor KO-mice. These data validate the hypothesis that H_3R-antagonists, through disinhibition of H_3-autoreceptors, enhancing synaptic histamine that in turn activates postsynaptic H_1-receptors promoting W. Interestingly amphetamine and modafinil, despite their potent arousal effects, appear unlikely to depend on histaminergic mechanism as their effects still occurred in HDC KO-mice. The present study thus distinguishes two classes of wake-improving agents: the first acting through non-histaminergic mechanisms and the second acting via histamine and supports brain H_3-receptors as potentially novel therapeutic targets for vigilance and sleep–wake disorders.

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Abbreviations: HA, histamine; HDC, histidine decarboxylase; KO, knockout; PS, paradoxical sleep; H_3R-agonist, H_3-receptor agonist; H_3R-antagonist, H_3-receptor antagonist/inverse agonist; SWS, slow wave sleep; W, wakefulness or waking; WT, wild type

0006-2952/$ – see front matter
doi:10.1016/j.bcp.2007.01.002

1. Introduction

The functional importance of histamine (HA) in sleep–wake regulation dates back to the 1930s when the prototypical antihistamine drugs were discovered. Now identified as H_1-receptor antagonists, the use of this class of drugs in the treatment of allergic diseases is frequently associated with sedation, drowsiness and slowed reaction time in humans. With the discovery, in the early 1980s, that histamine is a central neurotransmitter [1–3], it was hypothesized that blockade of histamine-mediated transmission could be responsible for these side-effects. Recent experimental data support the hypothesis that histaminergic neurons constitute a major wake-promoting system [4] within the brain arousal networks [5–9].

Histaminergic perikarya occur exclusively in the tuberomammillary nucleus (TMn) and adjacent areas of the posterior hypothalamus [10–13], a heterogeneous area crucial for waking as its destruction or inactivation induces hypersomnia [4–6]. TM neurons send inputs to various brain regions, notably those that control the sleep–wake cycle, such as the cortex, thalamus, preoptic and anterior hypothalamus, brainstem and forebrain cholinergic and monoaminergic structures [2–4,10–13]. Identified histaminergic neurons in the mouse [14] as well as presumed histaminergic cells in the cat [6,15], discharge tonically and specifically during wakefulness; this pattern of activity being the most wake-selective pattern identified in the brain to date. Histaminergic neurons stimulate or facilitate target neurons in large brain areas through postsynaptic H_1 and H_2 receptors [2,3], thus contributing to cortical activation [4]. Indeed, treatments that impair HA-mediated neurotransmission, e.g., blockade of HA synthesis or postsynaptic H_1 receptors, increase cortical slow waves and enhance sleep. In contrast, enhancement of histaminergic neurotransmission promotes waking [4,13,16,17]. Finally, Long-term abolition of HA synthesis in knockout (KO) mice impairs the cortical electroencephalogram (EEG) and has deleterious effect on both sleep and wake quality, thus causing permanent somnolence and behavioral deficits. Consequently, mice that lack brain HA are unable to remain awake when high vigilance is required, e.g. at lights off or placed in a new environment [16]. Together, these results indicate that HA-containing neurons have a key role in maintaining the brain awake under normal conditions and in the presence of behavioral challenges.

Since H_3-receptors control the release, synthesis and turnover of HA and the neuronal activity of histaminergic cells [15,18,19], it was hypothesized that the cortical activity and sleep–wake cycle could be modulated through H_3-receptor and consequently their ligands [20]. Consistent with this assumption, early studies in cats showed that sleep increased or decreased following, respectively, administration of H_3-receptor agonists or antagonist/inverse agonists. Thioperamide, an imidazole H_3R-antagonist, promoted cortical activation and waking while α-methylhistamine, a chiral H_3R-agonist and BP2-94, another H_3-receptor agonist, enhanced cortical slow activity and increased slow wave sleep [4,20]. Similar results were obtained using H_3R-agonists or antagonists in mice, rats and guinea pigs [16,21,22], although the effect of H_3R-agonists appeared to be compound- and species-dependent [23,24].

The robust effects of H_3-receptor ligands in sleep–wake control in animals supports a potential role in treating human sleep–wake disorders, notably the use of H_3R-antagonists to improve somnolence and vigilance deficiency of diverse pathophysiological origin. However, several important fundamental questions arise as regards to the characterization of their effects. For example, what are their effects on sleep–wake parameters as compared to those induced by the current wake-promoting compound modafinil [25–28] or classical psychostimulants? Is their waking effect mediated specifically by H_3-receptors and through HA-mediated neurotransmission? The latter question is particularly important as H_3-receptors also function as heteroreceptors that control the release and synthesis of other neurotransmitters in addition to HA including acetylcholine, dopamine, norepinephrine, serotonin and galanin [3,29], also involved in sleep–wake control [7–9].

In the present study, therefore, the effects of the H_3R-antagonists, thioperamide and ciproxifan, were studied on the cortical EEG and sleep–wake cycle in mouse, a species in which the effects of H_3R-ligands are less well documented, but of great interest in basic and preclinical investigations particularly because of increasing use of knockout (KO) models. The waking effects of H_3R-antagonists were compared with those induced by the atypical stimulant, modafinil and the classical psychostimulants, amphetamine and caffeine. Additionally, the pharmacological profile of ciproxifan was evaluated using pharmacological antagonism with the H_3R-agonist, imetit and in several KO mouse models in which HA-mediated neurotransmission was altered either in terms of synthesis or receptors.

2. Effects of modafinil, psychostimulants and H_3-receptor antagonists on the mouse cortical EEG and sleep–wake cycle

To compare the wake promoting effects of H_3R-antagonists versus modafinil and classical psychostimulants, C57/Black6/J genetic background mice ($n = 22$, Charles River, France) were implanted with electrodes to monitor the cortical EEG and sleep–wake cycle according to previously described methods [16]. Briefly, All mouse strains used in this study were housed individually in transparent barrels (Ø 20 cm, height 30 cm) in an insulated sound-proofed recording room maintained at an ambient temperature of $22 \pm 1\,^{\circ}C$ and on a 12 h light/dark cycle (lights-on at 7h00), food and water being available ad libitum. Polygraphic recordings were performed after administration of placebo or the drugs and scored as described [16] by 30 s epochs for wakefulness (W), slow wave sleep and paradoxical (PS or REM) sleep. Cortical EEG power spectra were analyzed for consecutive 30-s epochs within the frequency range of 0.4–60 Hz using a fast Fourier transformation routine by the CED-Spike 2 analysis system. Statistical evaluation was performed using ANOVA followed by Dunnett's *t*-test. Each animal served as its own control.

D-amphetamine (1, 4 and 8 mg/kg, Sigma, St. Louis, MO USA), caffeine (10, 30 and 100 mg/kg, Sigma), modafinil (10, 30 and 100 mg/kg, Cephalon, France), thioperamide (10, 30 and 100 mg/kg, Sigma) and ciproxifan (1, 3 and 10 mg/kg, Bioprojet)

Table 1 – Effect of wake-promoting compounds as indicated, on cumulated wake (CW) amount during 4 h and on latencies to slow wave sleep (SWS) and paradoxical sleep (PS)

Compound (mg/kg)	CW (0–4 h)	Latency to SWS	Latency to PS
Amphetamine			
1	111 (163) ± 7	55 (248) ± 8**	146 (213) ± 27**
4	197 (290) ± 7*	179 (805) ± 8**	250 (365) ± 20**
8	240 (354) ± 1**	262 (1182) ± 9**	360 (526) ± 13**
Caffeine			
10	116 (159) ± 13**	68 (278) ± 15	113 (116) ± 23
30	187 (258) ± 22**	140 (575) ± 33*	193 (197) ± 38
100	240 (331) ± 1**	424 (1737) ± 80**	754 (772) ± 87**
Modafinil			
10	68 (104) ± 11	35 (172) ± 9	58 (108) ± 9
30	93 (143) ± 18	56 (272) ± 19	108 (201) ± 18
100	205 (314) ± 20**	200 (978) ± 31**	255 (475) ± 39**
Thioperamide			
10	110 (134) ± 2	26 (261) ± 9	82 (123) ± 8
30	120 (146) ± 7*	41 (402) ± 12	86 (130) ± 7
100	164 (200) ± 22**	72 (710) ± 23**	259 (389) ± 55*
Ciproxifan			
1	102 (127) ± 12	53 (174) ± 13	91 (112) ± 6
3	126 (156) ± 14**	63 (208) ± 11	144 (177) ± 39
10	167 (208) ± 20**	96 (317) ± 30**	172 (210) ± 52**

Compounds were administered in methylcellulose 0.25% i.p. at 11.30 (light phase). The results are expressed both as the mean time (min ± S.E.M.) spent in waking (W) or to sleep onset, and as a percentage (in parentheses) obtained from the ratio of mean experimental values (compound treatment) over the mean control values (saline injection of the same animals). In the latter case, 100% indicates the control level or no change. Note that all compounds induce a dose-dependent increase in waking and concomitant delayed sleep latency (*,** $p < 0.05$ and 0.01; Dunnett's *t*-test as compared with placebo; $n = 8$).

were administered i.p. at the light phase (11 h30) when the animals slept most of the time at baseline (defined as sleeping period). As shown in Table 1 and Figs. 1 and 2, all compounds at the doses used increased the time spent awake. The wake effect, occurring as early as the first hour after dosing, was accompanied by delayed sleep latencies (Table 1; Figs. 1 and 2), the duration of the effect on waking being dose-dependant. The increase in wakefulness was at the expense of both slow wave sleep (SWS) and paradoxical sleep (PS). Compared with modafinil and psychostimulants, the effects of the two H_3R-antagonists had several characteristics:

2.1. *Prompt awakening effect*

The waking effect of H_3R-antagonists occurred quickly in the mouse and was promptly terminated, similar to that seen in the cat [4,20,30]. At a dose 10-fold higher than the minimally effective one, the waking effect was prolonged to two more hours whereas at an 8–10 fold higher dose, the waking effect of modafinil, amphetamine and caffeine was prolonged to an additional periods of 3, 4 and more than 7 h, respectively (Fig. 2). In addition to the bioavailability of H_3R-antagonists, their short-lasting effect probably reflects the fact that the HA released by H_3R-antagonists is rapidly eliminated and that there are no known transporter mechanisms involved in HA catabolism that could be affected by H_3R-antagonists. Indeed, released HA is instantly inactivated by the enzyme, histamine-*N*-methyltransferase. HA is also known to have a faster turnover rate than other neurotransmitters with the exception of acetylcholine [1,29]. H_3R-antagonists (e.g. ciproxifan) also have no interactions with monoamine transporters (data not shown). In contrast, the psychostimulant effects of amphetamine depend on an inhibition of the dopamine transporter (DAT) in addition to an enhancement of monoamine release and blockade of monoamine oxidase activity. Modafinil binds with moderate affinity to DAT (~4–7 μM) [31–33]. However, the dopaminergic mechanisms involved in the waking effect of modafinil may be relatively pronounced in the mouse [32] compared with other experimental species. The relative short lasting effect of H_3R-antagonists demonstrated here, if extrapolatable to humans, may be an advantage for their potential therapeutic use, i.e. maintaining daytime wakefulness, followed by normal nocturnal sleep.

2.2. *Quiet and alert waking*

In mice as in other species, psychostimulants such as amphetamine and caffeine markedly increased behavioral activity and locomotion in addition to EEG arousal, whereas no overt behavioral excitation occurs during wakefulness following modafinil, thioperamide and ciproxifan. Animals were quiescent for the majority of time presenting a level of activity similar to waking seen during baseline recording. The most notable difference seen in this study between H_3R-antagonists and other wake-promoting agents involved the qualitative aspect of waking, i.e., cortical EEG. Whereas all the compounds used caused a clear suppression of cortical slow waves (δ and slow θ bands, mainly 0.8–5 Hz), H_3R-antagonists like ciproxifan were distinct from other compounds due to their effect on cortical fast rhythms (β and γ bands, 20–60 Hz). Thus,

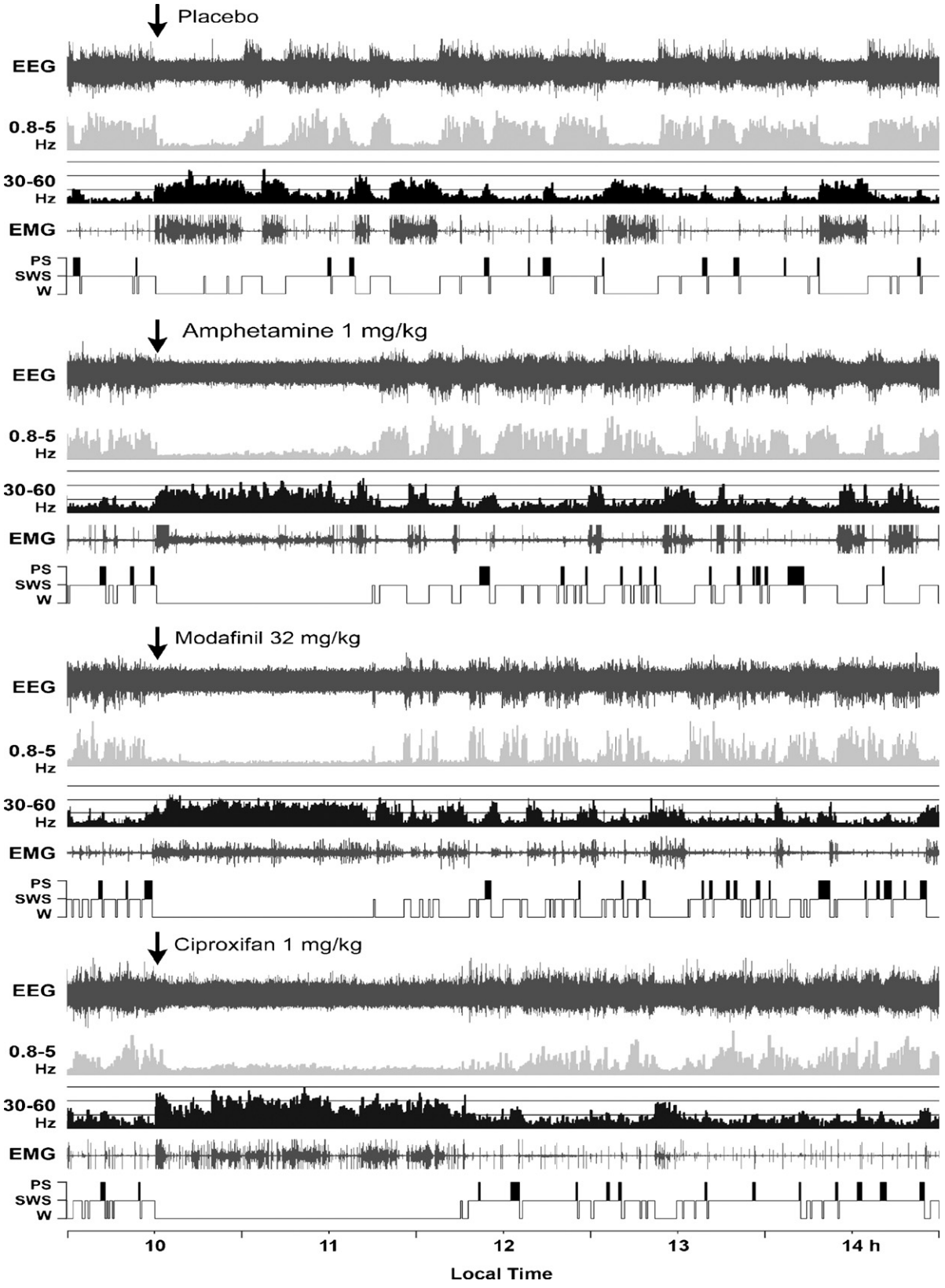

Fig. 1 – Effects of wake promoting compounds on cortical EEG and sleep–wake states in mice. Typical examples of polygraphic recordings, cortical EEG power density (μV^2) in δ band (0.8–5 Hz) and cortical fast rhythms ($\beta + \gamma$, 30–60 Hz), illustrating the waking state induced by intraperitoneal injection of amphetamine, modafinil and ciproxifan at doses indicated. Note that all compounds induce a suppression of cortical slow activity (0.5–8 Hz) accompanied with a continuous cortical fast rhythms (30–60 Hz), whereas only ciproxifan causes a marked enhancement of the fast rhythm amplitude. EEG, electroencephalogram; EMG, electromyogram; PS, paradoxical sleep; SWS, slow wave sleep; W, wake.

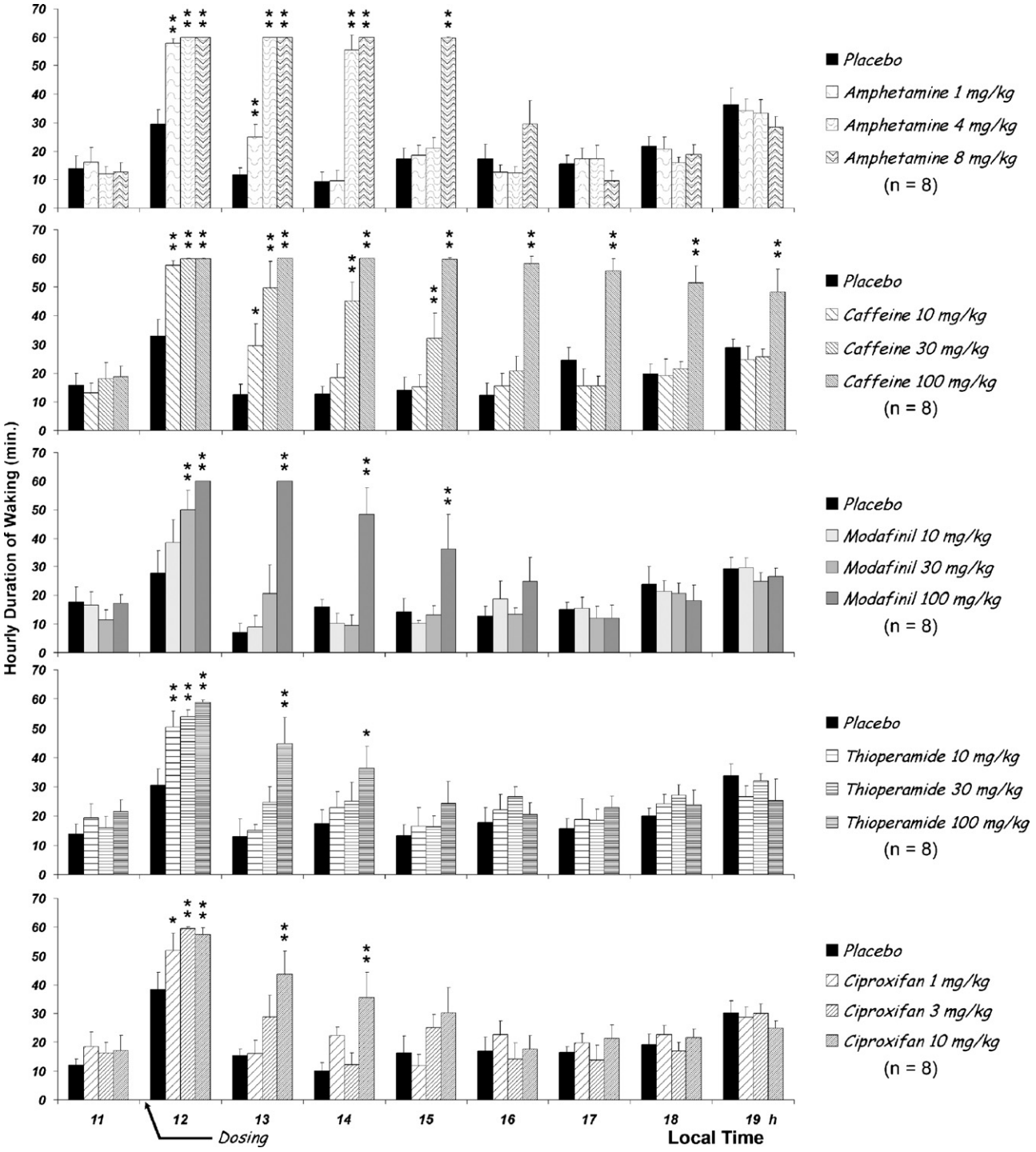

Fig. 2 – Hourly wake amount during 9 h following intraperitoneal injection (11:30 a.m. light phase) of increasing doses of wake promoting agents in C57/Black6/J mice. Note that the duration of waking effect induced by all compounds is dose-related ($^{*,**}p < 0.05$ and 0.01; Student's *t*-test as compared with placebo (methylcellulose 0.25%); $n = 8$).

amphetamine and modafinil enhanced waking behavior without increasing cortical fast activity. Conversely, the ''quiet'' waking induced by ciproxifan was accompanied by a marked enhancement in cortical fast rhythms (Fig. 1). Thus, the mean total power of cortical fast rhythms (20-60 Hz) during waking after ciproxifan dosing is increased by 9 ± 2% ($p < 0.05$, ANOVA) compared with that seen after placebo in the same mice ($n = 7$, data obtained from 120 consecutive samples of 30 s wake episodes after placebo or ciproxifan dosing in each mouse). Similar results have been obtained in the cat ([4,30] and data not shown).

The marked wake-improving effect of ciproxifan demonstrated in several species is consistent with the concept of a predominant role of histaminergic neurons in cortical

activation during waking. Because the occurrence of cortical fast rhythms is closely associated with the so-called higher mental activities, e.g., attention, alertness, and leaning, these results thus indicate that waking elicited by H_3R-antagonists is of a high level of vigilance and that the histaminergic system plays a role not only in waking, the basis for all other high brain functions, but also in some cognitive processes. These data also suggest that clinically suitable H_3R-antagonists might be designated as a therapeutic approach for vigilance disorders associated with cognitive deficiency [4,20,30,34]. Finally, ciproxifan, through activation of histamine neurons as demonstrated by their dense c-fos

Table 2 – Slow wave sleep (SWS) amount (min ± S.E.M.) during periods of significant arousal, immediate recovery and different recovery periods following wake-promoting compounds given i.p. at 11h30 (light phase), as indicated

Compound and dose (mg/kg)	Periods post-injection				
	Significant arousal	Immediate recovery	Recovery before 12 h	12–24 h recovery	Spontaneous arousal before lights-on (17–19 h)
Amphetamine					
1	0–2 h 37 (51) ± 3**	2–5 h 128 (101) ± 2	5–12 h 198 (100) ± 7	376 (99) ± 8	73 (99) ± 7
4	0–3 h 4 (3) ± 4**	3–6 h 123 (104) ± 3	6–12 h 189 (121) ± 6*	399 (105) ± 10*	86 (115) ± 6
8	0–4 h 0 (0) ± 1**	4–7 h 119 (102) ± 4	7–12 h 161 (135) ± 5**	440 (117) ± 6**	108 (149) ± 4**
Caffeine					
10	0–3 h 68 (59) ± 9**	3–6 h 122 (101) ± 8	6–12 h 186 (107) ± 17	377 (99) ± 22	73 (101) ± 11
30	0–4 h 49 (30) ± 14**	4–7 h 110 (96) ± 4	7–12 h 159 (120) ± 9*	409 (104) ± 17	91 (128) ± 10*
100	0–12 h 83 (19) ± 31**	†	†	391 (108) ± 23	71 (97) ± 8
Modafinil					
10	0–1 h 20 (51) ± 5	1–4 h 138 (106) ± 4	4–12 h 255 (97) ± 9	372 (96) ± 7	71 (106) ± 8
30	0–2 h 46 (60) ± 10*	2–5 h 130 (94) ± 4	5–12 h 227 (102) ± 6	371 (103) ± 17	67 (102) ± 10
100	0–5 h 67 (33) ± 14**	5–8 h 114 (109) ± 3	8–12 h 129 (111) ± 5	401 (96) ± 17	79 (107) ± 9
Thioperamide					
10	0–1 h 10 (42) ± 4	1–4 h 115 (95) ± 5	4–12 h 248 (101) ± 7	336 (97) ± 19	61 (96) ± 6
30	0–3 h 71 (66) ± 4**	3–6 h 107 (89) ± 4	6–12 h 165 (97) ± 5	365 (104) ± 16	73 (108) ± 7
100	0–4 h 74 (50) ± 15**	4–7 h 109 (97) ± 8	7–12 h 168 (117) ± 10	355 (103) ± 12	73 (106) ± 6
Ciproxifan					
1	0–1 h 8 (41) ± 5	1–4 h 118 (94) ± 5	4–12 h 225 (87) ± 14	363 (102) ± 26	67 (98) ± 10
3	0–2 h 30 (45) ± 5**	2–5 h 118 (95) ± 4	5–12 h 212 (98) ± 8	362 (93) ± 16	70 (101) ± 6
10	0–4 h 67 (46) ± 14**	4–7 h 111 (95) ± 4	7–12 h 154 (110) ± 9	395 (102) ± 9	78 (107) ± 6

The duration of each period is compound- and dose-dependent and was determined individually. The last post-injection period between 17 and 19 h corresponds to a spontaneous awakening period seen with C57/Black6 strain just before lights-on at baseline recordings. In parentheses are percentages of SWS duration compared with those obtained with placebo in the same animals. The 100% signifies the control level or no change. *Note*: (1) a dose-dependant SWS-suppressing effect of all compounds; (2) the presence of sleep rebound during different recovery periods after amphetamine or caffeine; (3) the absence of sleep rebound during any recovery period after modafinil, thioperamide or ciproxifan dosing (*,** $p < 0.05$ and 0.01; Dunnett's *t*-test as compared with placebo; $n = 8$).

expression after dosing, restored a sustained cortical activation in comatose or hypersomniac cats after acute or chronic brainstem transection, respectively [4]. This clear arousing effect suggests that drug-like H_3R-antagonists may have the ability to restore cortical activation in comatose or brain-traumatized patients.

2.3. *Absence of sleep rebound*

The long lasting waking elicited by amphetamine and caffeine, but not that induced by modafinil, was followed by a significant sleep rebound, mainly consisting of slow wave sleep (Tables 2 and 3) and by an increase in power spectral density of cortical slow activity (data not shown). These data are in agreement with those previously obtained in the cat [35,36] and rat [37,38]. Like modafinil, but unlike amphetamine or caffeine, the waking effects of thioperamide and ciproxifan were followed by a sleep–wake cycle with an amount of both slow wave sleep and paradoxical sleep similar to that seen during baseline recording, indicating no significant sleep rebound. The significance of the different effects of the studied agents on subsequent sleep remains unclear as the mechanisms and functions of sleep rebound are far from well understood. It has previously speculated that an overuse or exhaustion of catecholamines, such as the enhanced prolonged release associated with the use of amphetamine (but presumably not with that of modafinil), may be one cause of sleep rebound following the amphetamine-induced arousal and behavioral excitation [36]. One function of sleep rebound would, therefore, be to restore the physiological and functional levels of catecholaminergic neurons after over activity and also to allow the brain to recover from the deleterious effects of catecholamine systems during sustained waking. In support of this assumption, one of the few genes in the rat brain which is significantly induced and proportionally expressed after sleep deprivation is that of arylsulfotransferase, a final enzyme responsible for the catabolism of catecholamines in rodents [39,40]. Thus the absence of sleep rebound associated with modafinil could also be interpreted as

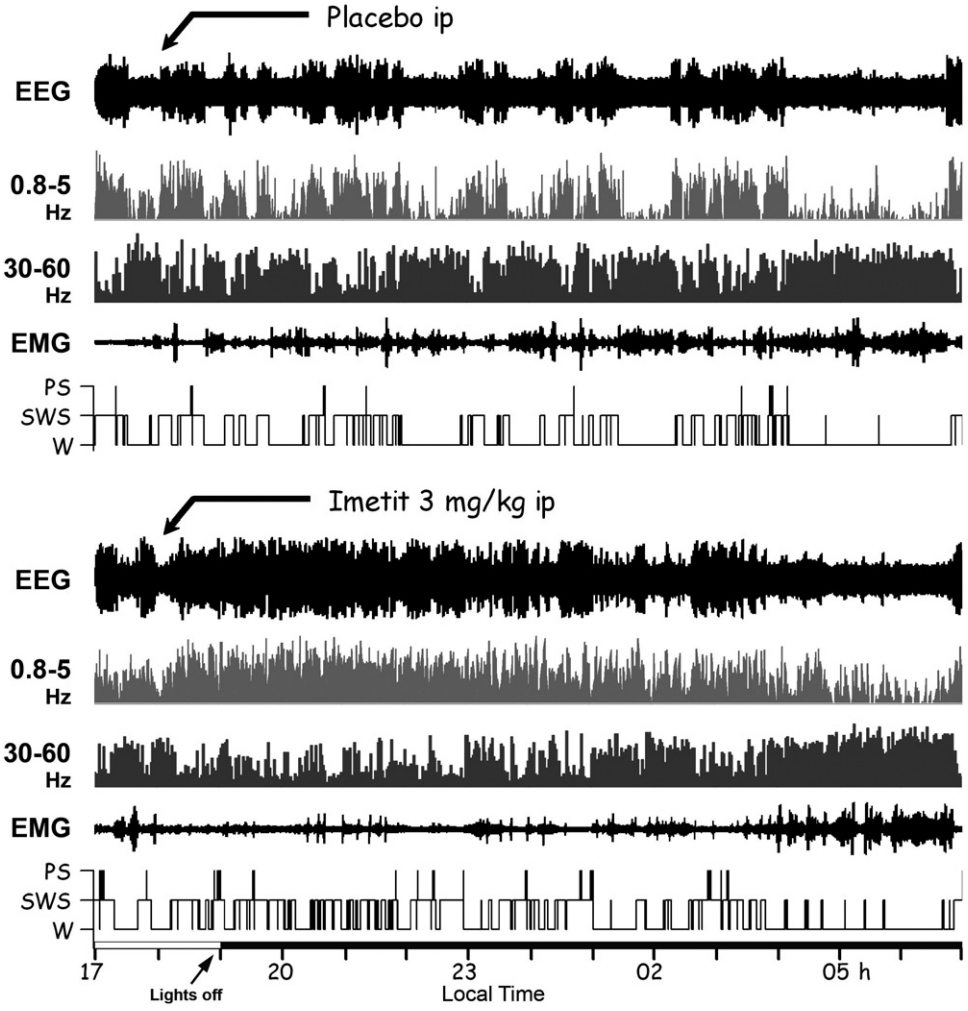

Fig. 3 – Typical examples of polygraphic recordings, cortical EEG power spectral density (μV^2) in different frequency bands and corresponding hypnograms illustrating the effects of intraperitoneal injection of imetit (at 18 h, indicated by the arrow) on the EEG and sleep–wake cycle in a mouse. Note that compared with the use of placebo, the compound enhances markedly cortical slow activity (0.8–5 Hz, δ and slow θ) and decreases fast rhythms ($\beta + \gamma$, 30–60 Hz), accompanied with an increase in slow wave sleep (SWS) (EEG, electroencephalogram; EMG, electromyogram; PS, paradoxical sleep; W, wake).

absence of catecholamine exhaustion as the waking effect of modafinil does not seem to depend on endogenous catecholamines [35]. Moreover, no signs of direct neuronal depolarization/excitation on target cells have been reported for modafinil, even though diffuse expression of immediate early gene c-fos [41], or enhanced histamine release [42] occurred with high doses of modafinil. These effects can be attributed to a direct consequence of the sustained waking induced by modafinil rather than a direct pharmacological targeted action per se. Indeed, c-fos expression is a state-dependent phenomenon, occurring most densely in large brain areas after spontaneous or induced wakefulness [43–45]. Alternatively, one of the major effects of modafinil is the induction of a marked decrease in GABA outflow in the critical brain regions involved in sleep–wake control including the posterior hypothalamus and preoptic area [46,47]. Wakefulness seen with modafinil could then result from a disinhibition of brain arousal systems, e.g., the histamine and orexin containing cells in the posterior hypothalamus known for their crucial role in the maintenance of waking [4–6,9]. A quiet waking state resulting from this disinhibitory mechanism would therefore have a different effect on the subsequent sleep rebound to that seen with amphetamine.

The reasons why H_3R-antagonist-induced waking is not associated with a sleep rebound remain to be determined. In any proposed hypothesis, the above-mentioned associated characteristics, including quiet waking, prompt and short-lasting effect, rapid HA turnover, the lack of an overuse of catecholamines and the lack of potent interactions with monoamine transporters may be contributory. Whatever these underlying mechanisms might be, the absence of sleep rebound observed with modafinil and H_3R-antagonists, is critical in the clinical setting in terms of quality of life outcomes.

3. Effects of the H_3-receptor agonist, imetit on the mouse cortical EEG and sleep–wake cycle and ciproxifan-induced waking

From this and other studies, it is clearly established that H_3R-antagonists promote waking and improve vigilance. An important corollary question is whether H_3R-agonists induce or facilitate sleep. It was also important to verify if the waking effect of H_3R-antagonists could be reversed by H_3R-agonists. To this aim, the effects of imetit (a potent and selective H_3R-agonist) were examined in the same mouse model ($n = 8$)

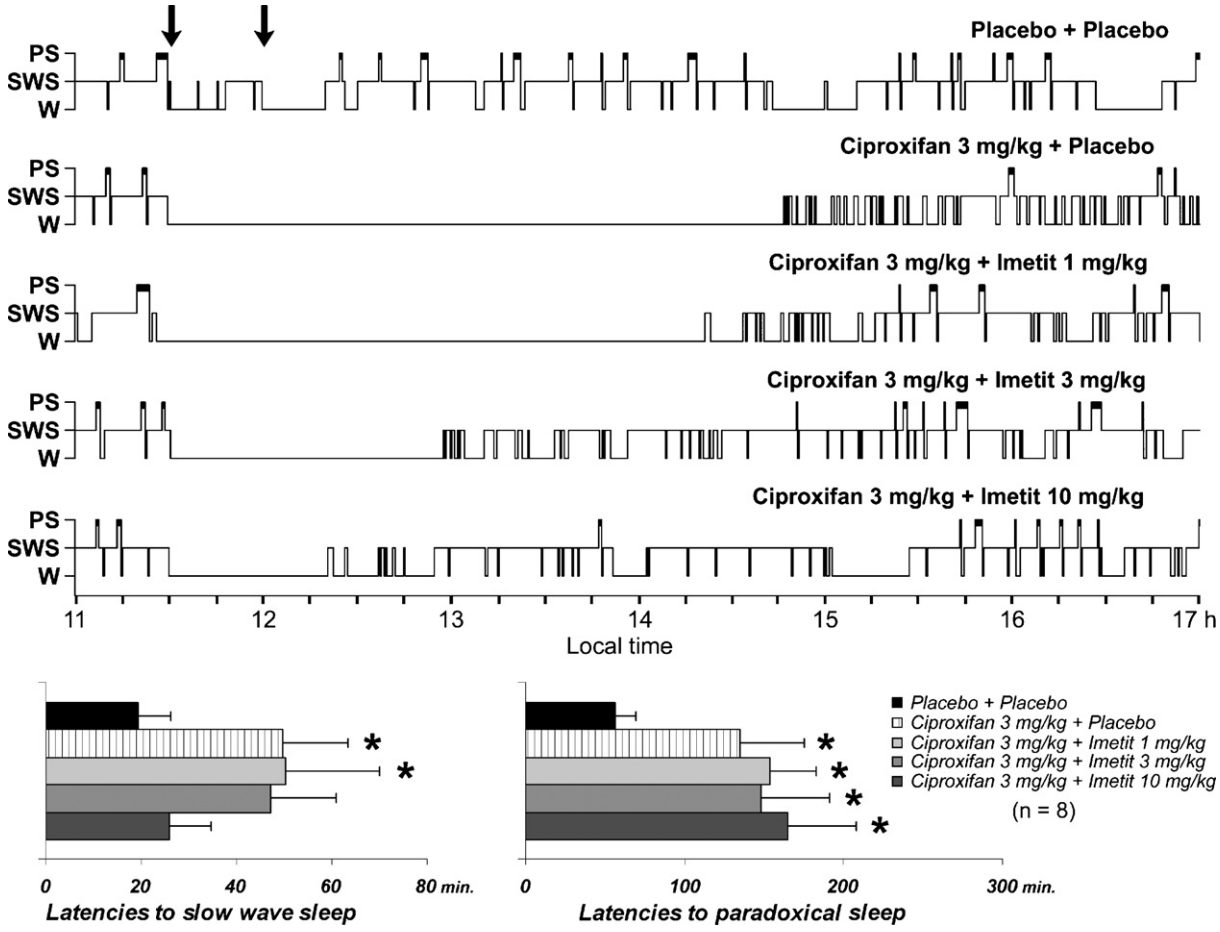

Fig. 4 – Effects of co-administration of ciproxifan with imetit on the sleep–wake cycle and latencies to slow wave sleep (SWS) and paradoxical sleep (PS) in the mouse. Representative 6 h hypnograms and histograms showing that (1) the waking effect of ciproxifan injection (i.p. at 11 h 30 indicated by the first arrow) is reversed by increasing doses of imetit (i.p. at 12 h, indicated by the second arrow); (2) the prolonged latency to SWS caused by ciproxifan is reversed by imetit at large doses while that to PS remained unchanged. Ordinates: sleep–wake stages (*$p < 0.05$; Dunnett's t-test after significant ANOVA, $n = 8$).

during lights-off phase, when the animals spent most of the time awake at baseline (defined as the waking period).

When administered alone before lights-off, imetit (1, 3 or 10 mg/kg i.p.) decreased cortical fast rhythms and markedly increased the power spectral density of the neocortical slow activity ($\delta + \theta$ ranges, mainly 0.8–5 Hz, Fig. 3) and spindles (8–15 Hz, not shown), resulting in a state of high voltage electrical activity (Fig. 3). The effects on the cortical EEG were manifested on a 4 h polygraphic recording as an increase in slow wave sleep and decrease in wake duration. Paradoxical sleep decreased slightly at all doses without reaching statistical significance (Figs. 3 and 5). Such effects were detectable at a dose of 1 mg/kg (although insignificant over the 4 h analyzed period) and increased at 3 and 10 mg/kg (Figs. 3 and 5). The data are consistent with those obtained in the cat using α-methylhistamine, a chiral H_3R-agonist [20]. These effects of imetit in the mouse were similar to those seen with BP2-94, another H_3-receptor agonist that in the cat induced a dramatic increase in the power spectral density of cortical slow activity, associated with a significant increase in slow wave sleep [4], a phenomenon similar to that seen during the recovery phase from sleep deprivation. H_3-receptor

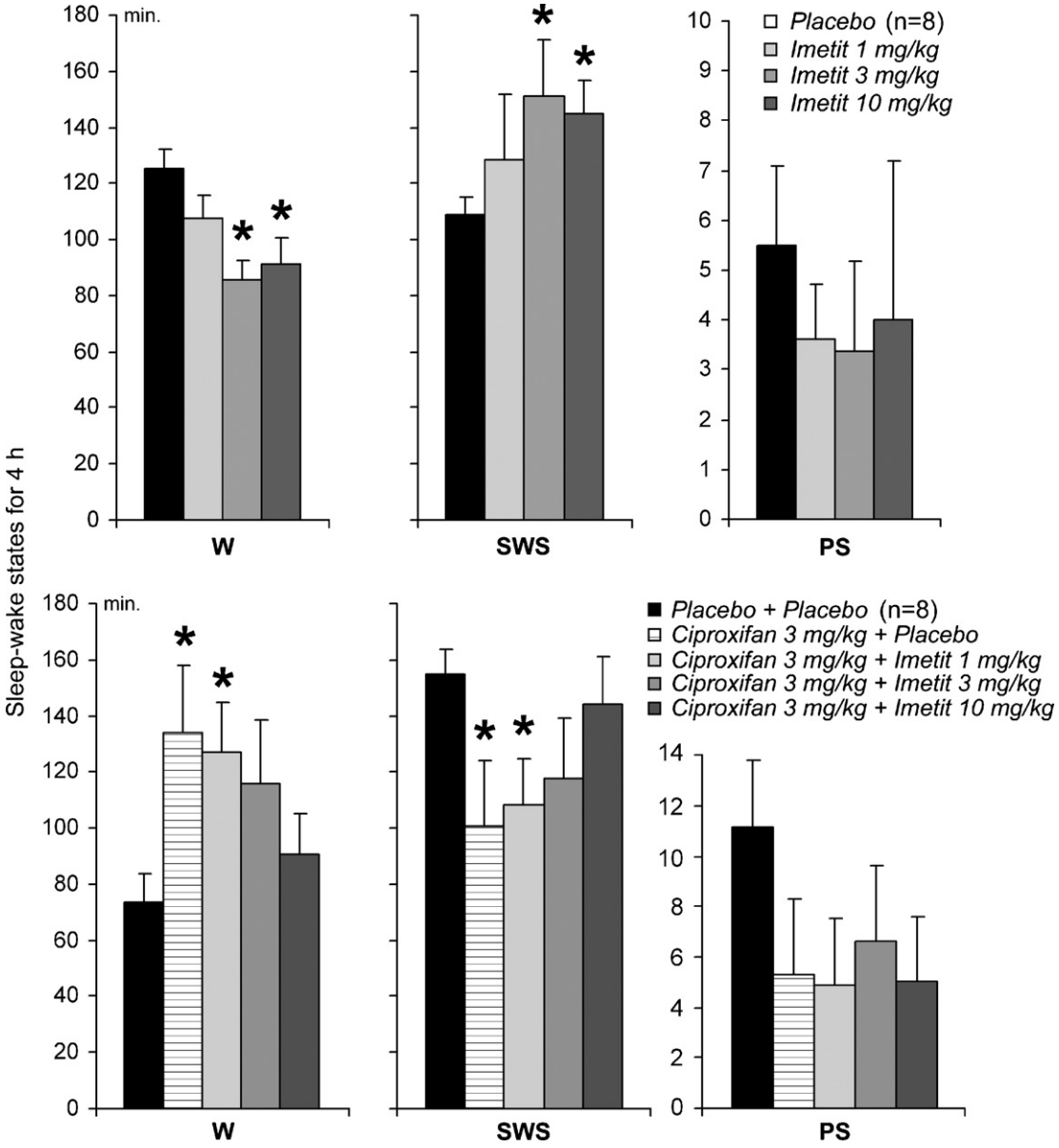

Fig. 5 – Quantitative variations of the sleep–wake cycle following administration of imetit alone or coupled with ciproxifan (i.p. dosing at 12 h, light phase). Histograms showing mean time (min) spent in each sleep–wake stage during 4 h after compound administration. *Note*: (1) in top panel, that imetit dosing (i.p., at 18 h just before the lights-off phase or waking period) caused a dose-related decrease in waking (W) and an increase in slow wave sleep (SWS) without reducing paradoxical sleep (PS); (2) in lower panel, ciproxifan (i.p., at 11h30 during light phase or sleeping period) caused a significant increase in W and a decrease in SWS; (3) in lower panel, the W-increasing and SWS-decreasing effects of ciproxifan were attenuated significantly by larger doses of imetit (i.p. at 12 h), namely 3 and 10 mg/kg. PS remains decreased although statistically non-significant ($^*p < 0.05$; Dunnett's *t*-test after significant ANOVA; $n = 8$).

agonists might therefore have beneficial effects, helping the brain to recover from fatigue, mental exhaustion or sleep deprivation due to diverse causes. They might also be potentially superior to the classical antihistamines, the H_1-receptor antagonists and other classes of hypnotics that induce slow wave sleep associated with prominent paradoxical sleep reduction, incompatible with physiological sleep, as their effect on paradoxical sleep might not occur or could be less prominent. Clinically suitable H_3R-agonists might thus be expected to improve qualitative and quantitative aspects of sleep in some types of insomnia, e.g., those resulting from anxiety, stress or neuropathology.

The arousal effects of ciproxifan (3 mg/kg, i.p.) observed during the sleep period were dose-dependently antagonized by the H_3-receptor agonist, imetit (1, 3 and 10 mg/kg, i.p. $n = 8$; Figs. 4 and 5) with a significant reduction of induced-waking occurring after 3 mg/kg imetit with wake and slow wave sleep duration returning to near control at 10 mg/kg dosing. Paradoxical sleep however, remained decreased after imetit treatment (Fig. 4). The delayed latency to slow wave sleep due to ciproxifan was reversed at 10 mg/kg whereas latency to paradoxical sleep was unchanged after all imetit doses (Fig. 4). These data are consistent with those from cats showing an antagonism of the waking effect of thioperamide by α-methylhistamine [20] supporting the concept of an H_3 receptor-dependent mechanism for their effects on EEG and sleep–wake parameters. However, it remains to be understood why imetit and other H_3R-agonists do not increase paradoxical sleep, as they do slow wave sleep, since a possible paradoxical sleep-permissive role of histamine neurons has been hypothesized in the mouse. This is based on a paradoxical sleep-off discharge pattern of HA-containing cells [14], on the one hand; and on the other hand, an increase in paradoxical sleep seen with acute or chronic suppression of histidine decarboxylase (HDC) using either the HDC inhibitor, α-fluoromethylhistidine or HDC KO mice [16]. In addition, both imetit and ciproxifan caused a slight decrease in paradoxical sleep (Fig. 5), making it possible that targets other than H_3-receptors or HA transmission may be involved or reflecting the inverse agonist profiles of these two compounds. Additional experiments are therefore necessary.

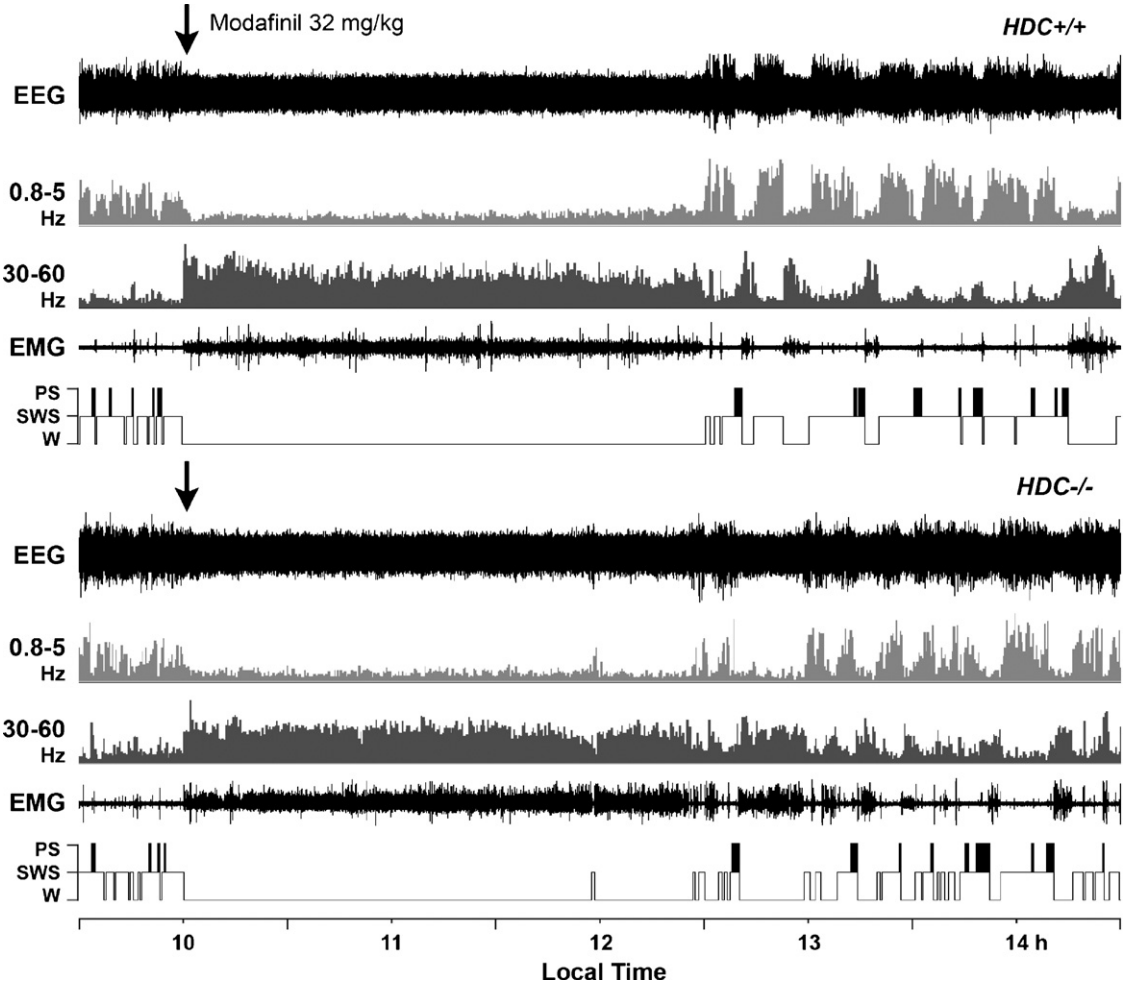

Fig. 6 – Effects of modafinil on cortical EEG and sleep–wake states in wild type (HDC+/+, upper traces) and histidine decarboxylase knockout (HDC−/−, lower traces) mice. Examples of polygraphic recordings, cortical EEG power spectral density (μV^2) in different frequency bands, and the corresponding hypnograms illustrating, in both genotypes, an identical awakening effect of modafinil (32 mg/kg, in 20% DMSO, i.p., 10:00 as indicated by arrow) accompanied with a suppression of cortical slow activity (0.8–5 Hz) and continuous cortical fast rhythms (30–60 Hz). EEG, electroencephalogram; EMG, electromyogram; PS, paradoxical sleep; SWS, slow wave sleep; W, wake.

4. Characterization of the wake-promoting agents with reference to histamine-mediated transmission using knockout mouse models

As HA neurons are thought to play a crucial role in maintaining cortical activation and waking, one may ask whether modafinil induces sustained wakefulness via activation of histaminergic neurons. The same question may be addressed regarding psychostimulants even though a predominant dopaminergic mechanism exists. The question has become more intriguing since reports in the rat of a c-fos expression in histaminergic tuberomammillary nucleus [41] and an increase in hypothalamic HA outflow [42], both seen with large doses of modafinil. In regard to H_3R-antagonists, one may also question the importance of histamine transmission in their arousal effects and if there is an involvement of other neurotransmitters also involved in waking [8,9] and controlled by H_3-receptors. To test the histaminergic hypothesis regarding the mechanisms of action of amphetamine, modafinil and ciproxifan in waking, their effects in KO mouse models in which histamine-mediated transmission is altered, e.g., HDC and H_1-, H_2- and H_3-receptor KO mice was studied. As previously reported, these KO mice are able to maintain, under the basal non-challenged conditions and despite qualitative change, a daily amount of waking near to that of wild type (WT) mice [16,48–50], probably due to the compensatory mechanisms elaborated by brain plasticity.

A group ($n = 9$) of 129Sv genetic-background inbred WT and HDC KO mice were recorded simultaneously, as previously described, to compare the sleep–wake effects of the W-promoting agents given i.p. during the light phase [16]. These mice were generated according to procedures previously described [51] and their genotypes confirmed using PCR. The doses used for amphetamine, modafinil and ciproxifan were 1, 32 and 1 mg/kg respectively because of their approximated equal potency in terms of wake induction. As presented above, all agents at the indicated doses caused, in all WT models (129Sv as well as C57/Black6/J genetic background), increased waking at the expense of slow wave sleep and paradoxical sleep as compared with placebo (Figs. 6 and 7; Table 3).

In the HDC-KO mice, the same amphetamine and modafinil doses increased waking and decreased slow wave sleep and paradoxical sleep during the sleeping phase, the effect being identical or slightly superior to that seen in WT animals. In contrast, the same ciproxifan dosing (or higher doses up to 10 mg/kg, data not shown) had no effect on either the cortical EEG or the sleep–wake states (Figs. 6 and 7; Table 3).

HDC-KO mice lack endogenous HA synthesis and HA-containing neurons in the brain [16,51]. The present data thus indicate that the sleep–wake effects of ciproxifan, but not those of amphetamine or modafinil, depend on histamine-mediated transmission. However, these data do not appear to support a direct excitation of histamine neurons in the mechanism of action of modafinil or amphetamine-like psychostimulants. C-fos expression in the histaminergic tuberomammillary nucleus and diffuse brain areas [41] or the HA release [42] seen in the rat after large doses of modafinil is thus likely to be the consequence of sustained waking rather than a modafinil-mediated excitation, as both c-fos expression and histamine release are positively correlated to waking [43–45,52]. Using c-fos as a marker in the cat supports this hypothesis. Indeed, examination of c-fos labeling after modafinil dosing but before an established long duration wake state revealed sparse c-fos expression in the histaminergic tuberomammillary nucleus and other brain regions [53]. Additionally in either normal [15] or

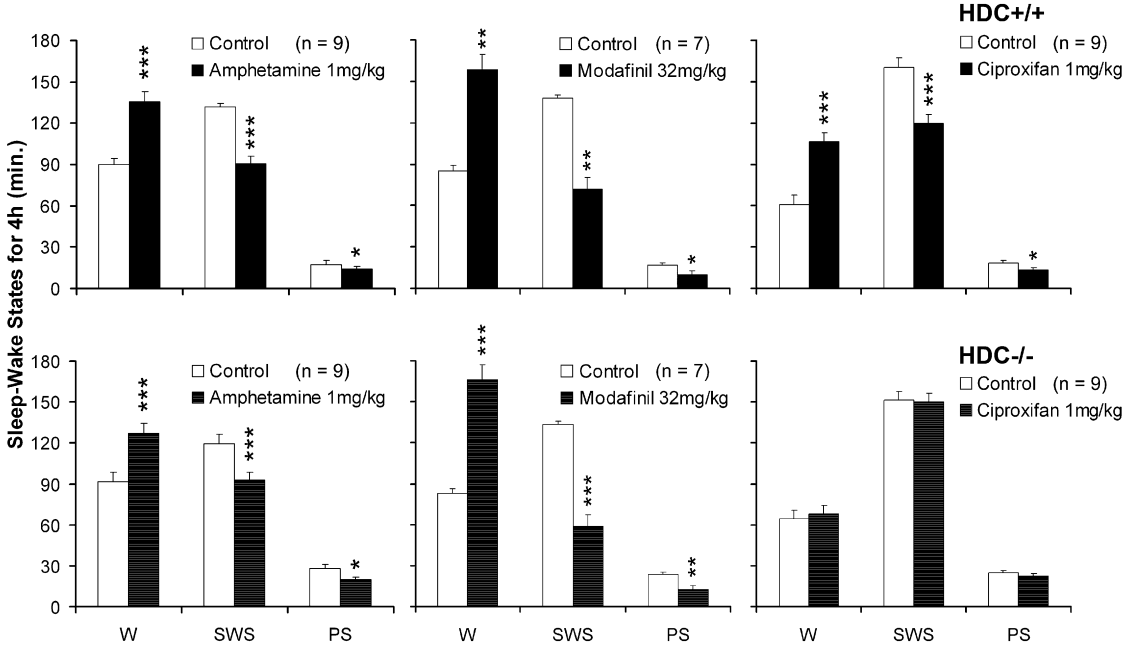

Fig. 7 – Effects of amphetamine, modafinil and ciproxifan on the sleep–wake states during 4 h in wild type (HDC+/+, upper) and histidine-decarboxylase knockout (HDC−/−, bottom) mice. Note that amphetamine or modafinil dosing (i.p.) produced a similar increase in waking (W) and a concomitant decrease in slow wave sleep (SWS) and paradoxical sleep (PS) in both mouse genotypes and that ciproxifan increased waking and decrease SWS and PS in HDC+/+ mice, but had no effect in HDC−/− mice. $^{*,**,***}p < 0.05$, 0.01 and 0.001 respectively, Student's t-test as compared with control.

Table 3 – Effects of amphetamine, modafinil and ciproxifan on wake amount in wild type and knockout mouse models

			Time post-injection		
	Genotype		0-4h	4-8h	8-12h
Amphetamine (1 mg/kg)	HDC +/+	n = 9	135 (150) ± 7***]°	68 (91) ± 7	141 (79) ± 9*
	HDC -/-		127 (139) ± 7***	70 (94) ± 5	138 (77) ± 10*
Modafinil (32 mg/kg)	HDC +/+	n = 7	159 (185) ± 9**]°	77 (104) ± 6	162 (93) ± 9
	HDC -/-		166 (200) ± 11***	70 (92) ± 3	178 (107) ± 8
Ciproxifan (1mg/kg)	HDC +/+	n = 9	107 (178) ± 7***]***	70 (94) ± 5	145 (102) ± 7
	HDC -/-		68 (107) ± 6°	75 (96) ± 4	125 (102) ± 9
	H1 +/+	n = 8	113 (186) ± 6***] **	78 (102) ± 8	179 (101) ± 8
	H1 -/-		70 (103) ± 8°	74 (105) ± 5	161 (99) ± 8
	H2 +/+	n = 8	116 (185) ± 11***]°	65 (94) ± 8	171 (110) ± 12
	H2 -/-		105 (193) ± 9***	69 (104) ± 5	168 (102) ± 10
	H3 +/+	n = 12	111 (150) ± 7**]**	67 (91) ± 4	156 (107) ± 6
	H3 -/-		72 (98) ± 4°	76 (97) ± 2	149 (105) ± 6

The results are expressed either as the mean time (min ± S.E.M.) spent awake, or as a percentage (in parentheses) obtained from the ratio of mean experimental values (compound treatment, i.p.) over the mean control values (saline injection) in the same animals. 100% indicates control level or no change. Three analyzed periods of 4 h are shown. Note that (1) amphetamine and modafinil increase waking in both $HDC^{-/-}$ and $HDC^{+/+}$ mice; (2) ciproxifan elicits significant waking effect in all wild type models and $H2^{-/-}$ mice but not in $HDC^{-/-}$ or $H1^{-/-}$ mice; (3) a significant sleep rebound was seen only with amphetamine, and not with other compounds, in both $HDC^{-/-}$ and $HDC^{+/+}$ mice ($^{\circ}p > 0.05$; $^{**,***}p < 0.01$ and 0.001; Student's *t*-test as compared with placebo (individual animal served as its own control) or between knock-out and wild type mice).

brainstem-transectioned [4] cats, expression of c-fos in histamine neurons has only been seen with ciproxifan and not with other waking substances including modafinil, as already mentioned, and psychostimulants like amphetamine and methylphenidate [53], indicating that only stimuli specific to the histaminergic system may induce c-fos expression within histaminergic cell bodies. However, it cannot be excluded that histaminergic neurons may be indirectly involved in modafinil-induced waking, as a significant decrease in GABA outflow in the posterior hypothalamus is seen in vivo after modafinil dosing [46,47] and so HA or orexin neurons located in this region could be disinhibited and so enhance waking.

Since only ciproxifan-mediated arousal depends on HA, this compound was further characterized using C57/Black6/J background KO mice devoid of H_1- ($n = 8$), or H_2- ($n = 8$) or H_3-receptors ($n = 12$). These mouse genotypes were generated, respectively, according to the previously described procedures and both WT and KO littermates were identified using PCR [54–56].

Ciproxifan (1 mg/kg, i.p.) increased waking (+50 to 86% over a 4 h recording) and cortical fast rhythms in all WT mouse groups during the sleeping period, whereas it had no effect in either H_1- or H_3-receptor KO littermates. Interestingly, the effects of ciproxifan on the cortical EEG and waking were intact in H_2-receptor KO-mice, the increase in waking being similar in KO (+93% over 4 h) than WT littermates (+85%) (Table 3). These data confirm the pharmacological selectivity of ciproxifan for H_3-receptors already demonstrated with imetit and the essential role of the H_3-receptors in its arousal effect. Although recent studies indicate that both ciproxifan and imetit may process weak activity at H_4-receptors [34,57,58] and although H_3-receptors also regulate the availability of neurotransmitters other than HA, e.g., norepinephrine, acetylcholine and 5HT, that are also involved in sleep–wake control, the results generated from KO mice indicated that the effect of ciproxifan on EEG and sleep–wake parameters selectively depend on H_3-receptor and histamine-mediated transmission. The fact that the waking effect of ciproxifan was observed in the H_2-receptor KO mouse but absent in the H_1-receptor KO genotype [48] confirms the dominant, if not exclusive, importance of H_1-receptors in the postsynaptic mechanisms of histaminergic arousal. Together, these data validate the earlier hypothesis [4,20] that H_3R-antagonists, via dis-autoinhibition of presynaptic H_3-receptors, enhance the turnover and activity of histaminergic neurons, increasing synaptic HA that in turn activates postsynaptic H_1-receptors, promoting wakefulness and improving vigilance.

5. Conclusions

Sleep–wake disorders constitute a major challenge of public health due to their high prevalence (19–37%) in the general population. Somnolence is associated with various pathological conditions including sleep apnea, excessive daytime sleepiness due to nocturnal insomnia, Parkinson's disease and narcolepsy or circumstances related to lifestyle, including daytime sleepiness due to voluntary sleep restriction or sleep deprivation resulting from night shift work, overwork or jet-lag. Novel, safe, efficacious and more specific therapeutic approaches are, therefore, in great demand in sleep medicine.

The present study has distinguished two classes of wake-promoting agents: those involving histamine and those that appear histamine-independent and supports the role of the brain H_3-receptors as potentially novel therapeutic targets for vigilance and sleep–wake disorders. Compared to current wake-promoting medications, H_3R-antagonists appear to possess several advantageous characteristics that might favor their development as novel therapeutics for the treatment of sleep–wake disorders especially somnolence:

5.1. *A well-defined mechanism of action*

A well-defined mechanism of action that is based on a clearly defined molecular target and the well-established role of HA neurons and the role of H_3-receptors in sleep–wake mechanisms.

5.2. *A more specified treatment*

HA is crucially involved in somnolence. Thus mice that lack HA are permanently somnolent [16] and narcoleptic dogs are HA deficient [59]. Moreover, narcoleptic patients have decreased HA levels in the CSF [60].

5.3. *Simultaneous cognitive improvement*

H_3R-antagonists may improve cognition either via promoting EEG activation and vigilance or by improvement of specific cognitive processes (e.g., learning and memory) via cholinergic or noradrenergic systems that are also regulated by H_3-receptors [30,61–63].

5.4. *A unique approach against narcolepsy*

Finally, H_3R-antagonists may represent a unique pharmacotherapy for the treatment of narcolepsy. In addition to their specific effects on the excessive somnolence, they may be also anticipated to inhibit or suppress narcoleptic attacks (direct onset of paradoxical sleep from waking or sleep onset REM periods called by some authors), an effect related to the permissive role exerted by histaminergic and other monoaminergic cells on paradoxical sleep. Indeed, HA cells in mice [14] and cats [6,15] exhibit paradoxical sleep-off activity and acute or chronic abolition of HA synthesis results in an increase in paradoxical sleep [16]. Thus, enhancement of histaminergic neuronal activity by H_3R-antagonists is anticipated to prevent the occurrence of paradoxical sleep and accordingly narcoleptic attacks in patients. Additionally, H_3R-antagonists are effective in treating cataplexy in a dog narcoleptic model [64]. The development of novel and selective H_3R-antagonists may thus lead to new therapies to treat the complex phenomenon of narcolepsy and other human sleep–wake disorders.

Acknowledgments

The authors wish to thank Colette Buda, Jean-Pierre Sastre and Gerard Guidon for their experimental and technical contributions, Pr H. Watanabe (Kyushu Univ. Fukuka, Japan) for providing the H_1- and H_2-receptor knockout mouse strains and Dr. H. Kotani (Banyu Pharmaceutical Co. Ltd, Japan) for providing the H_3-receptor knockout mouse strain and Dr. J.M. Lecomte (Bioprojet, Paris, France) for the kind gift of ciproxifan to JSL This work was supported by (1) INSERM U52, U480 and U628, Lyon, France; (2) Faculty of Medicine, Claude Bernard University, Lyon, France; (3) European Community, Fifth Framework Program Grant QLRT 826 (for JSL); (4) Cephalon, Inc.

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