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The stimulant effect of modafinil on wakefulness is not associated with an increase in anxiety in mice

A comparison with dexamphetamine

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Abstract. Modafinil is a new drug used in the treatment of narcolepsy. Its administration in mice induced a dose-dependent increase in locomotor activity. The effects of modafinil were compared with those of dexamphetamine on three tests that assessed the anxiety level (drugs were used at doses which induced a roughly similar stimulation of locomotor activity). Dexamphetamine increased the latency of exploration of a white compartment, increased thigmotaxis in an open-field and decreased the time spent in the open arms of an elevated plus-maze. None of these responses was significantly modified by modafinil. We conclude that modafinil does not share the anxiogenic effects of dexamphetamine.

Key words: Anxiety – Modafinil – Dexamphetamine – Black/white compartments – Elevated maze-plus – Thigmotaxis – Wakefulness

Several drugs decrease anxiety levels only as a consequence of their sedative effects. In contrast, mild or strong psychostimulant agents, such as caffeine or amphetamine, increase anxiety (Geller and Seifter 1960; Lapin 1993) and even trigger panic attacks (Charney et al. 1985). Therefore, when a new agent which stimulates wakefulness is developed, it is useful to be able to predict experimentally whether or not it displays anxiogenic properties.

The recently developed drug modafinil, a benzhydrylsulfinylacetamide derivative, stimulates wakefulness and appears to be useful in the treatment of hypersomnia as well as Gelineau's syndrome which corresponds to narcoleptic-catapleptic attacks (Billiard et al. 1987; Bastuji and Jouvet 1988). Modafinil displays a neurochemical profile different from that of amphetamine (Duteil et al. 1990a). Its mechanism of action is not well defined. It has been suggested that it could depend on the stimulation of a subtype of central alpha 1 adrenergic receptors (Billiard et al. 1987; Duteil et al. 1990b). The aim of the present study was to assess whether modafinil displays anxiogenic effects in mice and to compare the drug with dexamphetamine, which has been used for treating narcolepsy. The efficacy of dexamphetamine is significant but the use of the drug is accompanied by peripheral and central effects such as tachycardia, hypertension, tolerance, dependence, anorexia, "amphetamine psychosis", and anxiety (Brookes 1985). We have compared the effects of modafinil and dexamphetamine, administered at doses inducing roughly similar stimulation of locomotion, on three tests that assess the level of anxiety. They are the elevated plus-maze test (Pellow et al. 1985; Lister 1987), the black and white compartment test (Simon et al. 1992, 1993) and thigmotaxis in an open-field (Freixanet 1978; Treit et al. 1989; Simon et al. 1994). In the first test, anxiety is expressed by the relative time spent and entries into the open arms of the maze. In the second, anxiety is reflected by the latency of exploration of a white compartment, and the duration of the first stay in this white compartment. Finally, in the third test, anxiety is assessed by the degree of thigmotaxis, which corresponds to the movement of the animal along the walls of an open-field.

Materials and methods

Animals. Male Swiss albino mice (Charles River CD1, Saint Aubin lès Elbeuf, France), weighing 20–25 g were used. They were kept under standard conditions: 20 mice per cage (L = 40 cm, W = 25 cm, H = 18 cm), constant temperature (22 ± 1 °C), a 12–12 h day-night cycle (lights from 8 a.m. to 8 p.m.), and food and water ad libitum up to the time of the experiment. The experiments were carried out between 10 a.m. and 5 p.m. The animals were isolated in small individual cages for at least 30 min before testing. Each animal was used only once.

Black and white compartment test. The apparatus consisted of an enclosure divided into two compartments, each measuring L = 32cm, W = 22 cm, H = 18 cm. One compartment was dark (painted black and covered with a lid). The other compartment (not covered) was painted white and strongly lit by a 100-W light bulb set 50 cm above the floor. The compartments communicated through an opening (W = 5 cm, H = 5 cm) located at the base and in the middle of the partition wall.

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The testing procedure was as follows: after injection animals were placed in individual cages. Thirty minutes later they were introduced into the black compartment (the animal facing a corner of the wall opposed to the opening), and the compartment was covered. A chronometer was used to measure the delay before the animal entered for the first time entirely (four paws) into the white compartment. At this time a second chronometer was switched on and measured the duration of the first stay in the white compartment. By convention, if an animal did not leave the dark compartment during the first 6 min, the experiment was stopped. In contrast, if a mouse entered the white compartment immediately (latency < 5 s), the measure was not taken into account. This response was observed in less than 5% of tested animals, irrespective of drug treatment, and could correspond to an escape reflex by animals which failed to integrate the cues.

Elevated plus-maze test. The apparatus consisted of a wooden Greek cross placed 50 cm above the floor. The four arms were 18 cm long and 6 cm wide. Two opposite arms were surrounded by walls (6 cm high) (closed arms) while the two other were devoid of enclosing walls (open arms). The whole device was painted black and the room was brightly lit. Animals received the treatment 30 min before the test. The mouse was placed at the center of this maze, its head facing a closed arm. The measure (which lasted 5 min) was automated using an image analysis system (described further on). The number of entries and the time spent in the open arms were measured. The plus-maze was wiped clean after each animal.

Thigmotaxis assessment. The experimental device was a square open-field (40 × 40 cm) surrounded by walls (30 cm high) and covered by fine wire netting. All these elements were painted black, except for one of the walls which was in Plexiglas. The room was very dimly lit. The testing session lasted 10 min and was preceded by a 5-min period of habituation. The animals were injected 25 min before the introduction into the open-field. The thigmotaxis behavior (i.e. moving along the wall) was assessed by the ratio of the distance covered less than 5 cm away from the wall to the total distance covered and was expressed as a percentage. The measure was automated using an image analysis system (see further on).

Locomotor activity. Locomotor activity was measured with a Digiscan actimeter (Omnitech Electronics Inc., Columbus, Ohio, USA). The individual boxes (L = 20; W = 20; H = 30 cm) were placed in a dimly lit room. The horizontal activity was expressed by the total number of beams crossed by mice during the experiment. Mice were injected 30 min before their introduction into the actimeter.

Image analysis system. Some of the measures were carried out using the Videotrack 512 system (Viewpoint, Lyon, France). This system consisted of video cameras positioned above the experimental field, a video interface and a microcomputer. It converted the video input signals into binary images in such a manner that each animal corresponded to a white spot against a black background. Virtual windows on a computer screen corresponded to different areas of the experimental apparatus (for instance the peripheral zone of the open-field or the open arms of the elevated plus-maze).

Statistics. Statistical comparisons between groups were made with an ANOVA. Statistical comparisons of different groups to control groups were made with Duncan's test.

Drugs. Dexamphetamine sulfate (C.P.F., Melun, France) was dissolved in saline. Modafinil (a generous gift of Lafon Laboratories, Maisons-Alfort, France) was dissolved in dimethyl sulfoxide (DMSO) and then diluted in distilled water and Cremophor EL (BASF, Ludwigshaffen, Germany) (final concentration: 5% DMSO and 5% Cremophor EL).

All drugs were injected in a volume of 10 ml/kg. Doses always express the free base.

Results

Locomotor activity

The administration of (+) amphetamine (2–4 mg/kg SC) significantly increased the number of beams crossed in the actimeter. An increase in locomotor activity was also observed when mice were injected IP with modafinil, from a dose of 20 mg/kg. The effects induced by (+) amphetamine 2 mg/kg and modafinil 40 mg/kg dose were roughly similar (Fig. 1).

Black and white compartments test

The latency for entering the white compartment was significantly and dose dependently increased by (+) amphetamine (2–4 mg/kg SC). At a dose of 4 mg/kg, a reduction in the duration of the first stay in the white compartment was observed. In contrast, increasing doses of modafinil (20–80 mg/kg IP) failed to modify both responses (Table 1).

Elevated plus-maze

At all doses tested (+)amphetamine (2–4 mg/kg SC) significantly reduced the percentage of entries and time spent in the open arms. In contrast, these parameters were not significantly modified by 20, 40 or 80 mg/kg modafinil, whatever the route (SC or IP) of administration (Table 2).

Thigmotaxis

In mice tested in the open-field, an increase in the locomotor activity was observed with both (+)amphetamine and modafinil. This increase was comparable with those previously observed. (+)Amphetamine (2–4 mg/kg SC) dose-dependently increased the index of thigmotaxis, i.e.

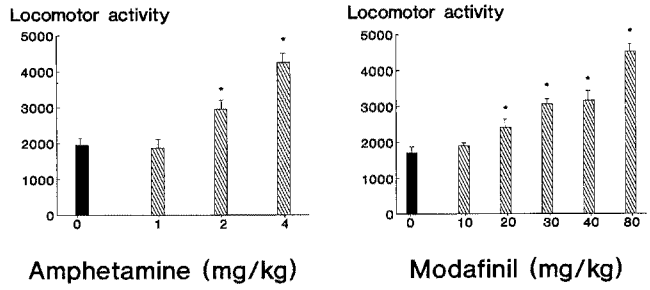

Fig. 1. Effects of increasing doses of dexamphetamine or modafinil on locomotor activity. Mice were injected SC with saline (0) or increasing doses of dexamphetamine (1, 2 and 4 mg/kg) (*left panel*) or were injected IP with vehicle (0) or increasing doses of modafinil (10, 20, 30, 40 and 80 mg/kg) (*right panel*), 30 min before their introduction into the actimeter. This introduction marked the beginning of measurements. The locomotor activity was assessed by the number of beams crossed during 10 min. Means ± SEM of 10–12 mice per group. Multiple comparisons (ANOVA): dexamphetamine: $F = 25.8$; $P < 0.001$; modafinil: $F = 26.1$;$P < 0.001$. Comparisons with control groups (Duncan's test): $^*P < 0.05$

Table 1. Effects of increasing doses of (+)amphetamine and modafinil on the black and white compartment test parameters. Mice were injected SC with saline or increasing doses of (+)amphetamine (1, 2, 4 mg/kg) or were injected IP with vehicle or increasing doses of modafinil (20, 40, 80 mg/kg). They were isolated in small individual cages for 30 min, then they were gently introduced into the black compartment. Means ± SEM of 12 mice per group (20 for control group). Multiple comparisons (ANOVA): dexamphetamine: $F = 16.5$; $P < 0.001$; modafinil: $F = 1.2$ $P > 0.05$

		White compartment entering latency (s)	White compartment first stay duration (s)
(+)Amphetamine	0	31 ± 5	8 ± 1
(mg/kg)	1	37 ± 11	9 ± 2
	2	144 ± 56*	6 ± 2
	4	316 ± 70*	4 ± 2*
Modafinil	0	28 ± 4	10 ± 1
(mg/kg)	20	39 ± 13	10 ± 2
	40	14 ± 2*	9 ± 2
	80	33 ± 9	9 ± 3

Comparisons with the control groups (Duncan's test): *$P < 0.05$

Table 2. Effects of (+)amphetamine and modafinil on the elevated plus-maze test. Mice were injected SC with saline or increasing doses of (+) amphetamine (1, 2, 4 mg/kg) or were injected IP with vehicle or increasing doses of modafinil (20, 40, 80 mg/kg) or were injected SC with vehicle or increasing doses of modafinil (20, 40, 80 mg/kg). They were isolated in small individual cages during 30 min, then they were set down at the center of the maze. Means ± SEM of 8–12 mice per group. Multiple comparisons (ANOVA): dexamphetamine: $F = 11,8$; $P < 0.001$ (% entries), $F = 10.9$; $P < 0.001$ (% time), modafinil IP: $F = 0.7$; $P > 0.05$ (% entries), $F = 1.6$; $P > 0.05$ (% time), modafinil SC: $F = 0.2$; $F > 0.05$ (% entries), $F = 1.1$; $P > 0.05$ (% time)

	% Entries made into open arms	% Time spent in open arms
Amphetamine (mg/kg SC)		
0	31.1 ± 4.7	7.8 ± 1.5
2	13.3 ± 4.3*	0.6 ± 0.2*
4	2.3 ± 1.1*	0.8 ± 0.4*
Modafinil (mg/kg IP)		
0	36.9 ± 4.2	13.7 ± 3.4
20	32.5 ± 5.3	8.8 ± 2.3
40	36.6 ± 3.9	7.9 ± 1.8
80	22.7 ± 6.1	10.6 ± 4.4
Modafinil (mg/kg SC)		
0	32.6 ± 3.8	8.7 ± 1.5
20	32.5 ± 5.3	8.8 ± 2.3
40	23.3 ± 4.9	7.0 ± 2.8
80	26.7 ± 3.5	8.5 ± 1.6

Comparisons with the control groups (Duncan's test): *$P < 0.05$

the relative distance travelled in the peripheral zone of the open-field. In contrast, this index was not modified in animals injected with modafinil (20–80 mg/kg IP) (Table 3).

Discussion

An increase of wakefulness, or a stimulation of vigilance, are often associated with an acute anxiety. To assess this contingent property of modafinil we have selected three

Table 3. Effect of (+)amphetamine and modafinil on thigmotaxis in an open field. Mice were injected SC with saline or increasing doses of (+) amphetamine (1, 2, 4 mg/kg), or were injected IP with vehicle or increasing doses of modafinil (20, 40, 80 mg/kg), then they were introduced in the open-field. The distances travelled were measured during 10 min, after a 5-min period of habituation. Mice were introduced into the open-field 25 min after the drug injection. Index of thigmotaxis = (distance travelled in the peripheric zone/total distance) × 100. Means ± SEM of 8–10 mice per group. Multiple comparisons (ANOVA): dexamphetamine: $F = 8.6$; $P < 0.001$; modafinil: $F = 0.9$; $P > 0.05$

	Total distance travelled (cm)	Index of thigmotaxis (%)
(+)Amphetamine (mg/kg)		
0	3442 ± 309	63 ± 3
2	6960 ± 1094*	75 ± 4*
4	9082 ± 1087*	82 ± 3*
Modafinil (mg/kg)		
0	3027 ± 221	65 ± 2
20	5838 ± 376*	70 ± 2
40	7417 ± 581*	65 ± 3
80	8921 ± 765*	64 ± 4

Comparisons with the control groups (Duncan's test): *$P < 0.05$

tests. We wanted them to meet two main requirements: a) they had to be specifically adapted to measure anxiogenic activities, as most of the tests classically used in psychopharmacology for the assessment of anxiety in rodents are intended to detect anxiolytic effects, and b) we wanted, if possible, to measure locomotor activities simultaneously. In the test we used, the tendency of mice to explore a new environment was opposed by their aversion to bright environment (in the black and white compartments test), to empty space (in the elevated plus-maze), or to the lack of tactile cues (in the maze plus elevated test or in the open-field). As our purpose was to measure anxiogenic effects, the animals were placed at the beginning of the experiments in the less anxiogenic locations. Thus, the higher the degree of anxiety, the longer the delay before entering the white compartment, or the open arms, or before leaving the peripheral zone.

A marked increase in locomotor activity has been observed in mice injected with modafinil as well as in those injected with (+)amphetamine. At the doses tested, this increase was similar with both drugs, in the elevated plus-maze or in the open-field. In contrast, for every parameter used to assess anxiety, the results obtained for (+)amphetamine and modafinil were different. Indeed (+)amphetamine appears to be anxiogenic since it increased the latency of exploration of the white compartment and increased thigmotaxis, whereas it decreased the number of entries, the distance travelled and the time spent in the open arms of the elevated plus-maze. The last result differs from those obtained in mice by Lister (1987) but not from those obtained by Lapin (1993). The inconsistencies could be due to the use of different strains of mice: NIH Swiss (Lister), SHR Swiss (Lapin) and CD1 Swiss (our study). In rats, the anxiogenic properties of dexamphetamine were clearly demonstrated (Geller et al. 1960). Contrary to dexamphetamine, modafinil did not significantly modify any of these parameters. Therefore we

conclude that in mice modafinil does not share the anxiogenic effects of dexamphetamine. This result is consistent with those observed with modafinil in monkeys (Hermant et al. 1991). The different mechanisms of action of the two drugs may account for these observations. (+) Amphetamine is a catecholamine uptake inhibitor (Ferris et al. 1972) and a potent dopamine releaser (Chiueh et al. 1974). In a previous study using the black and white compartment test, we have shown that anxious states could be underlined by an increase in dopamine transmissions (Simon et al. 1992, 1993), involving especially D_1 dopamine receptors. Voltammetry and behavioral studies showed that the stimulant effects of modafinil on wakefulness could involve $alpha_1$ and beta noradrenergic receptors but not dopaminergic receptors (Duteil et al. 1990; Rambert et al. 1990; Lin et al. 1992). This neurochemical difference may explain why, for a similar level of psychic stimulation, (+)amphetamine increased the level of anxiety, whereas modafinil failed to modify it.

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