SURINABANT, A NEW CB1 RECEPTOR ANTAGONIST, DISPLAYS EFFICACY IN ANIMAL MODELS OF ATTENTION DEFICIT / HYPERACTIVITY DISORDER

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Introduction

- There is growing evidence that CB1 receptor antagonists may be useful for the treatment of attention deficit / hyperactivity disorder (AD/HD).
- We investigated the effects of the novel CB1 receptor antagonist, surinabant (SR147778), in several experimental situations that have been claimed to model certain aspects of the AD/HD pathology: spontaneous hyperactivity in mice for the hyperkinetic syndrome, delayed choice paradigm for impulsivity, and distractibility for attention deficit in rats. An EcoG profile was performed to compare the effects of surinabant on electrocortical activity with that of the psychostimulants, amphetamine and methylphenidate.

Material and Methods

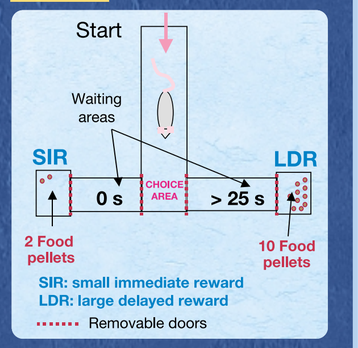
Spontaneous hyperactivity in BALB/c mice

- In a preliminary experiment, BALB/c mice (Iffa Credo, Les Oncins, France) were found to be still active after 2 hours of habituation (see the comparison with the other strains tested: C57BL/6, NMRI, DBA/2) [Fig.1A].
- BALB/c mice, weighing 16-20 g the day of testing, were individually placed into activity cages (20 cm diameter) equipped with two perpendicular photobeams, for a 1-hour period of habituation. Mice were then injected with the tested drug, put back into the activity cages and the number of interrupted photocell beams was measured during 1 hour. The effect of the compounds on muscular tone was also evaluated using a traction test: the mouse was suspended by the forepaws to an horizontal wire. The test was successfully accomplished when the hind paws touched the wire bar within 10 sec.
- Data were analyzed with a Kruskal-Wallis test.

Delayed choice paradigm in a T-maze in rats

- Male Wistar Rats (CERJ, France), 4 weeks old on their arrival, were housed 3-5 per cage. Tap water was provided *ad libitum*, food ration was 18-22 g per day and per animal.
- The T-maze [Fig. 2] is constructed from grey plastic tubing consisting of a starting runway and two arms each leading to a rectangular goal-box, one provided with the large reward (10 pellets, 45 mg Precision Foods pellets), and the other with the small reward (2 pellets). After being introduced into the starting runway, the rat reached the choice area. If the animal selected the arm leading to the large delayed reward (LDR), it was detained in this arm for a fixed duration (waiting delay – 25, 35, 45, 55, 65, 90 or 120 sec - adapted to each rat) before having access to the goal-box. If the animal selected the arm leading to the small reward (SIR), it had immediately access to the goal-box. Drug testing began when an animal selected the LDR on 0, 1 or 2 trial(s) out of 5 during one session and on 0 or 1 trial at the following session (control values, C1 and C2). Surinabant (dissolved in Tween80 + distilled water) or vehicle was injected ip, 30 min before test sessions (test values, T1 and T2). For each group of treatment, a paired Student t test was performed on the number of visits of the LDR arm during test sessions (T1+T2) vs control sessions (C1+C2).

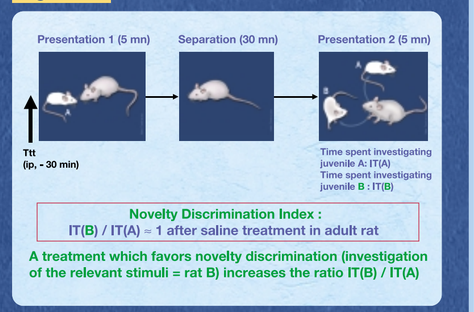
Figure 2



Spontaneous selective attention disturbance in rats

- Adult (160–200 g on arrival) and juvenile (3 weeks old, 45–50 g on arrival) male Wistar Han rats (Charles River, Saint-Aubin-les-Elbeuf, France) were housed individually, or five per cage, respectively.
- Experiments were performed during the dark phase. A first juvenile (A) was placed inside the home cage of an adult rat for a first presentation period (P1) of 5 min, and was returned to its home cage for 30 min. At the end of this 30 min inter-period interval, a second juvenile (B, novel) and the already known juvenile (A, familiar) were introduced into the adult home cage for a second 5-min period (P2) [Fig. 3]. Duration of investigative behaviour (nosing, sniffing, grooming, close following) expressed by the adult toward each of the two juveniles A and B (Investigation time (IT): IT(A) and IT(B), respectively) was recorded manually by an observer located in an adjacent room, using a video camera. During P2, a novelty discrimination index (NDI) is calculated: NDI = IT(B)/IT(A).
- Surinabant and methylphenidate were dissolved in Tween80 + saline and were injected ip, 30 min before P1.
- One way ANOVA was applied to the NDI.

Figure 3



Electrocorticoencephalogram (EcoG) profile in rats

- Male Sprague Dawley rats (Charles River, France) anesthetized with sodium pentobarbital, were mounted in a stereotaxic apparatus. Cortical electrodes were screwed on the bone over the sensori-motor, the visual cortice and the cerebellum (reference electrode), and then attached to a connector.
- After 3 weeks of post-operative recovery, rats were placed in a plexiglas cylinder (lights on : 07.00 a.m. to 07.00 p.m.). Recordings were performed from 10.00 a.m. to 04.00 p.m. during 3 consecutive days: control day (D1), drug day (D2), control day (D3).
- Analysis of the EcoG signal was performed automatically by means of a computerized system discriminating between the various sleep phases using spectral frequency analysis (Coherence, Deltamed Paris, France).
- Wakefulness is characterized by low voltage electrocortical activity (Θ rhythm; 4-8 Hz)
  - 4-6.5 Hz labels quiet wakefulness
  - 7-8 Hz labels wakefulness accompanied by locomotor activity
- Methylphenidate, *d*-amphetamine or surinabant, dissolved in saline with a drop of Tween80, were administered i.p., 15 min before recording, on day 2. Vehicle was administered on day 1 and day 3. The effects of the compound on the time spent in wakefulness were analyzed over a 6h period. Statistical analysis was carried out using one-way ANOVA applied to each hour, followed by Dunnett's tests (D2 or D3 vs D1).

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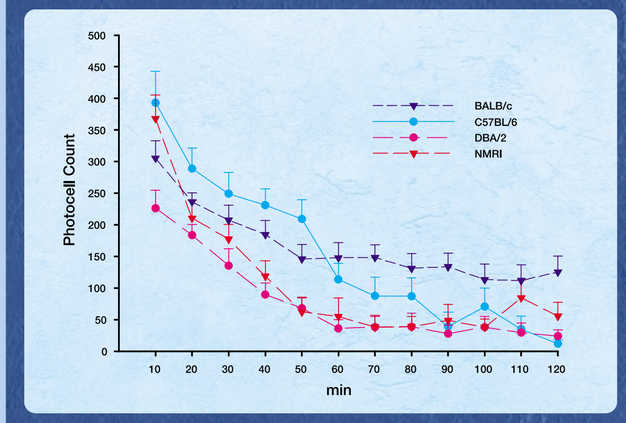
Conclusion

- **Together, these findings suggest that the CB1 receptor antagonist surinabant may represent a new therapeutic approach for the treatment of AD/HD, with an efficacy against the three main symptoms of this condition - namely the hyperkinetic syndrome, impulsivity and attention deficit. Furthermore, at high doses, surinabant should be devoid of amphetamine-like side effects, such as agitation.**

Results

Figure 1 A SPONTANEOUS LOCOMOTOR ACTIVITY IN DIFFERENT STRAINS OF MICE



BALB/c mice are still active after 2 hours of habituation.

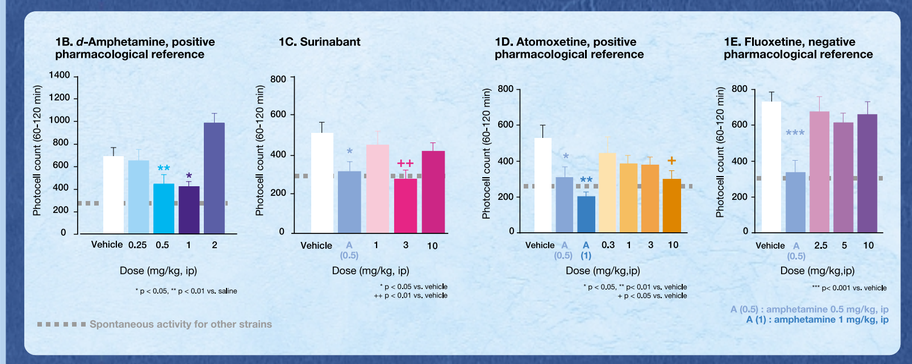
Table 1 HYPERKINETIC SYNDROME

	Doses (mg/kg ip)	Locomotor hyperactivity in BALB/c mice	Motor impairment (MED mg/kg)
• *d*-amphetamine	0.5-1	↘	> 1
• Surinabant	3	↘	> 10
• Atomoxetine	10	↘	> 20
• Fluoxetine	2.5-5-10	↔	> 10
• Diazepam	1-2-4	↘	1

↘ : decreased
↔ : not modified
MED : Minimal effective dose

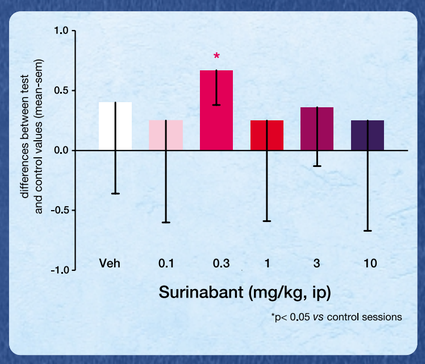
The hypolocomotor effect of surinabant, amphetamine or atomoxetine was not due to a non-specific change in muscular tone.

Figures 1 B, C, D, E HYPERKINETIC SYNDROME : SPONTANEOUS HYPERACTIVITY OF BALB/C MICE



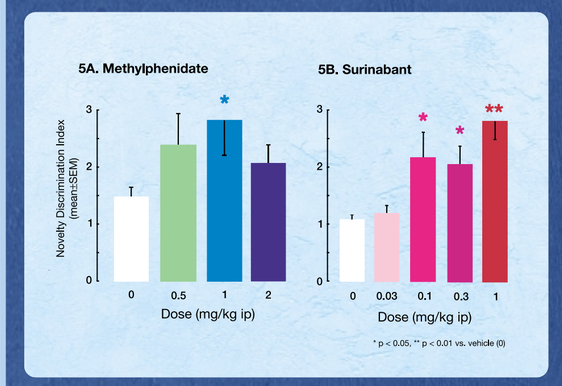
Surinabant (3 mg/kg, ip) was able to normalize spontaneous locomotor hyperactivity of BALB/c mice habituated to their environment [Fig. 1C], an effect shared by low doses of amphetamine (0.5-1 mg/kg) [Fig. 1B] and atomoxetine 10 mg/kg ip [Fig. 1D], but not by fluoxetine [Fig. 1E].

Figure 4 TOLERANCE TO A DELAYED REWARD



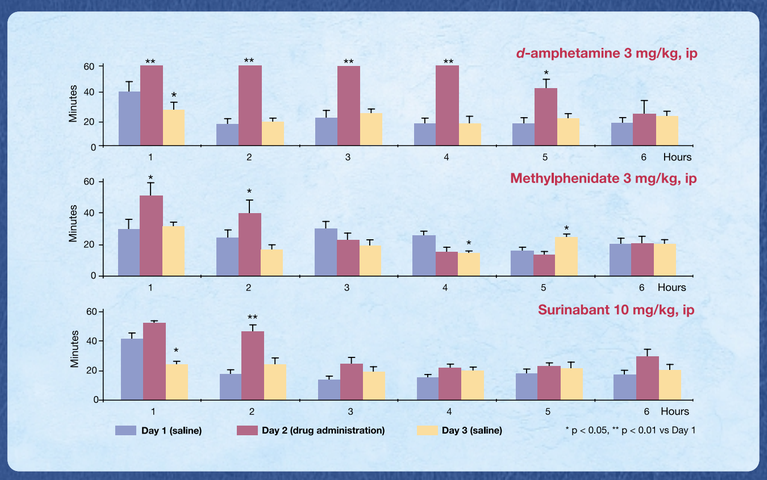
In the T-maze model of impulsivity in rats, surinabant (0.3 mg/kg, ip) increased the number of choices for a large but delayed reward vs. a small immediate one, an effect interpreted as an increased capacity to wait for a reward.

Figures 5 A, B SPONTANEOUS IMPAIRMENT OF SELECTIVE ATTENTION



Surinabant (0.1-1 mg/kg, ip), like methylphenidate, was found to increase selective attention of adult rats towards a novel juvenile, in the presence of a familiar one.

Figure 6 ECOG PROFILE: WAKEFULNESS DURATION AFTER D-AMPHETAMINE, METHYLPHENIDATE OR SURINABANT ADMINISTRATION IN ADULT RATS



A high dose of surinabant (10 mg/kg, ip) increased wakefulness duration, without any other EcoG modifications (no increase in the frequency of Θ rhythm).