THE CB1 RECEPTOR ANTAGONIST, RIMONABANT, REDUCED NICOTINE-SEEKING BEHAVIOUR MAINTAINED BY NICOTINE-ASSOCIATED CUES: BRAIN STRUCTURES INVOLVED

E. Kodas, C. Cohen, C. Louis and G. Griebel
Sanofi-Aventis, CNS Research Department, Bagneux, France

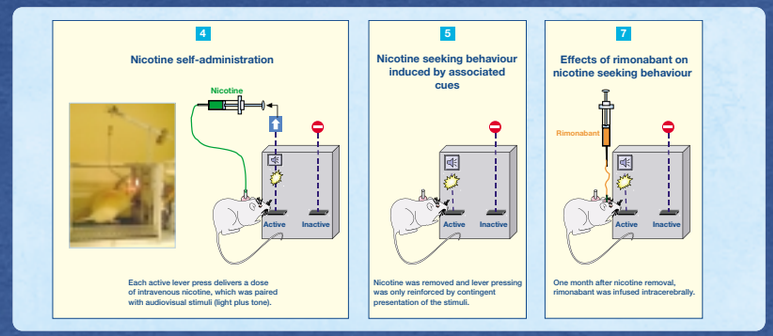
Introduction

- Environmental stimuli repeatedly associated with smoking acquire conditioned reinforcing properties. These stimuli become capable by themselves of inducing nicotine craving in abstinent smokers and lead to smoking relapse [1]. This feature of nicotine addiction has been extensively studied and modeled in animals. In rodents trained to self-administer nicotine, there is evidence indicating that cues previously associated to drug intake can also trigger by themselves nicotine-seeking behaviour [2].
- Rimonabant, a selective CB1 receptor blocker, has been demonstrated to reduce drugs intake [3,4]. Our group is the first having shown the effect of rimonabant in animal models of nicotine addiction. Firstly, rimonabant was shown to reduce intravenous self-administration of nicotine [5]. Secondly, rimonabant was demonstrated to reduce responding maintained by discrete cues associated with nicotine intake in rats [2]. Rats used in this latter experiment were previously subjected to extinction sessions during which cues-associated were presented but nicotine was not delivered.
- Subsequently to these findings, the purpose of the present investigation was to precise the cerebral regions involved in the nicotine-seeking-reducing action of rimonabant. The drug was directly infused into the shell of the nucleus accumbens (Shell Nacc), the basolateral amygdala (BLA) and the prelimbic cortex (PLCx), all brain structures heavily involved in drug seeking behaviour.

Material and Methods

EFFECTS OF RIMONABANT ON NICOTINE-SEEKING BEHAVIOUR

1. **SELECTION OF THE RATS:** Male Sprague-Dawley rats (200-220 g) were selected on their locomotor response to a stimulating dose of nicotine (0.6 mg/kg), in individual photocell activity cages equipped with two photocells placed perpendicularly. Only rats with an increase of locomotor activity of at least 80 photocell interrupts during 30 min session after nicotine injection were selected. During the entire procedure, animals were housed in single cage and restricted to a daily ration of 15-20 g of food.
2. **OPERANT TRAINING:** Animals were then trained to press the left lever in standard two-lever operant test chambers on a fixed-ratio 1 schedule of food reinforcement (5-8 days). Each left lever (active) press was reinforced by a food pellet. Responding on the right lever (inactive) had no consequence.
3. **CATHETERISATION OF THE JUGULAR VEIN:** Rats, anaesthetized with Zoletil® (60 mg/kg, i.p.), were then catheterised with a chronic silastic catheter in the right jugular vein.
4. **NICOTINE SELF-ADMINISTRATION:** Rats were trained to press on the left lever to receive intravenous nicotine (0.03 mg/kg/infusion), under conditions in which each infusion was paired with audiovisual stimuli presentation consisting of a 1sec tone and 20 sec light cues according to a concurrent fixed-ratio 1/time-out 20 sec schedule (session duration: 60 min/day, 12-15 days).
5. **CONDITIONED RESPONDING "EXTINCTION SESSION":** After nicotine self-administration acquisition (> 12 presses/h), rats were submitted to extinction sessions (15-20 days) i.e each active lever press was reinforced by contingent presentation of the stimuli previously associated to nicotine intake, but nicotine was not infused.
6. **IMPLANTATION OF GUIDE CANNULAE:** After conditioned responding has stabilized, guide cannulae (26 G, 15 or 12 mm length) were implanted bilaterally either into the shell Nacc (AP +1.7, ML ± 0.8, DV - 6.7 mm), the BLA (AP -2.87, ML ± 5.0, DV- 7.4 mm), or the PLCx (AP + 2.9, ML ± 1.0, DV = - 2.4 mm, tilt 7° angle), according to the atlas of Paxinos and Watson.
7. **EFFECT OF RIMONABANT ON CONDITIONED RESPONDING:** After a recovery period of 5 days, rats were trained again until they reached the same performance as before surgery (10-15 days).
   Rats received a bilateral intracerebral infusion of rimonabant (0.3 or 3 or 30 ng) dissolved in saline and 6% dimethylsulfoxide (DMSO) or vehicle, using stainless-steel microinjection cannulae (30 G). Rimonabant was infused in a volume of 0.5 µl/site at a rate of 0.25 µl/min. Three minutes later, conditioned responding was assessed for 1 h.
8. **HISTOLOGICAL CONTROL:** The correct location of the site of injection was microscopically checked for each rat using cresyl violet staining on brain slices.



EFFECTS OF RIMONABANT ON LOCOMOTOR ACTIVITY

- The effect of intracerebral infusion of rimonabant on locomotor activity was assessed in independent series of rats. These animals were housed, selected and implanted with guide cannulea according to the same procedures as those described above. Rats then received bilaterally either rimonabant (30 ng/0.5 µl/site) or vehicle and were placed in the activity cages. Locomotor activity was assessed during 1 hour.

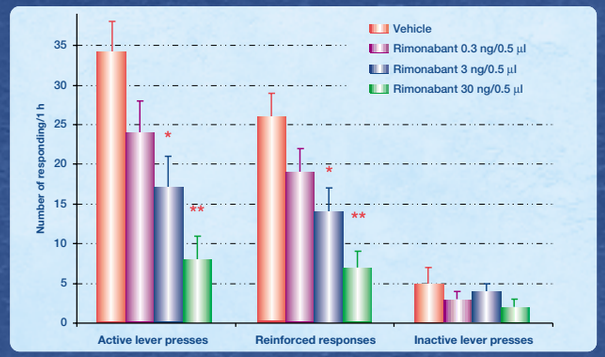
STATISTICAL ANALYSIS

- Data are expressed as the number of active lever presses, the number of inactive lever presses and the number of reinforced responses within each group. For each comparison, the control value was the mean number of presses performed by the vehicle group. Data obtained in the shell Nacc were analysed using one-way ANOVA followed by Dunnett's test. Data obtained in the BLA and the PLCx were compared using Student t-test.

Results

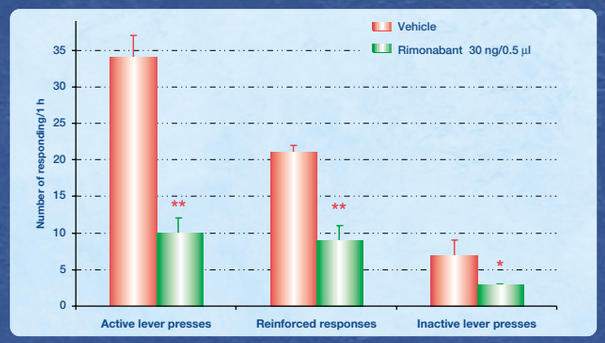
EFFECTS OF RIMONABANT ON NICOTINE-SEEKING BEHAVIOUR

Figure A RIMONABANT INFUSED INTO THE SHELL OF THE NUCLEUS ACCUMBENS REDUCED NICOTINE-SEEKING BEHAVIOUR IN A DOSE-DEPENDENT MANNER



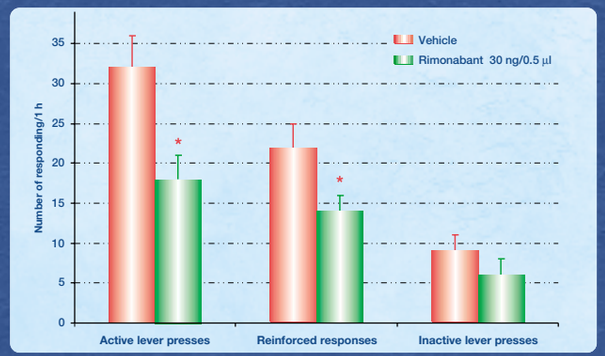
Each bar represents the mean (+ SEM) number of lever presses following infusion of rimonabant or vehicle (p<0.05, ** p<0.001, compared to vehicle animals). N = 7-11 rats per group.*

Figure B RIMONABANT INFUSED INTO THE BASOLATERAL AMYGDALA REDUCED NICOTINE-SEEKING BEHAVIOUR



Each bar represents the mean (+ SEM) number of lever presses following infusion of rimonabant or vehicle (p<0.05, ** p<0.001, compared to vehicle animals). N = 7 rats per group.*

Figure C RIMONABANT INFUSED INTO THE PRELIMBIC CORTEX REDUCED NICOTINE-SEEKING BEHAVIOUR



Each bar represents the mean (+ SEM) number of lever presses following infusion of rimonabant or vehicle (p<0.05, compared to vehicle animals). N = 12 rats per group.*

EFFECTS OF INTRACEREBRAL INFUSION OF RIMONABANT ON LOCOMOTOR ACTIVITY

Tableau 1 RIMONABANT DID NOT ALTER LOCOMOTOR ACTIVITY WHEN INFUSED INTO TESTED REGIONS

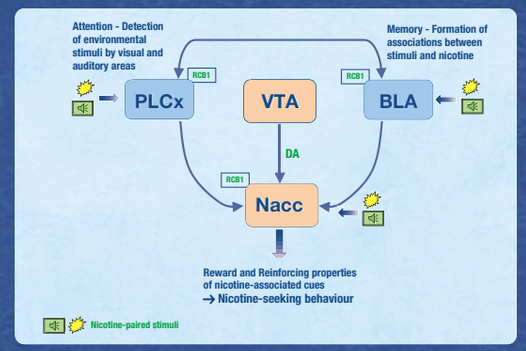
Mean of total number of photocell counts ± SEM after rimonabant or vehicle infusion. Locomotor activity was measured during one hour. N = 7-9 rats per group and per region.

Drugs \ Regions	Shell Nucleus accumbens	Basolateral amygdala	Prelimbic cortex
• Vehicle	377 ± 52	364 ± 26	344 ± 34
• Rimonabant 30 ng	412 ± 56	373 ± 61	402 ± 57

Rimonabant did not alter locomotor activity when infused into tested regions.

Conclusion

- **The shell of the Nacc, the BLA and the PLCx are targets of rimonabant for its anti-motivational effects towards nicotine seeking.**
- **Together with recent preclinical and clinical findings, these results confirm that rimonabant represents a promising therapeutic treatment for smoking cessation.**



Acknowledgments

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References

1 • Cagguila et al., Psychopharmacology 2002; 163(2):230-7
2 • Cohen et al., Neuropsychopharmacology 2005; 30(1):145-55
3 • Solinas et al., J Pharmacol Exp Ther. 2003; 306(1):93-102
4 • De Vries et al., Nat Med. 2001; 7(10):1151-4
5 • Cohen et al., Behavioural Pharmacology 2002; 13:451-463

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