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Short communication

Behavioral sensitization to heroin by cannabinoid pretreatment in the rat

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Abstract

The behavioral consequences of acute heroin challenge (0.5 mg/kg, s.c.) were measured in rats previously submitted to repeated administration of increasing doses of the synthetic cannabinoid receptor agonist, *R*(+)-[2,3-dihydro-5-methyl-3-[(morpholinyl)methyl]pyrrolo-[1,2,3-de]-1,4-benzoxazinyl]-(1-naphthalenyl) methanone mesylate (WIN55212.2) (first day 2 mg/kg, second day 4 mg/kg, third day 8 mg/kg) or vehicle. Heroin administration to rats pretreated with vehicle produced catalepsy. The same dose of heroin in WIN55212.2-pretreated rats was followed by a marked increase of locomotor activity with stereotyped and non-stereotyped behaviors. These effects were blocked by the opioid receptor antagonist, naloxone. These findings indicate that pretreatment with WIN55212.2 produces cross-sensitization to heroin in the rat. These changes might reflect long-lasting changes of receptor population or transcriptional mechanisms in the mesolimbic system. © 2001 Published by Elsevier Science B.V.

Keywords: Drug abuse; WIN55212.2; Heroin

Natural cannabinoids and synthetic drugs acting on central cannabinoid receptors (CB1) share many neurobiological properties with drugs of abuse, and opiates in particular. Cannabinoids activate mesolimbic dopamine neurons in the ventral tegmental area (Gessa et al., 1998), and increase extracellular dopamine levels in the shell of the rat nucleus accumbens through a μ_1 opioid receptor-mediated mechanism (Tanda et al., 1997). Moreover, cannabinoids ameliorate the signs of opiate withdrawal in morphine-dependent mice (Vela et al., 1995), and chronic administration of cannabinoids produces neuroadaptative changes within the limbic system that resemble those found for opiates and ethanol (Rodriguez de Fonseca et al., 1997). In the present study, we further investigated the interactions between cannabinoid and opioid systems by measuring whether pretreatment with the synthetic cannabinoid receptor agonist, *R*(+)-[2,3-dihydro-5-methyl-3-[(morpholinyl)methyl]pyrrolo [1,2,3-de]-1,4-benzoxazinyl]-(1-naphthalenyl) methanone mesylate (WIN55212.2), modifies the behavioral response to heroin challenge in the rat.

Male Sprague–Dawley rats (Harlan, Italy), weighing 250–270 g at the beginning of treatment, were housed in pair cages under standard temperature and humidity, on a 12-h light/dark cycle (light on 0700–1900 h), with free access to food and water. All procedures were carried out according to the guidelines of the National Institutes of Health for care and use of laboratory animals, and were approved by the local Ethical Committee. WIN55212.2 (Sigma, Italy) was dissolved as described previously (Tanda et al., 1997). Rats were given i.p. twice a day for 3 consecutive days increasing doses of WIN55212.2 (first day 2 mg/kg, second day 4 mg/kg, third day 8 mg/kg) or vehicle. All treatments were performed in the home cage. Heroin (Salars, Italy) was dissolved in saline with 0.05% acetic acid. Fourteen days after the end of treatment with WIN55212.2 or vehicle, rats were challenged with either vehicle or heroin (0.5 mg/kg, s.c.). The specificity of effects of heroin on opioid receptors was tested by pretreating rats with naloxone (0.1 mg/kg, s.c.) 10 min prior to heroin. Immediately after the administration of heroin or vehicle, rats were placed in open-field perspex activity cages (Ugo Basile, Italy), and locomotor activity was measured for 50 min as the number of 'bridges' that each animal made or broke with its paw between the 30 evenly spaced stainless steel bars of the cage floor. Simul-

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Table 1
Behavioral data for the different groups of animals

Group	Motor activity	Still (min)	Catalepsy (min)	Non-stereotyped (min)	Stereotyped (min)
Vehicle–vehicle	577 ± 89	23 ± 6	0	10 ± 4	3 ± 3
Vehicle–heroin	$229 \pm 31^{a,b}$	$0^{a,b}$	$32 \pm 7^{a,b}$	8 ± 4	4 ± 3
WIN–vehicle	601 ± 71	21 ± 7	0	9 ± 5	4 ± 3
WIN–heroin	$1074 \pm 109^{a,c,d,f}$	$0^{a,d,f}$	0^{c}	$32 \pm 9^{c,e}$	18 ± 4^{a}
Vehicle–naloxone–heroin	543 ± 49	20 ± 5	0	11 ± 5	6 ± 4
WIN–naloxone–heroin	598 ± 72	23 ± 7	0	10 ± 5	4 ± 2

Data represent means $\pm$ S.E.M., $n = 6$ for each group. See text for details of the group names.
[a] $P < 0.05$ different from vehicle–vehicle.
[b] $P < 0.05$ different from vehicle–naloxone–heroin.
[c] $P < 0.05$ different from vehicle–heroin.
[d] $P < 0.05$ different from WIN–naloxone–heroin.
[e] $P < 0.05$ different from WIN–vehicle.
[f] Bonferroni's *t*-test statistic.

taneously with motor activity counts, the following behavioral patterns were rated by an observer who was blind to treatments: opiate catalepsy (frozen posture and trunk rigidity), non-stereotyped activities (forward locomotion and rearing), and stereotyped activities (confined gnawing, grooming, licking, and sniffing). The period of time spent by each rat on each behavior was recorded as described previously (Cadoni and Di Chiara, 1999). Data were expressed as means $\pm$ S.E.M. The statistical analysis was carried out by means of a one-way analysis of variance followed by Bonferroni's *t*-test for multiple comparisons.

The results of the study are summarized in Table 1. Acute administration of heroin to vehicle-pretreated rats (vehicle-heroin group) produced catalepsy for most of the observation period; non-stereotyped and stereotyped activities in these animals were limited to the beginning and the end of the 50-min observation period. Conversely, acute administration of heroin to rats pretreated with WIN55212.2 (WIN–heroin group) was followed by a marked increase of non-stereotyped and stereotyped activities, with no catalepsy. In both cases, the effects of acute heroin administration were blocked by naloxone (vehicle–naloxone–heroin and WIN–naloxone–heroin groups, respectively). Finally, pretreatment with WIN55212.2 per se (WIN–vehicle group) did not produce behavioral differences from the control rats (vehicle–vehicle group). The different behavioral patterns observed were accompanied by significant differences of locomotor activity scores. Vehicle–heroin rats had significantly lower scores than did vehicle–vehicle rats, whereas WIN–heroin rats had significantly higher scores than did vehicle–vehicle, vehicle–heroin and WIN–vehicle groups (Table 1). Again, pretreatment with naloxone blocked the effects of heroin under all conditions.

These results show that pretreatment of rats with the synthetic cannabinoid receptor agonist WIN55212.2 in the rat significantly modifies the behavioral response to subsequent heroin challenge. Specifically, the effect consisted of a marked increase of the locomotor activity score for stereotyped and non-stereotyped activities, together with lack of catalepsy (Table 1). The effects of heroin were blocked by naloxone, suggesting that these behavioral changes were mediated through the action of the drug at endogenous opioid receptors. These changes were measured 14 days after the end of treatment with WIN55212.2, when pretreatment with WIN55212.2 per se (WIN–vehicle group) did not produce any behavioral change (Table 1). They were similar to the effects reported previously for morphine (Cadoni and Di Chiara, 1999) or heroin (Pontieri et al., 1997) sensitization. Thus, it is likely that the results obtained reflect long-lasting neuroadaptative changes in brain structures underlying the reinforcing properties of opiates (Di Chiara and North, 1992) occurring as the consequence of repeated exposure to WIN55212.2. Given the previous evidence of WIN55212.2-induced stimulation of dopamine transmission in the nucleus accumbens through opioid receptor-mediated mechanisms (Tanda et al., 1997), it is likely that repeated injection of the drug produces long-lasting changes of opioid system in receptors or in intracellular post-trascriptional mechanisms that contribute to the development of cross-sensitization to heroin.

While there is support from epidemiological data (Kandel, 1984), the question as to whether previous exposure to cannabinoids may predispose to heroin addiction is still debated at present. The present results show that pretreatment with WIN55212.2 produces cross-sensitization to subsequent administration of heroin, suggesting a link between cannabinoid and heroin abuse.

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