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Physiology & Behavior 69 (2000) 547–554

PHYSIOLOGY & BEHAVIOR

Inhibitory effects of the cannabinoid agonist HU 210 on rat sexual behaviour

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Received 1 October 1999; received in revised form 30 November 1999; accepted 14 January 2000

Abstract

The present study investigates the effects induced by the cannabinoid agonist HU 210 (25–100 μg/kg, administered intraperitoneally [i.p.]) on the following parameters: (a) sexual behaviour of male rats, categorised on the basis of seven consecutive mating pretests as sexually active (SA) and sexually inactive (SI) and (b) sexual receptivity of ovariectomised female rats displaying hormonally induced heat. The data obtained show that HU 210, administered in acute or subchronic mode (once daily for 7 and 14 days), impaired the copulatory pattern of SA rats in a dose- and mode-dependent manner, decreasing their sexual drive, mainly as represented by an increase in mount and intromission latencies, and affecting ejaculation mechanisms (represented as a decrease in intromission frequency and increase in ejaculation latency). After subchronic treatment with the highest dose had been suspended for 2 weeks, SA males' performance was still impaired. In SI rats, acute injections of the drug (25 and 50 μg/kg, i.p.) at the higher dose increased contact latency and decreased genital exploration time towards the female. Acute HU 210 (25–100 μg/kg, i.p.) also inhibited female sexual behaviour, potently reducing lordosis quotient and lordosis intensity.

Keywords: Cannabinoids; Huzio; Sexual Behavior; Rat

1. Introduction

The discovery of multiple cannabinoid (CB) receptors [15,34,36] and of drugs that interact selectively with them [9,37,38,46] has stimulated research into the neurochemical mechanisms underlying various expressions of animal behaviour that might be modulated by this system [43]. However, despite some reports of the aphrodisiac effects of marijuana and others that describe cannabis-induced human sexual dysfunctions [5,43], there are few properly controlled studies relating CB effects to sexual behaviour [16,29]. Acute administration of Δ^9-tetrahydrocannabinol (Δ^9THC), the main active component of marijuana [30], interferes with rat copulatory behaviour [42], decreasing the percentage of copulating animals and increasing the latency periods to mount and intromit [44]. Δ^9THC also affects the development of reproductive functions in male mice [11], suppressing adult copulatory activity and reducing fertility-inhibiting testicular steroidogenesis [10]. As far as we know, no further data exist on the activity of novel CB compounds on sexual drive, ejaculatory mechanisms, and receptivity of rats. A modification of these behaviours would be amply justified because one of the main neurotransmitters involved in CB activity is dopamine (DA) [6,16,45,46], and it is well known that DA exerts a key role in modulating sexuality [7,12,20,41].

Our work was therefore designed to examine the influence exerted by the potent synthetic CB agonist HU 210 [(−)11-OH-Δ^8-THC-DMH] [37,38] on the sexual behaviour of male and female rats. HU 210 was found to be sevenfold more potent than Δ^9THC at binding to the neuronal CB receptor and about 12-fold more potent than (+)11-OH-Δ^8-THC-DMH in inhibiting adenylate cyclase [37]. Stereoselectivity of the compound was demonstrated in biological measures in animal tests [40], and its activity on DAergic systems has been documented [13,26].

This study was conducted with (a) male rats categorised on the basis of seven consecutive mating pretests as sexually active (SA) and or sexually inactive (SI) [24] and (b) ovariectomised female rats displaying hormonally induced heat.

2. Materials and Methods

2.1. Animals

The subjects were male and female Wistar rats (Harlan Nossan, Udine, Italy) weighing 230–250 g at the outset. They were housed in same-sex groups of six, with food and water provided ad lib, on a 12-h light cycle (from 0700 to 1900 h) for at least 2 weeks prior to the start of the tests.

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0031-9384/00/$ – see front matter
PII: S0031-9384(00)00214-6

All female rats were ovariectomised under ketamine plus xylazine anesthesia (120 + 2 mg/kg intraperitoneally [i.p.]) and brought into oestrus with estradiol benzoate (30 μg/rat, s.c.), followed 48 h later by progesterone (0.5 mg/rat, s.c.), and used 4–5 h thereafter. They were screened with sexually experienced males; only those exhibiting good sexual receptivity (solicitation behaviour and lordosis in response to mounting) and no rejection behaviour were used. The regulations in effect regarding the care of animals for scientific purposes (Community European Ethical Council 86/609; Italian Decree Law 27/01/92, #116) were strictly complied with.

2.2. General behavioural procedure

All the experiments were performed between 0900 and 1400 h in a sound-attenuated, air-conditioned room, where the animals were monitored by trained observers unaware of the experimental design. The controls were handled in the same way as the treated animals.

2.2.1. Evaluation of male sexual behaviour

The male rats were transferred singly to an observation cage (40 × 30 × 34 cm) into which, after a 3-min adaptation period, a receptive female was introduced. Male copulatory behaviour was evaluated as in previous works [22,23]. The parameters considered were as follows: mount and intromission latencies (ML and IL: the time from the introduction of the female until the first mount and intromission, respectively), mount and intromission frequencies (MF and IF: the number of mounts and intromissions preceding ejaculation), ejaculation latency (EL: the interval between the first intromission and ejaculation), and postejaculatory interval (PEI: the time between the first ejaculation and the next intromission). After the PEI, the test was considered complete. Tests were discontinued when IL or PEI were greater than 15 min or when EL was greater than 30 min; in this case, ML, IL, and PEI values were arbitrarily put at 900 s and EL at 1800 s. Only those animals that completed at least the last four training mating tests, out of the seven conducted at 4-day intervals, were considered sexually active (SA) ($n = 25$). Those that never mounted or intromitted during these tests were considered sexually inactive (SI) ($n = 18$), and those that displayed discontinuous activity were discarded. In SI rats, the following parameters were evaluated in Tests 6 and 7: (a) latency to the first contact (CL), calculated as the time from the introduction of the female on the opposite side of the cage until the first voluntary contact by the male and (b) total time spent in genital exploration by the male (GET), which was recorded from the introduction of the female until the 15th minute [24].

2.2.2. Experimental protocol for male sexual behaviour

After having verified the consistency of the SA and SI rats' sexual behaviour in Tests 6 and 7, both categories of animals were divided into subgroups (not statistically different for any of the parameters considered), which were treated acutely with vehicle or with HU 210 at the doses of 25 and 50 μg/kg, or, only in the case of SA rats, of 100 μg/kg, 40 min before Test 8. SA or SI rats were transferred singly to glass observation cages, and their sexual behaviour towards a receptive female, presented 3 min later, was monitored. Thereafter, SA animals were returned to their home cages and received vehicle or HU 210 as before at the same doses, once daily for 14 consecutive days.

The copulatory behaviour of SA rats, assigned to the different treatment groups, was evaluated after 7 days (Test 9) and 14 days (Test 10) of subchronic treatment as well as at 2 weeks after the last injection (abstinence; Test 11).

2.2.3. Evaluation of female sexual behaviour

During behavioural tests, naive females received eight mounts in the observation cages (40 × 30 × 34 cm); data from the first two were discarded. We considered the following as indices of sexual responsiveness: the Lordosis Quotient (LQ) = (number of lordosis response score of 2 or 3 per 6 mounts) × 100 and the Lordosis Rating (LR) = (sum of 0, 1, 2, or 3 response scores per 6 mounts) [4]. The intensity of each lordosis was rated by the observers unaware of the animal treatment, using a 4-point scale (score, 0–3) [33]: 0, *absence of lordosis*; 1, *marginal lordosis* (slight spinal flexion, slightly raised head, and hips with tailbase elevated from the floor); 2, *normal lordosis* (spinal flexion, head at an approximate angle of 30° from the horizontal, front paws placed slightly forward, and hind legs straightened up stiffly); and 3, exaggerated lordosis (pronounced spinal flexion, head at an angle of 45° or more from the horizontal) [33]. During the test period, the percentage of animals displaying proceptive behaviour, namely ear wiggling and hopping, was also recorded.

2.2.4. Experimental protocol for female sexual behaviour

After having verified the sexual responsiveness of all females ($n = 24$; time 0), they were divided into 4 subgroups (not statistically different for LQ and LR), which were treated acutely with vehicle or HU 210 at the doses of 25, 50, or 100 μg/kg and were observed 30, 60 and 120 minutes later.

2.3. Drugs and treatments

HU 210 (Tocris–Cookson, Bristol, UK) was dissolved in saline with a drop of Tween 80 (0.1%); ketamine (Farmaceutici Gellini, Aprilia, Italy) and xylazine (Bayer, Milano, Italy) were freshly dissolved in saline. All the solutions had a concentration that allowed the administration of 1 mL/kg i.p. Estradiol benzoate and progesterone (Sigma Chemical Co., St. Louis, MO) were dissolved in corn oil, and both were injected subcutaneously (s.c.), 0.2 mL per rat. The doses and the pretreatment times used for HU 210 were chosen on the basis of a previous work [25].

2.4. Statistical evaluation

Data are presented as means ± SEM of the values recorded in the animals of each treatment group and, in the case of SA male rats, as the number of animals displaying a

specific behaviour (ML, IL, EL, and PEI) after treatment. The values of the mating parameters (expressed in seconds or in the number counted) in Pretest 7 were compared with those obtained in Tests 8, 9, 10, and 11 after acute, subchronic treatment as well as during abstinence and were analyzed using Kruskall–Wallace test (KW) followed by Mann–Whitney U-test. The CL and GET of SI rats in Test 7 were compared with those presented by the same animals in Test 8, after acute treatment with vehicle or HU 210, and analyzed by ANOVA followed by Student–Newman–Keuls (SNK) test and Student's *t*-test for paired data. In the case of experiments with females, the values of LQ and LR were analyzed by ANOVA or by ANOVA for repeated measures, followed by SNK test. The percentage of animals displaying proceptive behaviour was analysed by χ^2 test. For all statistical evaluations, the level of significance was set at $p < 0.05$.

3. Results

Figure 1 shows that HU 210 at the dose of 25 μg/kg did not significantly modify the copulatory behaviour of SA rats in any of the experimental conditions, although there was a trend to increase ML, IL and EL after subchronic treatments.

At 50 μg/kg (Fig. 2), the drug impaired some animals'

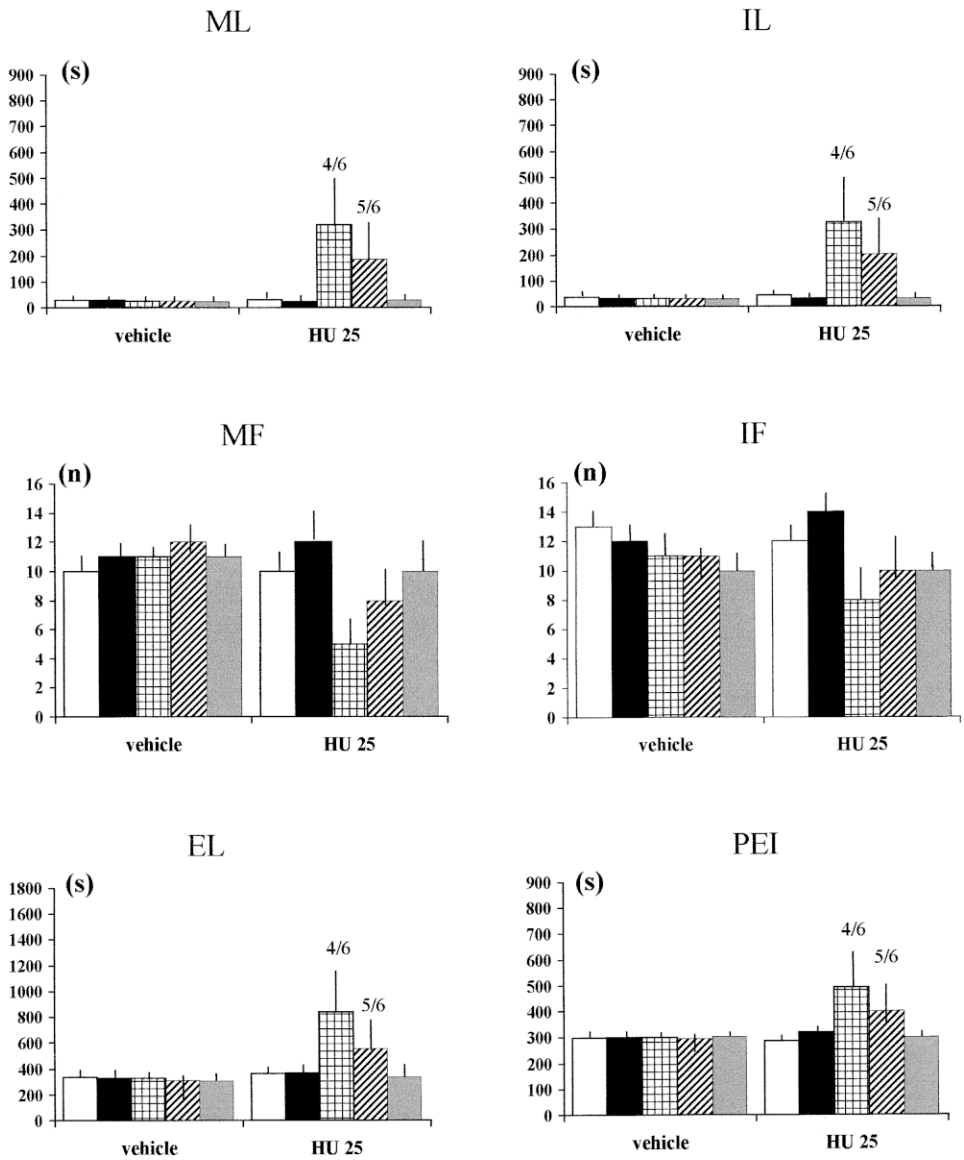

Fig. 1. Effect of HU 210 at 25 μg/kg on copulatory behaviour of sexually active male rats. HU 210 (HU) or vehicle were injected intraperitoneally 40 min before the tests. Tests were as follows: Test 7 (unshaded columns) = base, Test 8 (completely solid columns) = acute treatment, Test 9 (cross-hatched columns) = 7 days of subchronic treatment, Test 10 (diagonally hatched columns) = 14 days of subchronic treatment, and Test 11 (lightly solid columns) = abstinence (14 days). ML, IL, EL = latency to the first mount, intromission, and ejaculation, respectively; MF, IF = mount and intromission frequency, respectively; PEI = postejaculatory interval. Histograms are the means ± SEM of the values recorded in the treatment groups. The number of animals displaying the behaviour in question is reported over the histograms.

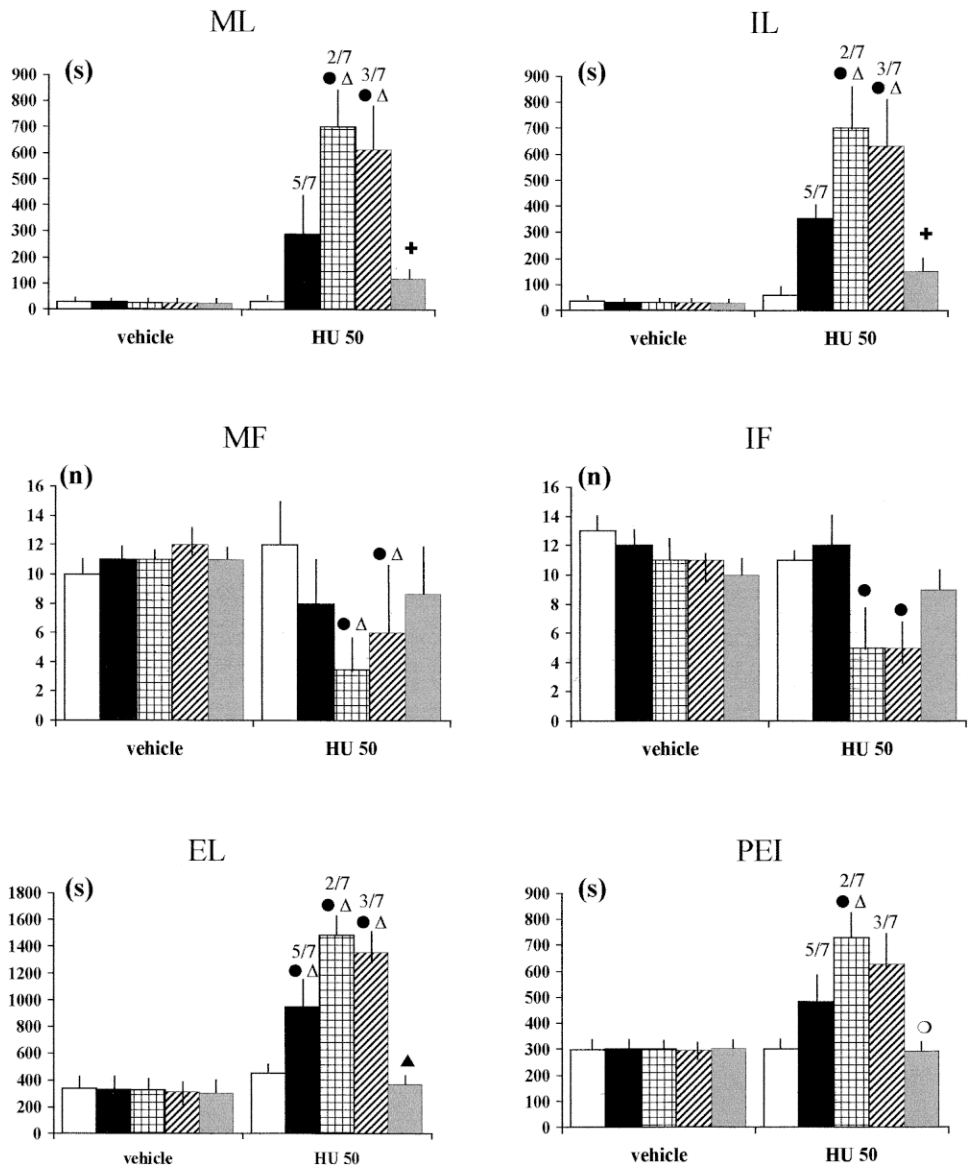

Fig. 2. Effect of HU 210 at 50 μg/kg on copulatory behaviour of sexually active male rats. Tests were as follows: Test 7 (unshaded columns) = base, Test 8 (completely solid columns) = acute treatment, Test 9 (cross-hatched columns) = 7 days of subchronic treatment, Test 10 (diagonally hatched columns) = 14 days of subchronic treatment, and Test 11 (lightly solid columns) = abstinence (14 days). ML, IL, EL = latency to the first mount, intromission, and ejaculation, respectively; MF, IF = mount and intromission frequency, respectively; PEI = postejaculatory interval. Histograms are the means ± SEM of the values recorded in the treatment groups. The number of animals displaying the behaviour in question is reported over the histograms. Significantly different from the same animals: filled circle in Test 7; unfilled circle in Test 9; filled plus sign in Tests 9 and 10; and filled triangle in Tests 8, 9, and 10 (Kruskall–Wallace followed by Mann–Whitney *U*-test). Unfilled triangle, significantly different from respective vehicle-treated rats (Mann–Whitney *U*-test).

ability to perform the mating tests, exerting a significant interference in all the parameters considered, namely ML (KW: H = 16.2, $p = 0.003$), IL (KW: H = 16.2, $p = 0.003$), MF (KW: H = 12.83, $p = 0.01$), IF (KW: H = 11.5, $p = 0.02$), EL (KW: H = 20.8, $p < 0.001$), and PEI (KW: H = 11.9, $p = 0.02$). The negative effect on mating was more marked after subchronic than after acute treatment, when only EL was significantly affected compared with the basal value. A complete recovery was achieved after 2 weeks of abstinence.

HU 210 at 100 μg/kg (Fig. 3) potently counteracted copulatory behaviour in SA rats, in all experimental conditions, significantly affecting ML (KW: H = 28.3, $p < 0.001$, IL (KW: H = 30.3, $p < 0.001$), MF (KW: H = 31.2, $p < 0.001$), IF (KW: H = 30.2, $p < 0.001$), EL (KW: H = 37.5, $p < 0.001$), and PEI (KW: H = 41.8, $p < 0.001$). After acute injection of the CB, only two of six animals displayed a few mounts and intromissions, but none reached ejaculation during the test period. Sexual impairment grew more marked after 7 days of subchronic treatment, which abolished mating in all rats. The HU 210 (100 μg/kg) –induced effects wore off during abstinence, during which only IL was found still to be prolonged.

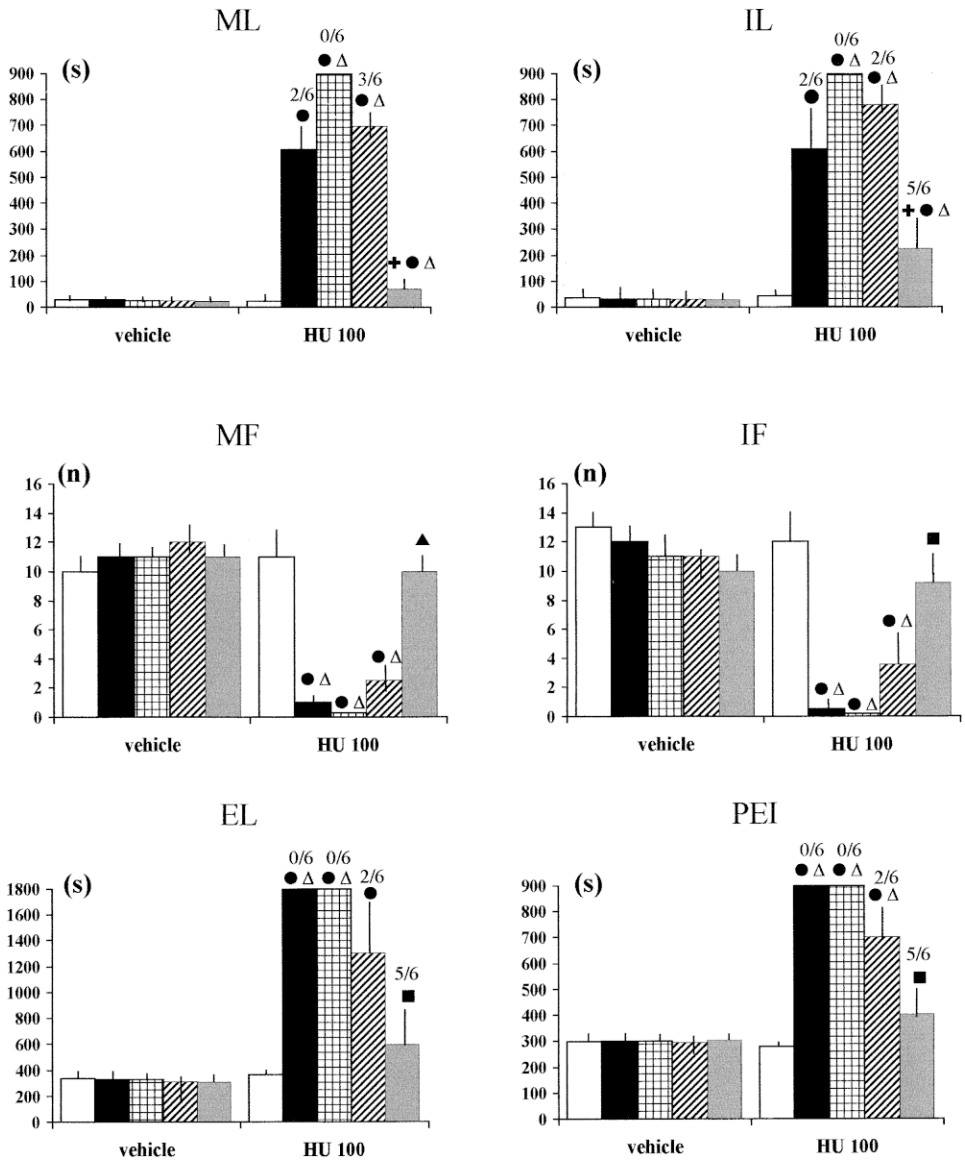

Fig. 3. Effect of HU 210 at 100 μg/kg on copulatory behaviour of sexually active male rats. Tests were as follows: Test 7 (unshaded columns) = base, Test 8 (completely solid columns) = acute treatment, Test 9 (cross-hatched columns) = 7 days of subchronic treatment, Test 10 (diagonally hatched) = 14 days of subchronic treatment, and Test 11 (lightly solid columns) = abstinence (14 days). ML, IL, EL = latency to the first mount, intromission, and ejaculation, respectively; MF, IF = mount and intromission frequency, respectively; PEI = postejaculatory interval. Histograms are the means ± SEM of the values recorded in the treatment groups. The number of animals displaying the behaviour in question is reported over the histograms. Significantly different from the same animals: filled circle in Test 7; filled square in Tests 8 and 9; filled plus sign in Tests 9 and 10; and filled triangle in Tests 8, 9, and 10 (Kruskall–Wallace followed by Mann–Whitney *U*-test). Open triangle, significantly different from respective vehicle-treated rats (Mann–Whitney *U*-test).

In SI rats, CL and GET were evaluated after acute HU 210 at 25 and 50 μg/kg (Table 1). Only the higher dose had an adverse effect on SI sexual behaviour, significantly increasing CL [$F(2, 15) = 22.8$, $p < 0.001$] and reducing GET [$F(2, 15) = 11.2$, $p = 0.001$)] with respect to vehicle- and HU- (25 μg/kg) treated animals.

Fig. 4 reports the influence of HU 210 (25–100 μg/kg) on LQ and LR in female rats. HU 210 at 25 μg/kg reduced both LQ and LR from 60 min onwards [$F(3, 15) = 6.9$, $p = 0.004$; $F(3, 15) = 6.7$, $p = 0.004$, respectively]. Inhibition of female sexual behaviour was confirmed at 50 and 100 μg/kg because the two doses produced similar reductions after 30 min onwards, both on LQ [$F(3, 15) = 10.7$, $p < 0.001$ and $F(3, 15) = 24.1$, $p < 0.001$, respectively] and LR [$F(3, 15) = 10.3$, $p < 0.001$ and $F(3, 15) = 47$, $p < 0.001$, respectively].

Table 2 shows that the disruption on proceptive behaviours produced by the two highest doses (HU 210: 50 and 100 μg/kg) started at 30 min ($\chi^2 = 15.1$, $p = 0.002$; $\chi^2 = 18.9$, $p < 0.001$).

Table 1
Effect of acute HU 210 on contact latency (CL) and genital exploration time (GET) of sexually inactive (SI) male rats

Test number	Treatment (μg/kg)	CL (s)	GET (s)
7	—	8 ± 3	98 ± 22
8	Vehicle	7 ± 2	103 ± 18
7	—	5 ± 2	110 ± 15
8	HU 25	6 ± 2	90 ± 20
7	—	7 ± 2	86 ± 16
8	HU 50	222 ± 45*#	6 ± 4*#

HU 210 (HU) or vehicle was i.p. injected 40 min before the 8th test. Values are the means ± SEM of CL and GET for animals in each treatment group ($n = 6$).

* Significantly different from the same animals in Test 7 (Student's *t*-test for paired data);

Significantly different from vehicle- and HU 25–treated rats in Test 8 (ANOVA followed by Student–Newman–Keuls test).

4. Discussion

This study demonstrates that the CB agonist HU 210 exerts sexual inhibition in male and female rats. Because our tests were conducted during the light phase, the possibility that the effects obtained after HU 210 are dependent on the experimental procedure adopted cannot be excluded, although it has been found that after appropriate training, copulatory tests were regularly performed in the light phase by a high percentage of rats. Similar behavioural modifications were obtained after drug treatments in the nocturnal active phase [18,21,31].

HU 210–induced effects on copulation were noticeable, although in the light of the results obtained on subsequent tests with females, it could be expected that male sexual behaviour also might have been maximally compromised later than tested. Sexual impairment in the mating tests involved both the precopulatory phase, the most common measures of which are ML and IL, and the consummatory phase, mainly represented by IF and EL [2,41]. In the case of the SA males, subchronically treated at 100 μg/kg, normal IF and EL, associated with prolonged ML and IL, after 2 weeks of abstinence, would suggest that at this dose, the effect was long lasting and that ejaculatory mechanisms recover before sexual arousal. One plausible reason for the sexual failure induced by the drug is that it strictly depends on its sedative activity [25,38], which, as is known, is shared by all CBs [16,43,46]. However, sedation cannot entirely justify the phenomenon because sexual disruption worsened after subchronic treatment, whereas it has been demonstrated that tolerance does develop to the inhibition of motor activity produced by CBs and HU 210 [16,26,43].

The adverse effect of HU 210 on the sexual drive of SA male rats was confirmed in SI animals, in whom CL and GET, as useful indicators of their modest sexual activity [24,31], were enhanced and reduced, respectively, by acute HU 210 at 50 μg/kg.

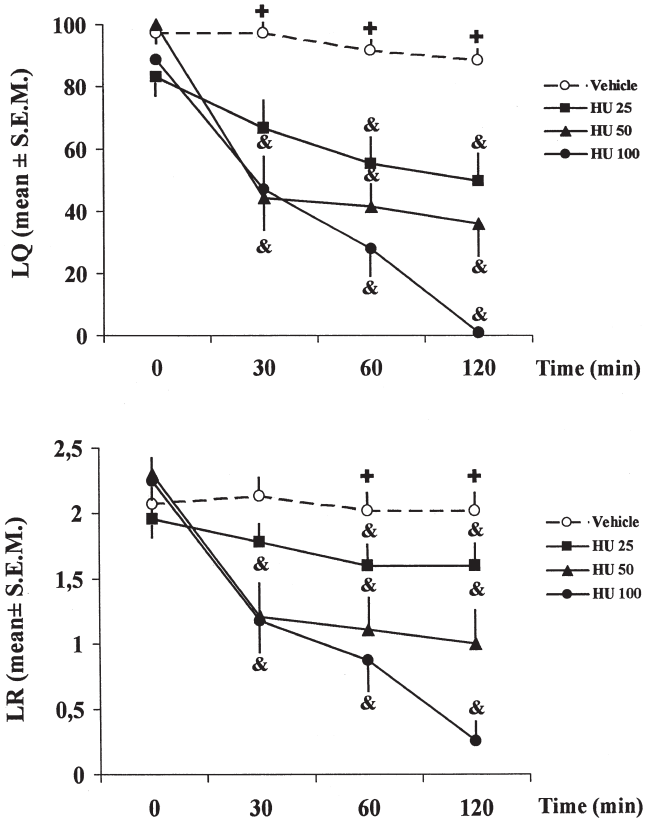

Fig. 4. Effect of HU 210 on lordosis quotient (LQ) and on lordosis rating (LR) in female rats. HU 210 (HU) or vehicle was injected intraperitoneally immediately after the first test (time 0) and subsequent tests were performed 30, 60, and 120 min later. Values are the means ± SEM of the LQ and LR displayed by the animals ($n = 6$) in each treatment group. Ampersand, significantly different from the same animals at time 0 (ANOVA for paired data followed by SNK test); filled plus sign, significantly different from all treatment groups in the same test (ANOVA followed by SNK test).

Therefore, although findings of enhancement of DAergic neurotransmission after CBs would fit in with the popular reputation of marijuana and hashish as sexual stimulants, our results show that the influence of HU 210 on male rat sexual behaviour was quite different from that expected as

Table 2
Effect of acute HU 210 on proceptive behaviour in female rats

Treatment (μg/kg)	Pretest[a]	30 min	60 min	120 min
Vehicle	4/6	5/6	4/6	4/6
HU 25	5/6	5/6	4/6	4/6
HU 50	5/6	1/6*	0/6*	0/6*
HU 100	5/6	0/6*	0/6*	0/6*

HU 210 (HU) or vehicle were i.p. injected immediately after the first test (time 0), and posttests were performed 30, 60, and 120 min later.

[a] Data are presented as number of animals displaying proceptive behaviour per group.

* Significantly different from the same treatment group at time 0 and from all treatment groups in the same test (χ^2 test).

dependent on DA central activation [1,3,21,23,28]. Not only were ML and IL, which reflect sexual arousal in SA rats, prolonged by the drug, but ejaculatory mechanisms were inhibited, unlike that which occurs after acute DA agonists, which typically reduce both IF and EL, thus facilitating ejaculation [22,41]. Present experiments demonstrate that the reduction in IF by HU 210 depends on the failure of a certain percentage of rats to display intromissions and that EL consequently was strongly delayed. On the whole, our results are in line with the reported negative influence exerted by Δ^9-THC in male rat copulatory behaviour [44]. As Murphy et al. [44] also described a concurrent modification in rat neuroendocrine responses, it is quite possible that the complex set of hormonal changes provoked by HU 210 administration [8] similarly played a crucial role in mating impairment.

Our work shows that HU 210 potently inhibits female sexual behaviour as well. Here again, any neurochemical interpretation must remain purely speculative for the present. It is known that while lordosis, a behavioural response requiring immobility, is inhibited or suppressed by DA and DA agonists, active components of female receptivity (proceptive behaviour) are facilitated by the same [7]. HU 210 inhibited lordosis, similar to action of DA agonists at high doses that increase DA function and motor activity [7], but in the case of the CB, locomotor stimulation is to be excluded as a cause of low sexual responsiveness. Neither can a state of marked hypoactivity explain the phenomenon, for some females were seen actively to reject the males and react aggressively when mounted. Moreover, HU 210 antagonism of proceptive behaviour is in accordance with the sedative properties of the CB but is incompatible with an involvement of DA stimulation. Because it is known that anxiety can have disruptive effects on normal behaviour and animal performance in several tests [27], the possibility cannot be excluded that the negative influence on male and female rat sexual behaviour also depends on a highly charged emotional state brought about by the drug. The correlation between CBs and anxiety or emotionality has long been proposed [35,39] and is supported by experimental findings on animals, where CBs induce a potent secretion of adrenocorticotropin hormone [17] and corticotropin-releasing factor (CRF) [14], both of which play key roles in stress [19]. Moreover, the attenuation exerted by the CRF antagonist D-phenyl CRF12-41 on rat anxiogenic responses to HU 210 [14] strongly suggests the mediation of endogenous CRF systems in these effects. Abnormal behavioural patterns, which are accepted as pointers of modified emotionality or of a state of fear, have been elicited in rodents by high doses of HU 210 [32], which also produced a deterioration of rat learning in a water maze task [27].

In conclusion, regardless of any premature neurochemical or anthropomorphical interpretation of the phenomena observed, our findings show that the CB agonist HU 210 negatively interferes with male and female rat sexual behaviour and that the effect is dose and time related.

Acknowledgments

This work was supported by grants from Ministero della Università e della Ricerca Scientifica e Tecnologica.

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