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Effects of the cannabinoid receptor agonist, HU 210, on ingestive behaviour and body weight of rats

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Abstract

The effect of the synthetic cannabinoid receptor agonist, (−)11-hydroxy-Δ^8-tetrahydrocannabinol-dymethylheptyl (HU 210), on rat body weight and eating and drinking behaviour was examined. In Experiment 1, the drug (25, 50 or 100 μg/kg), sub-chronically administered for 4 days, produced a dose- and time-dependent loss of body weight that, at the highest dose, was not regained by 7 days after the drug was stopped, and remained markedly below that of vehicle-treated animals. In Experiment 2, food and water intakes, which were evaluated in fasted rats, tested as in Experiment 1, were significantly inhibited only by the dose of 100 μg/kg, and this effect was still present 7 days after the last injection of the drug. The possibility that the effects observed are not directly dependent on the control of appetite and might be ascribable to stress-related phenomena is discussed.

Keywords: Cannabinoid; HU 210; Ingestive behaviour; Body weight, rat

1. Introduction

The identification of central cannabinoid CB_1 receptors for cannabinoids (Devane et al., 1988; Herkenham et al., 1991; Mailleux et al., 1992) and of endogenous ligands such as anandamide (Mechoulam et al., 1998), indicates the existence of a brain cannabinoid system, whose physiological role still remains unraveled. One of the most common effects of marijuana or hashish intoxication in humans is an increased appetite (Abel, 1971; Greenberg et al., 1976; Foltin et al., 1988; Mattes et al., 1994), particularly for sweet foods (Foltin et al., 1988). However, the modulation of feeding by the cannabinoid system is not well established and considerable discrepancies emerge from the studies of cannabinoid influence on animal ingestive behaviour. While some authors reported hyperphagia in rats after Δ^9-tetrahydrocannabinol (Trojniar and Wise, 1991; Williams et al., 1998), others agree on a clear anorexic effect elicited in animals by the same compound and by new synthetic cannabinoids (Dewey, 1986; O'Connell et al., 1987). Again, it has been demonstrated that anandamide and Δ^9-tetrahydrocannabinol have no significant influence on chow consumption in ad lib fed rats (Crawley et al., 1993; Graceffo and Robinson, 1998).

As previous studies had shown marked body weight loss in the animals treated with the potent cannabinoid CB_1 receptor agonist, (−)11-hydroxy-Δ^8-tetrahydrocannabinol-dimethylheptyl (HU 210) (Little et al., 1989; Howlett et al., 1990), the purpose of the present work was to investigate in more detail the possible modifications exerted by this novel cannabinoid on rat body weight, simultaneously assessing feeding and drinking behaviour. HU 210 is a synthetic compound, which was found to be sevenfold more potent than Δ^9-tetrahydrocannabinol for binding to the neuronal cannabinoid CB_1 receptor and about 12-fold more potent than (+)[11-hydroxy-Δ^8-tetrahydrocannabinol-dimethylheptyl] for inhibiting adenylate cyclase (Howlett et al., 1990). Stereoselectivity of the drug had been demonstrated in animals earlier (Mechoulam et al., 1988).

2. Materials and methods

2.1. Animals

The subjects were male Wistar rats (Harlan Nossan, Correzzana, MI, Italy) weighing 230–250 g at the outset.

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They were housed in individual plexiglass cages with food and water ad libitum, and on a 12-h light cycle, from 0700 to 1900 h, under controlled environmental conditions (temperature 22 ± 2°C; humidity 60%) for at least 1 week prior to the beginning of the tests. The regulations in force regarding the care of animals for scientific purposes (CEE Council 86/609; Italian DL 27/01/92 no. 116) were strictly complied with.

2.2. General behavioural procedure

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

2.3. Experiment 1: evaluation of body weight

Thirty-two naive rats were randomly assigned to four treatment groups ($n = 8$). Their body weight was recorded before and after intraperitoneal (i.p.) injections with HU 210 at 25, 50 and 100 μg/kg or vehicle. Four evaluations were performed, all at 0900 h, namely, 24 h after the 1st treatment, 1, 4 and 7 days after the last injection of a 4-day sub-chronic treatment. Body weight was expressed as percent of that on the day prior to the start of treatments.

2.4. Experiment 2: evaluation of food and water intake

Thirty naive rats were randomly assigned to four treatment groups that were treated as in Experiment 1; the number of animals for each group is specified in the legend of Figs. 2–4. Each group was tested three times: (1) after acute treatment, (2) after sub-chronic treatment (once daily for 4 days) and (3) 7 days after the last injection of sub-chronic treatment. On each test day, after food and water deprivation for 20 ± 2 h, the rats were i.p. injected with vehicle or HU 210 at the various doses and immediately afterwards, returned to their home cages where preweighed food pellets and water bottles were provided 50 min later. Food and water intakes were evaluated 1, 2 and 24 h after presentation by weighing food pellets, spillage and water bottles; in the 3rd test, cumulative feeding and drinking were monitored only 24 h later. Food and water intakes were expressed in grams of food and water ingested.

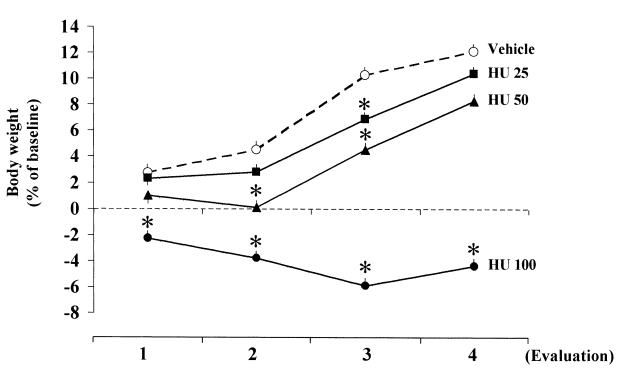

Fig. 1. Effect of HU 210 on body weight in rats. Vehicle or HU 210 (HU) at 25, 50 or 100 μg/kg were administered i.p. once daily for 4 days. Evaluations: 1 = 24 h after the first injection; 2, 3 and 4 = 1, 4 and 7 days after the last injection, respectively. Each point is the means ± SEM for eight rats. * Significantly different from vehicle-treated rats (ANOVA followed by Student–Newman–Keuls' test).

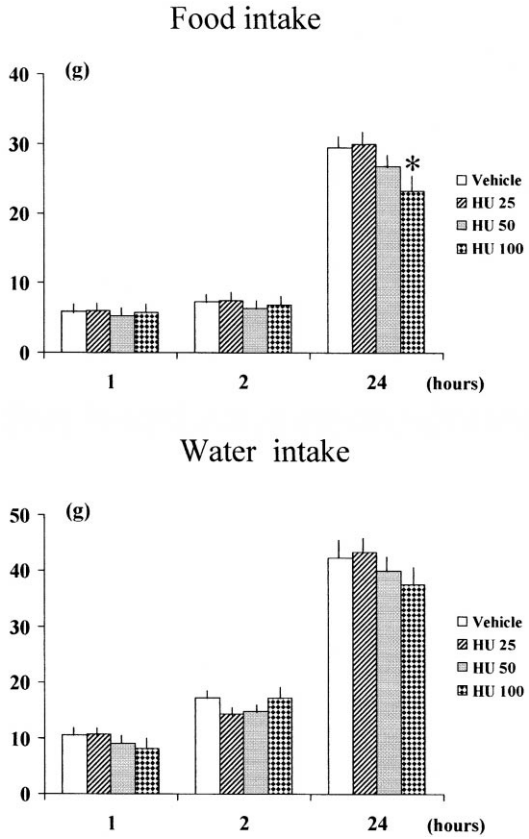

Fig. 2. Effect of acute HU 210 on food and water intake of rats (test 1). Vehicle or HU 210 (HU) at 25, 50 or 100 μg/kg were administered i.p. 50 min before food and water presentation to fasted rats and evaluations were performed 1, 2 and 24 h later. Each histogram is the means ± SEM for seven (vehicle and HU 25) or eight (HU 50 and HU 100) rats. * Significantly different from vehicle-treated rats (ANOVA followed by Student–Newman–Keuls' test).

2.5. Drugs

HU 210 (Tocris Cookson, Bristol, United Kingdom) was freshly prepared, being dissolved in vehicle [suspension containing a drop (0.1%) of Tween 80 and distilled water] at concentrations that allowed the administration of 1 ml/kg. The doses used were chosen on the basis of previous experiments (Ferrari et al., 1999a).

2.6. Statistical evaluation

The values are presented as means ± SEM and were analysed with an analysis of variance (ANOVA) followed by Student–Newman–Keuls' test with the level of significance set at $P < 0.05$.

3. Results

3.1. Experiment 1: evaluation of body weight

Fig. 1 shows that HU 210 produced a dose-dependent loss of body weight. The effect of the highest dose (100 μg/kg), which was already visible 24 h after the acute injection [$F(3,28) = 13.5$, $P < 0.001$], was shared with 50 μg/kg after sub-chronic treatment [$F(3,28) = 26.88$, $P < 0.001$] and with all doses 4 days after the last treatment [$F(3,28) = 130$, $P < 0.001$]. Seven days after the stoppage of the drug dose, the body weight of the rats injected with HU 210 at 100 μg/kg was not regained and remained markedly below that of vehicle-treated animals [$F(3,28) = 43.76$, $P < 0.001$].

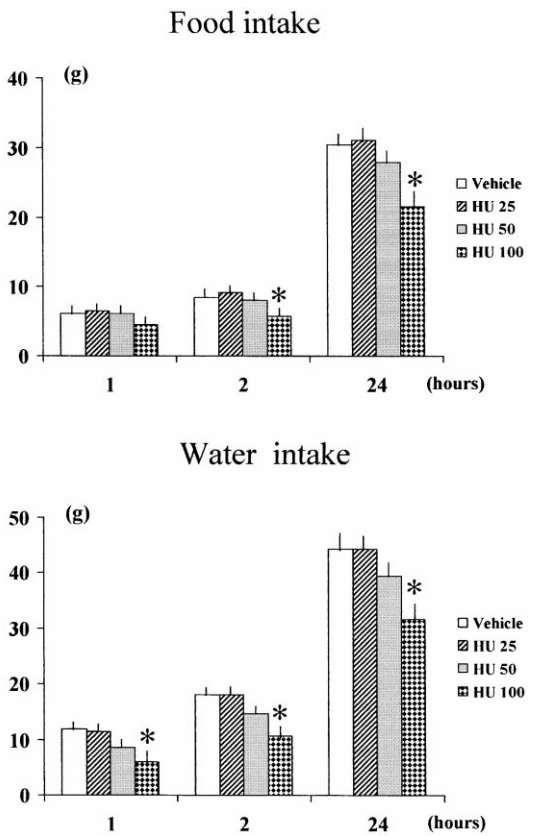

Fig. 3. Effect of sub-chronic HU 210 on food and water intake of rats (test 2). Vehicle or HU 210 (HU) at 25, 50 or 100 μg/kg were administered i.p. once daily for 4 days. Fifty minutes after the last injection, food and water were presented to fasted rats and evaluations were performed 1, 2 and 24 h later. Each histogram is the means ± SEM for seven (vehicle and HU 25) or eight (HU 50 and HU 100) rats. * Significantly different from vehicle-treated rats (ANOVA followed by Student–Newman–Keuls' test).

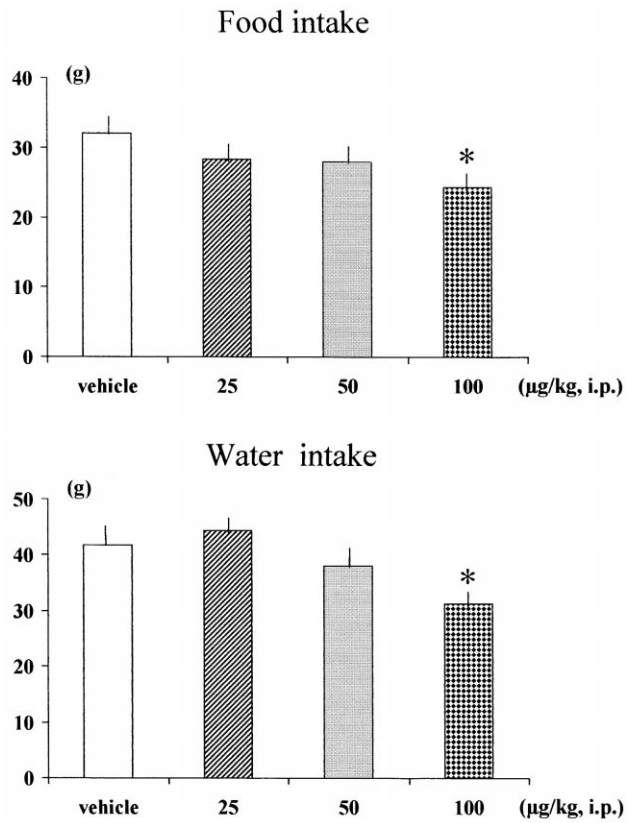

Fig. 4. Effect of HU 210 on food and water intake of rats, 7 days after the last injection of a sub-chronic treatment (test 3). Vehicle or HU 210 (HU) at 25, 50 or 100 μg/kg were administered i.p. once daily for 4 days. Seven days after the last injection, food and water were presented to fasted rats and the evaluation was performed 24 h later. Each histogram is the means ± SEM for seven (vehicle and HU 25) or eight (HU 50 and HU 100) rats. * Significantly different from vehicle-treated rats (ANOVA followed by Student–Newman–Keuls' test).

3.2. Experiment 2: evaluation of food and water intake

As shown in Fig. 2, after acute treatments, food intake was inhibited only by the dose of 100 μg/kg, 24 h after food presentation [$F(3,26) = 4.3$, $P = 0.014$], while water intake was not significantly affected.

After sub-chronic treatments (Fig. 3), the inhibition of food consumption by HU 210 at 100 μg/kg appeared 2 h after food presentation [$F(3,26) = 5.1$, $P = 0.007$] and was still seen at 24 h [$F(3,26) = 8.6$, $P < 0.001$]. Water intake (Fig. 3) was reduced by this same dose at all three test periods [$F(3,26) = 5.3$, $P = 0.005$], [$F(3,26) = 5.8$, $P = 0.003$], [$F(3,26) = 5.8$, $P = 0.004$].

Fig. 4 shows that 7 days after the last cannabinoid administration at 100 μg/kg, and 24 h after food and water presentation, the inhibition of feeding and drinking was still present [$F(3,26) = 5.8$, $P = 0.004$; $F(3,26) = 6.6$, $P = 0.002$, respectively].

4. Discussion

Evidence has accumulated that cannabinoid CB_1 receptors are involved in the regulation of feeding behaviour and body weight. As previously pointed out, while it is reported that cannabinoids possess appetite-stimulating properties in humans, experiments in rats have shown inconsistent effects, with increase, suppression or no effect on ingestion being produced.

Among recently available cannabinoid CB_1 receptor-selective agonists, HU 210 shared a multiplicity of typical pharmacological effects (Dewey, 1986; Compton et al., 1992; Romero et al., 1995), namely, antiemesis (Ferrari et al., 1999a), a decrease in psychomotor performance (De Fonseca et al., 1994; Giuliani et al., 2000) and in body temperature (Ovadia et al., 1995), psychotropic activity (Mechoulam et al., 1988; Ferrari et al., 1999b) and enhancement of vocalization (Ferrari et al., 1999a,c).

The main result of present study was that acute and sub-chronic HU 210 (25–100 μg/kg) induced a dose-dependent loss of body weight, and the marked decrease obtained after the highest dose was long-lasting, as it was still present 7 days after the end of the sub-chronic treatment. When food and water intakes were measured, it was found that both were significantly affected by HU 210 only at 100 μg/kg.

It has been reported that smoking active marijuana increases food intake and body weight; however, the latter effect appears to be a temporary phenomenon, as chronic cannabis users maintain a body weight significantly below that of a control group (Foltin et al., 1988). Acute and chronic administration of cannabinoids elicits a characteristic set of endocrine effects in rodents (Kubena et al., 1971), suggesting an alteration of the hypothalamic mechanisms controlling pituitary hormone synthesis and release (Dewey et al., 1970, Calderon et al., 1988). In particular, Δ^9-tetrahydrocannabinol, HU 210 and anandamide induce potently the secretion of corticosterone, adrenocorticotropin hormone (ACTH) and corticotropin releasing factor (CRF) (Kubena et al., 1971; Calderon et al., 1988; De Fonseca et al., 1996). Abnormal function of the hypothalamic–pituitary–adrenal axis has been linked to anorexia (Donohoe, 1984) and inhibition of feeding is induced in various animal species by CRF and ACTH, which extensive studies indicate to be modulators of stress-related disturbances of food intake (Morley and Levine, 1982; Vergoni et al., 1990). In view of the correlation between cannabinoids, stress, CRF and ACTH, that has long been proposed (Zuardi et al., 1982; Dewey, 1986) and supported by experimental findings in animals (Maclean and Littleton, 1977; De Fonseca et al., 1996; Ferrari et al., 1999c; Giuliani et al., 2000), a cannabinoid-induced anorexic effect would not be surprising. In our experiments, facilitation of ingestive behaviour was not seen at any dose, differing from what was reported from other studies (Trojniar and Wise, 1991; Williams et al., 1998), with animal hyperphagia being consistent with both human data and rodent appetite suppression and weight loss produced by the cannabinoid antagonist, *N*-Piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-3-pyrazole-carboxamide (SR 141716A) (Arnone et al., 1997; Colombo et al., 1998). A reduction in feeding and drinking was obtained after HU 210 only at 100 μg/kg; this finding is consistent with the marked weight loss of the animals treated with this dose but might reasonably be ascribable to other non-specific effects incompatible with the normal expression of ingestive behaviour. Sedation has been reported after acute and sub-chronic HU 210 at 100 μg/kg, but was not seen 7 days after the suspension of the drug at this dose (Giuliani et al., 2000). On the contrary, animal behavioural studies suggest the persistence of an anxiogenic-like state when treatment with the drug at high doses is discontinued (Ferrari et al., 1999c; Giuliani et al., 2000). As far as we know, the possible occurrence of HU 210-induced gastrointestinal distress and peritoneal lesions, which could contribute to lower ingestive behaviour and body weight, was not investigated. Therefore, in accordance with Dewey (1986), ''additional studies into the effect of cannabinoids on gastrointestinal function are needed; the result of these studies might shed light on the discrepancy in the effects of cannabinoids on food intake in animals and man.'' As there is some evidence for (1) biphasic dose–response modulation by Δ^9-tetrahydrocannabinol of rodent eating (Glick and Milloy, 1972) and (2) stimulant effect on preference for sweet calories (Sofia and Knobloch, 1976), it cannot be excluded that more appropriate test situations and/or HU 210 doses lower than those used in our study could reveal a facilitatory influence on feeding. However, at present, despite the number of similarities between the effects of cannabinoids in experimental animals and in man, our results further support the suggested limitations of rodent models for predicting cannabinoid modulation of feeding (Graceffo and Robinson, 1998). The potent and long-lasting reduction of body weight exerted by HU 210 seems, at least partially, unrelated to ingestive behaviour and the underlying mechanisms need to be explored in future research.

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