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SPECIAL REPORT

Role of the endocannabinoid system in MDMA intracerebral self-administration in rats

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I.c.v. self-administration of MDMA (0.01–2 μg per infusion), alone and in combination with CP 55,940 (0.4 μg infusion^{-1}), was studied on an operant responding procedure. On the basis of individual preference for one of two levers, developed during training, rats were allowed to self-administer vehicle from the preferred lever and MDMA from the other. Pressings on the MDMA associated-lever, except for the maximal unit dose, progressively increased. The combination of CP 55,940 with MDMA (1 μg infusion^{-1}) reduced the number of drug-associated lever pressings compared to the single drugs. Pre-treatment with SR 141716A (0.5 mg kg^{-1} i.p.), 15 min before each daily session, significantly increased MDMA self-administration. These findings suggest that MDMA self-administration is under endogenous tonic control by the endocannabinoid system.
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Abbreviations: CEPH, cerebrospinal fluid; CEPHC, cannabinoid vehicle; MDMA, 3,4 methylenedioxymethamphetamine; THC, Δ^9-tetrahydrocannabinol

Introduction 3,4 Methylenedioxymethamphetamine (MDMA), a ring-substituted amphetamine with hallucinogenic and sympathomimetic properties, is a recreational drug available on the street under the name of 'ecstasy' or 'adam'. Studies to predict its potential for recreational use by humans have been done with different animal species and tests. MDMA (3–320 μg infusion^{-1}) is self-administered i.v. by rhesus monkeys (Beardsley *et al*., 1986) and baboons (Lamb & Griffiths, 1987), enhances lever pressing for rewarding brain stimulation (Reid *et al*., 1996), and establishes a dose-dependent (0.2–20 mg kg^{-1}) conditioned place preference in rats (Bilsky *et al*., 1990). It is common for MDMA users to consume cannabis to alleviate the negative feelings they experience when the MDMA-related euphoria is diminishing (Croft *et al*., 2001).

Natural and synthetic cannabinoids appear atypical as drugs of abuse since their ability to lower the threshold for electrical self-stimulation (Gardner & Vorel, 1998; Arnold *et al*., 2001) and to support self-administration (Tanda *et al*., 2000; Fattore *et al*., 2001; Braida *et al*., 2001b) or conditioned place preference in rats (Gardner & Vorel, 1998; Valjent & Maldonado, 2000; Braida *et al*., 2001a) is still controversial. The present study examined the involvement of cannabinoid system on MDMA self-administered by rats through an intracerebroventricular (i.c.v.) route. This method (Braida *et al*., 1998) presents advantages such as a durable preparation, the possibility of simultaneous choice between the addicting drug and vehicle, and the avoidance of peripheral effects.

Methods *Animals* Male Wistar rats (Charles River, Calco, Como, Italy) weighing 350±10 g, were housed singly in cages, under standard laboratory conditions with a 12 h light/dark cycle (lights on at 0800 h). Food was given *ad libitum*, but water was allowed only for 1 h session^{-1} and then for 10 min afterwards throughout the experiment. All experiments were conducted in accordance with the Italian Government Decree No.94/2000A.

Experimental procedure for i.c.v. self-administration This method has been described before (Braida *et al*., 1998). Briefly, animals were individually trained for 1 h a day to press two active levers in an operant chamber to obtain water as reinforcer for 1 week in a continuous reinforcement schedule. One week after implantation with i.c.v. double guide stainless steel cannulas, animals were trained daily to receive an infusion of 2 μl 8 s^{-1} of vehicle each time they pressed either lever, through a bilateral injection cannula inserted in the double guide and connected to two infusion pumps. Each infusion delivered MDMA vehicle (sterile cerebrospinal fluid (CEPH)) or cannabinoid vehicle (CEPH, ethanol, cremophor, 18 : 1 : 1 (CEPHC)) depending on the self-administration pattern.

Drugs and treatment When a stable baseline was reached (at least 5 days with no more than 15% difference across the session), drug sessions were carried out. On the basis of individual preference, the preferred lever was always associated with the vehicle and the non-preferred one with the drug. One group of six rats was allowed to self-administer increasing concentrations (0.01, 0.1, 1 and 2 μg 2 μl per infusion) of MDMA (Sigma, St Louis, U.S.A.). Within this group, each unit dose was given in a counterbalanced order and only when the baseline response for the preceding unit dose was stable. Two further groups of six animals each, which had reached 5 days of stable baseline with the unit

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dose of MDMA that produced the maximal response to lever pressing or CP 55,940 [(-)-cis-3-[2-hydroxy-4-(1,1-dimethyl-heptyl) phenyl] trans−4-(3-hydroxypropyl) cyclohexanol] (Tocris Kookson Ltd, Bristol, U.K.) (0.4 μg 2 μl per infusion), were allowed to self-administer the combination of the two drugs. For the antagonism studies, one further group of six rats received an i.p. injection of cannabinoid vehicle 15 min before each daily session of MDMA self-administration (1 μg 2 μl per infusion). When 5 days of stable baseline were reached, the animals received an i.p. injection of SR 1414716A[N-piperidino-5-(4-chlorophenyl)1-(2,4)dichlorophe nyl)-4 methylpyrazole 3-carboxamide] (a kind gift from Sanofi, Montpellier, France) (0.5 mg kg^{-1}) 15 min before each daily session. The unit dose of CP 55,940 and the dose of SR 141716A were chosen on the basis of a previous study (Braida *et al*., 2001b) in which the cannabinoid agonist produced the maximal reinforcing effect that was completely blocked by the cannabinoid antagonist, in the same test.

Results Rats trained to press both levers during training did not change their operant responding before and after surgery (data not shown). Figure 1(A) shows the time course for one representative rat during the training and testing procedure. For the sake of brevity, only the last 10 days for each self-administration period are shown. There was a progressive increase in the number of pressings of the less preferred lever when MDMA was delivered at different concentrations. The highest concentration produced a gradual decrease in the number of pressings delivering MDMA and an increase of those delivering vehicle. The mean number of pressings on the lever delivering vehicle or increasing concentrations of MDMA is shown in Figure 1(B). There was a progressive increase in the number of pressings on the MDMA lever. The maximal reinforcing unit dose was 1 μg per infusion while drug-associated lever pressing was less frequent with 2 μg^{-1} infusion. The corresponding mean daily intake of MDMA was linearly related to the log of the self-administered unit doses (R^2 value = 0.99). The estimated ED_{50} ($\pm$confidence limits) (μg 2 μl per infusion) was 0.87 ($\pm$0.03). I.c.v. self-administration of MDMA or CP 55,940 alone, at the maximal reinforcing unit dose, significantly increased the number of drug-associated and reduced the number of vehicle-associated lever pressings (Figure 2). The combination of CP 55,940 with the maximal reinforcing unit dose of MDMA, simultaneously delivered by pressing the same lever, significantly reduced the mean number of drug associated lever pressings in comparison with the single drugs. Pre-treatment with SR 141716A, administered peripherally, did not *per se* affect operant responding in comparison with vehicle. The cannabinoid antagonist significantly increased MDMA-associated lever pressings and decreased vehicle-associated lever pressings in comparison with the drug alone.

Discussion Our data demonstrate, for the first time, the acquisition and dose-related responding for i.c.v. self-administration of MDMA. The biphasic effect for the number of bar pressings (increase with 0.01 – 1 μg per infusion and decrease with 2 μg per infusion) indicates that rats tend to adjust the dose during sessions by modifying the response frequency (Koob, 1993). A similar pattern was also observed for opiates and CP 55,940 (Braida *et al*., 2001b) using the same test. The results with the combination of CP 55,940 and MDMA indicate that infusion of the cannabinoid agonist alters i.c.v. MDMA self-administration, significantly reducing MDMA intake. Similar results were recently seen when CP 55,940 was combined either with heroin or etonitazene using the same test (Braida *et al*., 2001b). In addition, i.v. pre-treatment with another synthetic cannabinoid, WIN 55,212-2, reduced i.v. self-administration of cocaine in rats (Fattore *et al*., 2001). The decrease in response seems to follow the effect of changes in the unit dose of the reinforcer, suggesting a synergistic action of cannabinoid agonists on the reinforcing properties of MDMA and other drugs of abuse. The possibility that non-specific effects were responsible for the lower mean number of bar pressings with the combination can be excluded. No signs of motor sedation were observed since the drop in mean number of pressings on the drug-associated lever was counterbalanced by an increase in those of the

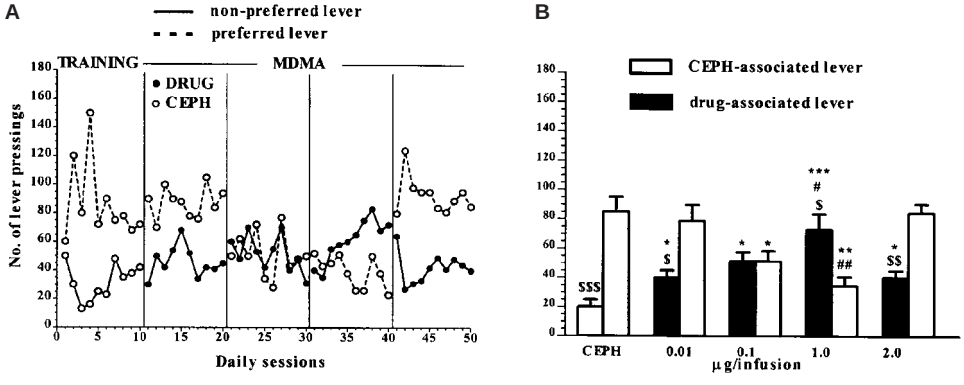

Figure 1 Effect of increasing concentrations of MDMA on operant responding. (a) Number of pressings, in a free choice situation, by one representative rat during a 1 h daily session on the preferred and non-preferred lever. Cerebrospinal fluid (CEPH) was delivered i.c.v. by pressing the lever found preferred during training. MDMA was delivered i.c.v. by pressing the lever found non-preferred during training. (b) Number of pressings on the drug- or vehicle-associated lever. Results are mean ($\pm$s.e.mean) of the last five daily sessions after 15 – 20 days of acquisition, with six rats per group. *$P<0.05$, **$P<0.01$, ***$P<0.001$ *vs* the corresponding CEPH group; \$$P<0.05$, \$\$$P<0.01$, \$\$\$$P<0.001$ *vs* the corresponding vehicle-associated lever, #$P<0.05$, ##$P<0.01$ *vs* the corresponding 0.01 and 2.0 dose groups (ANOVA followed by *post-hoc* Tukey's test).

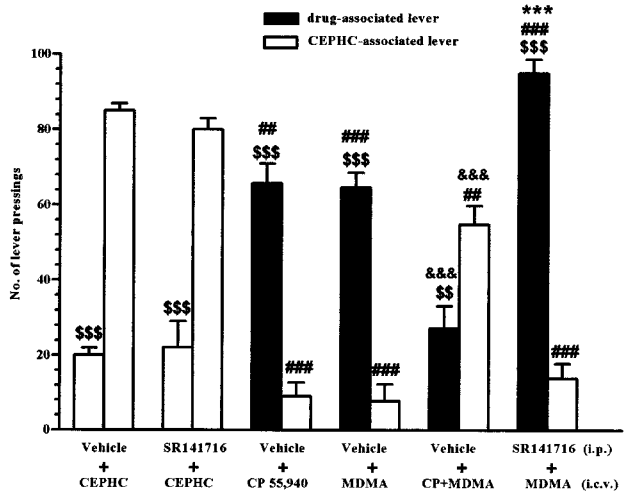

Figure 2 Mean operant responding (±s.e.), in a free-choice situation, to the drug and the cannabinoid vehicle (CEPHC) lever pressing during the last five stable daily sessions of 15–20 days of acquisition of six rats per group. Drug-lever pressing delivered 1 μg 2 μl per infusion of MDMA or 0.4 μg 2 μl per infusion of CP 55,940 or the combination. MDMA and CP 55,940 were dissolved in CEPHC. Vehicle (SR 141716A vehicle) or SR 141716A (0.5 mg kg^{-1}) were given i.p. 15 min before each daily session. \$\$$P<0.01$. \$\$\$$P<0.001$ *vs* the corresponding CEPHC associated-lever pressing; ##$P<0.01$; ###$P<0.001$ *vs* the corresponding vehicle + CEPHC and SR 141716A + CEPHC; ***$P<0.001$ *vs* corresponding MDMA alone; &&&$P<0.001$ *vs* corresponding CP 55,940 and MDMA alone, and SR 141716A + MDMA (ANOVA followed by *post-hoc* Tukey's test).

vehicle. Although a decrease in the rate-enhancing effect of MDMA itself cannot be ruled out, the increase in operant responding induced by SR 141716A on MDMA self-administration, suggests a reduction in sensitivity to the motivation. The mechanism regulating MDMA's reinforcing effect might therefore be influenced by the endogenous cannabinoid system. Recent studies have confirmed the involvement of the endocannabinoids in drug abuse: enhanced formation of anandamide in rats chronically treated with Δ^9-tetrahydrocannabinol (THC) (Di Marzo *et al*., 2000), an increase after HU210 and a decrease after SR 141716 treatment in relapse to cocaine seeking (De Vries *et al*., 2001), inhibition of morphine self-administration in mutant CB1 receptor knockout mice (Cossu *et al*., 2001), and an attenuation of some manifestations of morphine abstinence in mice pre-treated with THC (Valverde *et al*., 2001). A possible intrinsic effect of SR 1417116A can be ruled out since, when administered alone, the compound did not modify operant responding in comparison with that seen with pre-treatment with its vehicle. Pre-treatment with the receptor cannabinoid antagonist has also been shown to reduce the reinforcing value of electrical brain self-stimulation (Deroche-Gamonet *et al*., 2001), operant responding for beer (Gallate & McGregor, 1999) and alcohol (Serra *et al*., 2001) in rats. The mechanism by which MDMA and cannabinoids interact is difficult to explain. MDMA raises the dopamine level by increasing release, inhibiting uptake and by MAO inhibition (Morland, 2001). On the other hand, cannabinoids participate in the regulation of dopamine synthesis, release and turnover (Gardner & Vorel, 1998). The overlapping expression of cannabinoid and dopamine receptors recently found in some brain areas, including the nucleus accumbens (Hermann *et al*., 2002), raises the possibility that stimulation of cannabinoid receptors have other effects in addition to MDMA reinforcing properties.

In conclusion, the present study provides the first evidence that the mechanism regulating MDMA's reinforcing effects is under an endogenous cannabinoid control. The positive interaction of concurrent CP 55,940 and MDMA may help explain the combined use of marijuana and MDMA by multi-drug users in order to overcome the unpleasant effects that are often felt as the initial euphoria dissipates (Croft *et al*., 2001).

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