Published in final edited form as:

Neuropharmacology. 2012 July ; 63(1): 161–168. doi:10.1016/j.neuropharm.2011.10.018.

CANNABINOID FACILITATION OF BEHAVIORAL AND BIOCHEMICAL HEDONIC TASTE RESPONSES

M.A. De Luca^{1,2,§}, M. Solinas^{3,§}, Z. Bimpisidis¹, S.R. Goldberg⁴, and G. Di Chiara^{1,2,5}

¹Department of Toxicology, University of Cagliari, Italy

²INN, National Institute of Neuroscience and Centre of Excellence “Neurobiology of Addiction”

³Institut de Biologie et Physiologie Cellulaires, CNRS UMR 6187, University of Poitiers, France

⁴Preclinical Pharmacology Section, Intramural Research Program, National Institute on Drug Abuse, NIH, DHHS, Baltimore MD, USA

⁵CNR, Institute of Neuroscience, Cagliari Section

Abstract

Cannabinoid receptor agonists are known to stimulate feeding in humans and animals and this effect is thought to be related to an increase in food palatability. On the other hand, highly palatable food stimulates dopamine (DA) transmission in the shell of the n. accumbens and this effect undergoes one trial habituation. In order to investigate the relationship between the affective properties of tastes and the response of accumbens shell DA we studied the effect of delta-9-tetrahydrocannabinol (THC) on behavioral taste reactivity to intraoral infusion of appetitive (sucrose solutions) and aversive (quinine and saturated NaCl solutions) tastes and on the response of in vivo dopamine (DA) transmission n. accumbens shell to intraoral sucrose. Rats were implanted with intraoral cannulae and the effect of systemic administration of THC on the behavioral reactions to intraoral infusion of sucrose and of quinine or saturated NaCl solutions were scored. THC increased the hedonic reactions to sucrose but did not affect the aversive reactions to quinine and NaCl. The effects of THC were fully blocked by the CB1 receptor inverse agonist/antagonist rimonabant given at doses that do not affect taste reactivity to sucrose. In rats implanted with microdialysis probes and with intraoral cannulae, THC, made sucrose effective in raising dialysate DA in the shell of the n. accumbens. As in the case of highly palatable food (Fonzies, sweet chocolate), the stimulatory effect of sucrose on shell DA under THC underwent one trial habituation. Altogether, these findings demonstrate that stimulation of CB1 receptors specifically increases the palatability of hedonic taste without affecting that of aversive tastes. Consistent with the ability of THC to increase sucrose palatability is the observation that under THC pretreatment sucrose acquires the ability to induce a release of DA in the shell of the accumbens and this property undergoes adaptation after repeated exposure to the taste (habituation).

Keywords

Cannabis; taste; hedonic; aversive; dopamine; microdialysis; nucleus accumbens

Correspondence should be addressed to: M.A. De Luca, University of Cagliari, Dipartimento di Tossicologia, Via Ospedale 72, 09124 Cagliari, Italy. Phone: +39-070-6758633, Fax: +39-070-6758665, deluca@unica.it.

[§]These authors contributed equally to the paper

1. Introduction

It has long been known that in humans Cannabis increases appetite and consumption of food, particularly sweet food (Abel, 1975; Foltin et al., 1988; Yeomans and Gray, 2002). The ability of cannabinoid agonists to enhance food intake has also been demonstrated in laboratory animals. For example, in rats, the natural cannabinoid agonist delta-9-tetrahydrocannabinol (THC) increases food intake (Williams et al., 1998) and the consumption of sweet solutions (Gallate et al., 1999). THC also increases the reinforcing properties of food in a progressive ratio paradigm (Solinas and Goldberg, 2005).

On the other hand, cannabinoid CB1 receptor inverse agonists/antagonists such as rimonabant (SR-14716A) reduce intake of food or sweet solutions (Arnone et al., 1997; Colombo et al., 1998; Freedland et al., 2000; McLaughlin et al., 2003; Simiand et al., 1998; Thornton-Jones et al., 2004). Thus, cannabinoid antagonists or inverse agonists have been proposed as medications for the treatment of obesity (Berry and Mechoulam, 2002; Piomelli, 2005). These findings have led to the hypothesis that the endogenous cannabinoid system plays a role in the regulation of appetite and food intake (Cota et al., 2003; Solinas et al., 2008) and that the endogenous cannabinoid system is involved in reward processes that mediate the incentive or hedonic value of food (Williams and Kirkham, 1999; Kirkham, 2003).

Brain dopamine (DA) neurotransmission is tightly coupled to natural and drug reward (Schultz, 2002; Di Chiara et al., 2004; Wise, 2008). Thus, drugs of abuse of the most diverse pharmacological classes, including psychostimulants, narcotic analgetics, nicotine, cannabinoids, ethanol and benzodiazepine anxiolytics, increase DA transmission in rats as well as in humans preferentially or, depending on doses and conditions, selectively in the ventral striatum/n. accumbens (Di Chiara, 1990; Robinson et al., 2003; Volkow et al., 2003; Fillenz, 2005; Chang and Haning, 2006). Further studies in rats indicate that this property of drugs of abuse is specifically related to its shell subdivision (Pontieri et al., 1995; Tanda et al., 1997; Lecca et al., 2006). Palatable food also stimulates DA transmission in the n. accumbens shell and core and in the prefrontal cortex and this property is related, not only to palatability but also to relative novelty of the food and to motivational need state (hunger). Thus, a single exposure to the palatable food or taste results in habituation of the DA response specifically in the n. accumbens shell, an observation that has been suggested to indicate a role of shell DA in associative learning (Bassareo et al., 2007). Although sweet taste is the quintessential reward (Myers et al., 2005), the intraoral infusion of 20% sucrose, under our experimental conditions, does not stimulate NAc shell DA transmission (Bassareo et al., 2002, 2003). Under appropriate conditions, however, intraoral sucrose does stimulate DA transmission in vivo (Hajnal et al., 2004).

The facilitatory effects of THC on food intake and food reinforcement might be due to an increase of the incentive-motivational properties of food (Bindra, 1968) or of the hedonic properties of its taste (palatability) or both.

Results from parametric studies and microstructural analysis of sucrose consumption have been interpreted to indicate that THC increases food palatability (Higgs et al., 2003; Miller et al., 2004). In order to eliminate the possible confound of active consumption, the taste reactivity paradigm has been introduced (Grill and Norgren, 1978). In this test rats are implanted with intraoral cannulas and the behavioral reactions to intraoral infusion of small volumes of a solution of a taste stimulus (or gustatory stimulus) is recorded. Rats show typical behavioral reactions to appetitive and aversive tastes that are thought to be homologous to the taste reactions of human and non-human primates (Rosenstein and Oster, 1988; Steiner et al., 2001). It has been proposed that the taste-reactivity paradigm provides a

direct estimation of the affective value, either appetitive/hedonic or aversive, of taste stimuli (Berridge, 2000).

Taste reactivity studies show that THC administration increases the hedonic reactions to sucrose taste and reduces the aversive reactions to quinine, consistently with an increase in palatability (Jarrett et al., 2005, 2007). However, the time-course of the effect of THC on sucrose-induced hedonic taste reactions is peculiar, since become significant only 120 min after THC; moreover, the intensity of these is rather mild. These characteristics contrast with the time-course and extent of the effect of THC on sucrose consumption, that is almost immediate and rather pronounced as well as with the known behavioral and biochemical effects of systemic THC, including those on DA release in the n.accumbens estimated by microdialysis (Tanda et al., 1997). The peculiar aspects of the effects of THC on taste reactivity might be due to the specific procedure utilized by Jarrett et al. (2005 and 2007). For example, rats were given ad libitum for 5 days before the test a 32% sucrose solution in place of water as the sole fluid source. Moreover, THC was administered 72 hours before the test. We therefore investigated the effect of THC on taste reactivity in rats naive to the taste stimulus as well as to THC or to its antagonist rimonabant.

With these premises, we investigated the effect of THC on taste reactivity to sucrose and to quinine and saturated NaCl applied in rats naive to the taste stimulus and to THC. In addition, we investigated the ability of the cannabinoid CB1 inverse agonist/antagonist rimonabant to reverse the effects of THC. Another aspect that we have investigated in the present study is the influence of THC on the DA releasing properties of sucrose in the shell and core of the n.accumbens and in the prefrontal cortex (PFCX) and on the adaptive changes (habituation) of this response induced by a single pre-exposure to the taste stimulus (Bassareo et al., 2002).

2. Methods

2.1 Animals

Experimentally naive, male Sprague-Dawley rats (Charles River, Wilmington, MA and Charles River, Calco, Italy) weighing 275–300g were housed in a temperature- and humidity- controlled room with a 12-h light/dark cycle (light from 7:00 A.M. to 7:00 P.M.). Experiments were conducted between 10:00 am and 4:00 pm. Animals used in this study were maintained in facilities fully accredited by the American Association for the Accreditation of Laboratory Animal Care and all experimentation was conducted in accordance with the guidelines of the Institutional Care and Use Committee of the National Institute on Drug Abuse, National Institutes of Health, and the Guide for Care and Use of Laboratory Animals (NIH Publications No. 8023, revised 1978) and the Council of the European Communities (86/609/EEC).

2.2 Materials

THC (50 mg/ml in ethanol from the National Institute on Drug Abuse, Rockville, MD) was dissolved in a solution of 40% (w/v) of cyclodextrin in water (RBI/SIGMA, Natick, MA) and administered intraperitoneally (i.p.) in a volume of 2.0 ml/kg at the doses of 0.5 or 1.0 mg/kg; rimonabant (SR-141716A free-base; National Institute on Drug Abuse) was suspended in 0.3% Tween-80 in saline and administered at the dose of 1.0 mg/kg i.p. in a volume of 2.0 ml/kg.

THC was given 30 min before the start of the behavioral experiment whereas rimonabant was given 30 min before the THC injection (60 min before the behavioral experiment). Rats were infused intraorally (i.o.) with water or with hedonic or aversive taste solutions. Hedonic taste solutions consisted of sucrose solutions (5% or 20%, zucchero di Sardegna

semolato, SADAM SpA Villasor, Cagliari). Aversive taste solutions were obtained dissolving quinine HCl (5×10^{-4} M, Quinine hydrochloride, Sigma, Milano, Italy) and NaCl (saturated solution, NaCl, Farmitalia Carlo Erba, Milano, Italy) in tap water.

2.3 Behavioral experiments

2.3.1 Oral catheter preparation and surgery—Oral catheters were made of a 22-gauge stainless steel needle and polyethylene (PE) tubing (Polyethylene tubing, Portex limited, Hythe, Kent, England) (ID 0.58 mm and OD 0.96 mm). The 22-gauge stainless steel needle was cut on one side (length 2 cm) and inserted in the PE tubing that ended with a perforated circular disk.

For surgical implantation of catheters, rats were anaesthetized with sodium pentobarbital (31 mg/kg, i.p.) and chloral hydrate (142 mg/kg, i.p.) anesthesia (NIDA Pharmacy, Baltimore, MD) and the oral catheter was inserted at the level of the first molar, then the PE tubing was passed along the skull where it was fixed to the skull by glasionomeric cement (CX-Plus, Shofu Inc., Tokyo, Japan).

2.3.2 Behavioral reactions—The taste reactivity test has been utilized as an operational estimate of the hedonic valence (positive or negative) and hedonic impact of tastes (Grill and Norgren, 1978; Bassareo et al., 2002).

After surgery, rats were individually housed in the same Perplex cages of the behavioural experimental sessions to familiarize them with the test chamber for 18h. The day of the experiment, behavior was recorded during the intraoral (i.o.) infusion of the solutions. The oral catheter was connected by polyethylene tubing to an infusion pump (CMA 400 Syringe Pump, Sweden) which delivered water, 5% or 20% sucrose, saturated NaCl, or quinine HCl 5×10^{-4} M at a rate of 0.2 ml/min for 5 min (total amount of 1 ml). During the taste reactivity test, animals were videotaped and three classes of taste reactivity patterns were scored: positive hedonic (ingestive), negative hedonic (aversive), and neutral. Positive hedonic reactions were characterized by lateral tongue protrusions, rhythmic tongue protrusion, and paw licks; aversive reactions were characterized by gapes, chin rubs, face washing, forelimb flails, paw tread, and locomotion; neutral reactions were characterized by: rhythmic mouth movements and passive drip of the solution. Each lateral and rhythmic tongue protrusion gape, chin rub, forelimb flails, and paw tread was counted as an individual reaction and each one was assigned a value of 1 point. Other reactions were assigned a value of 1 point if the duration was between 1 and 5s and 2 points if the duration was more than 5s.

2.4 Microdialysis experiments

2.4.1 Probe preparation and surgery—Vertical concentric dialysis probes, with a dialysing portion of 1.5 mm for NAc and 3 mm for PFCX, were prepared with AN69 fibers (Hospal Dasco, Bologna, Italy), as previously reported (De Luca et al., 2011).

Rats were anaesthetized with chloral hydrate (400 mg/kg i.p.) (Carlo Erba, Italy) and placed in a stereotaxic apparatus. The skull was exposed and a small hole drilled to expose the dura on one side; this was removed, and the microdialysis probe was inserted vertically at the level of the NAc shell or core according to the atlas of Paxinos and Watson (1998) (coordinates shell: A, 2.2; L, 1.0 from bregma; V, -7.8 from dura; core: A, 1.4; L, 1.6 from bregma; V, -7.6 from dura; PFCX: A, 3.7; L, 0.8 from bregma; V, -4.8 from dura). In the same session, an oral catheter was implanted as described above (see behavioral experiments).

2.4.2 Analytical procedure—On the day following surgery, the probes were connected to an infusion pump and perfused with Ringer's solution (147 mM NaCl, 4 mM KCl, 2.2 mM CaCl_2) at a constant rate of 1 $\mu\text{l}/\text{min}$. Dialysate samples (10 μl) were taken every 10 min and injected into an HPLC equipped with a reverse phase column (C8 3.5 μm , Waters, Mildford, MA, USA) and a coulometric detector (ESA, Coulochem II, Bedford, MA) to quantify DA. The first electrode of the detector was set at +130 mV (oxidation) and the second at -175 mV (reduction). The composition of the mobile phase was: 50 mM NaH_2PO_4 , 0.1 mM $\text{Na}_2\text{-EDTA}$, 0.5 mM n-octyl sodium sulfate, 15% (v/v) methanol, pH 5.5. The sensitivity of the assay for DA was 5 fmol/sample. Basal DA values were the means of three consecutive samples differing by no more than 10%.

2.4.3 Histology—At the end of the experiment, rats were transcardially perfused with 50 ml saline and 150 ml of a 10% formaldehyde solution. The probes were removed and the brains were cut by vibratome into serial coronal slices (20 μm). The location of the probes was determined using the atlas of Paxinos and Watson (1998).

2.5 Statistics

Statistical analysis was carried out by Statistica for Windows. Difference in behavioral score and in the levels of extracellular DA between groups was analyzed by analysis of variance (ANOVA) for repeated measures. Results from treatments showing significant overall changes were subjected to post hoc test of Newmann-Keuls (behavioral score) or Tukey (DA levels); statistical significance was set at $p < 0.05$.

3. Results

3.1 Experiment 1. Effect of 0.5 and 1.0 mg/kg THC on behavioral reactions to 20% sucrose

In the first experiment we studied the effect of two doses of THC (0.5 and 1.0 mg/kg i.p.) on behavioral reactions to intraoral infusion of 20% sucrose solution. Fig. 1A shows the time-course of hedonic reactions and Fig. 1B shows the total hedonic reactions elicited by intraoral infusion of 20% sucrose solution in rats pretreated with THC (0.5 and 1.0 mg/kg i.p.; $N = 4$ and $N = 6$, respectively) and vehicle ($N = 9$). THC at a dose of 1 mg/kg, but not 0.5 mg/kg, significantly increased reactions to sucrose. Two-way ANOVA of hedonic scores at different times (Fig. 1A) revealed a significant effect of THC dose [$F_{(2, 16)} = 14.28$; $p < 0.001$] and time [$F_{(3, 48)} = 7.13$; $p < 0.001$]. One way ANOVA of total score (Fig. 1 B) revealed a significant effect of THC dose [$F_{(2, 16)} = 14.28$; $p < 0.001$].

3.2 Experiment 2. Effect of 1.0 mg/kg THC on behavioral reactions to intraoral 5% and 20% sucrose

In the second experiment we investigated the relationship between sucrose concentration and the hedonic-enhancing properties of THC. Fig. 2 A, B, C shows the time-course of hedonic reactions and Fig. 2 D the total hedonic reactions to intraoral water and sucrose solution at two different concentrations (5% or 20%) after THC pretreatment (1.0 mg/kg i.p.; THC water $N = 4$, THC suc 5% $N = 6$, THC suc 20% $N = 5$) and vehicle pretreatment (Veh water $N = 7$, Veh suc 5% $N = 6$, Veh suc 20% $N = 9$). Intraoral infusion of water produced a very low hedonic score both in vehicle- and THC-pretreated rats (Fig. 2 A). In contrast, both 5% sucrose solution (Fig. 2 B) and 20% sucrose solution (Fig. 2 C) produced significant hedonic scores in THC treated rats, but the higher sucrose concentration produced a more intense and longer-lasting effect. For water, two-way ANOVA of hedonic scores revealed a significant effect of time [$F_{(3, 27)} = 3.6$; $p < 0.02$]. For sucrose 5%, two-way ANOVA of hedonic scores revealed a significant effect of treatment [$F_{(1, 10)} = 7.83$; $p < 0.02$], time [$F_{(3, 30)} = 3.4$; $p < 0.03$] and a significant treatment x time interaction [$F_{(3, 30)} = 4.53$; $p < 0.009$]. For sucrose 20%, two-way ANOVA of hedonic scores revealed a

significant effect of treatment [$F_{(1, 13)} = 21.61$; $p < 0.001$] and time [$F_{(3, 39)} = 4.16$; $p < 0.01$]. Two-way ANOVA of total scores revealed significant effect of THC [$F_{(2, 32)} = 37.98$; $p < 0.001$], sucrose concentration [$F_{(1, 32)} = 21.68$; $p < 0.0001$] and a significant THC x sucrose concentration interaction [$F_{(2, 32)} = 11.2$; $p < 0.0002$].

3.3 Experiment 3. Effect of THC on behavioral aversive reactions to quinine and saturated NaCl solutions

Fig. 3 A, B shows the time-course of the aversive reactions and Fig. 3C the total aversive reactions after intraoral administration of 5×10^{-4} M quinine HCl or saturated NaCl solutions in rats pretreated with THC (1.0 mg/kg i.p.; quinine N = 4, NaCl N = 6) or vehicle (quinine N = 4, NaCl N = 5). Two-way (Fig. 3 A, B) or one-way ANOVA (Fig. 3 C) of aversive scores did not reveal any significant effect.

3.4 Experiment 4. Effect of the CB1 inverse agonist/antagonist rimonabant on THC induced potentiation of hedonic reactions to sucrose

Fig. 4 A shows the time-course and Fig. 4 B shows the total hedonic score after intraoral administration of 20% sucrose in rats pretreated with rimonabant (1.0 mg/kg i.p., 30 min before THC or vehicle; Veh-Veh N = 9, rimonabant-Veh N = 6, Veh-THC N = 8, rimonabant-THC N = 9). After rimonabant administration, nor scratching behaviour and grooming were observed. Pretreatment with rimonabant completely eliminated the increased hedonic reactions to sucrose induced by THC. Two-way ANOVA of hedonic score at different times (Fig. 4 A) revealed a significant effect of treatment [$F_{(3, 28)} = 15.02$; $p < 0.0001$] and time [$F_{(3, 84)} = 5.07$; $p < 0.001$]. One way ANOVA of total score (Fig. 4 B) revealed significant effects of treatment [$F_{(3, 28)} = 15.03$; $p < 0.0001$].

3.5 Experiment 5. Effect of THC on dialysate DA after intraoral 20% sucrose

Basal value of DA, expressed as fmoles/10ul sample (mean $\pm$ SEM), were: NAc shell 52 ± 5 (N = 36), NAc core 54 ± 4 (N = 9), PFCX 15 ± 2 (N = 10). Consistent with previous studies (Bassareo et al., 2002, 2003), intraoral administration of sucrose produced a significant increase of DA levels in the PFCX but not in the NAc shell of rats treated with vehicle. Administration of 1 mg/kg of THC i.p., per se, produced a significant extracellular release of DA in the NAc shell and in the PFCX, but not in the NAc core. When DA levels had returned to basal levels, intraoral administration of 20% sucrose produced significant increases in DA levels in the NAc shell only in THC-treated rats. DA levels in the PFCX also increased following sucrose administration but this increase was not different from Veh-treated rats. Two-way ANOVA revealed significant effects of THC treatment [$F_{(1, 8)} = 13.61$; $p < 0.006$], time [$F_{(5, 40)} = 2.7$; $p < 0.03$] and treatment x time interaction [$F_{(5, 40)} = 2.44$; $p < 0.05$] in the NAc shell and only a significant effect of time [$F_{(5, 40)} = 3.2$; $p < 0.01$] in the PFCX.

3.6. Experiment 6. Effect of the CB1 inverse agonist/antagonist rimonabant on THC facilitated release of NAc shell DA response to sucrose

In this experiment, we investigated whether rimonabant could eliminate the THC-facilitated NAc shell DA responsiveness to sucrose. Fig. 6 shows that the DA response to THC and to 20 % sucrose was completely abolished by the cannabinoid CB1 inverse agonist/antagonist rimonabant (1.0 mg/kg i.p., 30 min before THC). Two-way ANOVA of the DA response to THC revealed a significant effect of THC treatment [$F_{(1, 9)} = 18.27$; $p < 0.002$] and treatment x time interaction [$F_{(6, 54)} = 3.31$; $p < 0.007$]; two-way ANOVA of the DA response to sucrose revealed a significant effect of treatment [$F_{(1, 9)} = 6.39$; $p < 0.03$], time [$F_{(12, 108)} = 2.21$; $p < 0.01$] and treatment x time interaction [$F_{(12, 108)} = 2.94$; $p < 0.01$].

3.7. Experiment. 7 Effect of repeated intraoral sucrose on THC facilitated release of NAc shell DA response to sucrose

In this experiment, we investigated whether THC could alter DA responses in the NAc shell to repeated administration of intraoral sucrose. In vehicle-treated rats, a first or a second sucrose administration did not alter DA levels in the NAc shell (Fig. 7 A, B). In THC-treated rats, sucrose produced a significant increase in DA levels in the NAc shell after the first administration (even when sucrose was given 2 h after THC administration) but not after a second administration of sucrose, suggesting that DA responses in the NAc shell to sucrose undergo habituation (Fig. 7 C–D). For the THC-Wat-Suc group, one-way ANOVA revealed significant effects of hour [$F_{(2, 12)} = 15.29$; $p < 0.01$] and time [$F_{(5, 30)} = 8.78$; $p < 0.0001$] and an hour X time interaction [$F_{(10, 60)} = 2.47$; $p < 0.05$]. For the THC-Suc-Suc group, statistical one-way ANOVA revealed significant effects of hour [$F_{(2, 12)} = 30.34$; $p < 0.0001$] and time [$F_{(5, 30)} = 7.17$; $p < 0.01$] and an hour X time interaction [$F_{(10, 60)} = 3.24$; $p < 0.01$]. For the Veh-Suc-Suc group one-way ANOVA revealed significant effects of hour [$F_{(2, 6)} = 6.31$; $p < 0.05$]. For the Veh-Wat-Wat group, one-way ANOVA revealed no significant effects.

4. Discussion

The main findings of this study are threefold: first, THC dose-dependently induces a rapid and robust potentiation of the hedonic reactions to sweet taste in a cannabinoid CB1 receptor-dependent fashion; second: intraoral sucrose, otherwise ineffective per se, given after systemic THC, becomes capable to increase dialysate DA in the shell of the n. accumbens; third, this effect undergoes habituation after repeated exposure to sucrose.

The main conclusion of the present study is consistent with the conclusion of the study of Jarrett et al. (2005), i.e. that THC increases ingestive reactions to intraoral sucrose. However, while in the study of Jarrett et al. (2005) the THC-induced potentiation of hedonic reactivity was limited to 32% as no potentiation was observed to 10% and 20% sucrose, we obtained a significant increase of hedonic responses to 20% and even to 5% sucrose. Moreover, while Jarrett et al. (2005) report an increase in hedonic reactions after THC at 120 min after THC but not 30 min and 60 min thereafter, in our study the increase of ingestive reactions to sucrose was maximal 30 min after THC, leveling off, but being still significant, at 60 min and 120 min. A further difference with Jarrett et al. (2005) is the degree of potentiation of the hedonic scores induced by THC, that was rather mild in the case of Jarrett et al. (2005) but robust in the present study, amounting to about three times the control scores.

A difference is also observed between the present study and the study of Jarrett et al. (2007). In this case the difference is even more substantial, since we failed to observe the reduction in the aversive reactions to quinine solutions after administration of THC they observed. Thus, our observations indicate that the effect of THC on taste reactions specifically affects the processing of hedonic information and excludes that its effect is related to a general influence on arousal or incentive motivation.

The reason for the discrepancies between the present study and those of Jarrett et al. (2005 and 2007) is unclear but, as pointed out in the Introduction, might be related to procedural differences such as the fact that we performed our tests in rats naive to sucrose and quinine and to THC, while Jarrett et al. (2005 and 2007) pre-exposed the animals ad libitum to a 32% sucrose solution as the sole fluid for 5 days and to a 0.05% quinine sulfate solution for 3 days before the start of the taste reactivity tests and in addition administered a dose of 1.0 mg/kg of THC, 72 hours before the taste reactivity experiment. We avoided such pre-exposures because we intended to study in parallel the adaptive responses of brain DA to

repeated sucrose exposure and also because repeated exposure to the taste stimulus over many days could result in associative as well as non-associative mechanisms that might alter the effect of THC. As a matter of fact, in our conditions the effect of THC was more robust and consistent with the time course of other effects of THC as well as with other observations of the literature obtained in other paradigms. For example, our observation that THC potentiated hedonic reactions to 5% and to 20% sucrose, are consistent with the stimulatory effect of THC on the microstructural aspects of licking for a 10% sucrose solutions reported by Higgs et al. (2003) and attributed by these authors to an increase in palatability.

Our results, therefore, provide strong evidence that THC specifically increases palatability of a sweet reward and provide an explanation of what has been known for centuries, the property of cannabinoids to enhance appetite and food consumption (Abel, 1975). This effect has been confirmed in controlled clinical studies in humans (Abel, 1975; Foltin et al., 1988; Yeomans and Gray, 2002; Berry and Mechulam, 2002) and has been demonstrated in experimental animal models (Cota et al., 2003; Fride et al., 2005; Di Marzo and Matias, 2005; Solinas et al., 2008). Some studies have also shown that cannabinoid-induced appetite is selective for foods rich in sugar and fat, suggesting that the effects of THC consistently with an increase in the palatability of food (Arnone et al., 1997; Simiand et al., 1998; but see Rowland et al., 2001; Verty et al., 2004). In addition, THC-induced increases in motivation to obtain food pellets (that are sweeter than normal chow) under a progressive-ratio schedule depends on the actual consumption of the food, suggesting that the gustatory component of food is important for THC's effects on food-reinforced behavior (Solinas and Goldberg, 2005). Finally, Mahler et al. (2007) found that intra-shell injections of the endogenous cannabinoid anandamide also increased hedonic reactions to sucrose.

Natural rewarding stimuli, especially when they are novel and unfamiliar, share with drugs of abuse the ability to increase DA levels in the NAc shell, but this effect undergoes slowly reversible habituation after a single exposure (Bassareo et al., 2002). The second main finding of this study, is that THC administration, through activation of CB1 receptors, enables oral sucrose to increase DA in the shell, but not in the core, of the nucleus accumbens and this effect shows the same adaptive property of a highly palatable food since undergoes habituation after a single exposure to the food taste. Proneness to habituation of n. accumbens shell DA response after repeated exposure is a property of a non-drug reward like palatable food, that distinguishes it from a drug of abuse, whose ability to increase DA in the shell is resistant to habituation after repeated exposure. This indicates that the release of DA in the shell elicited by sucrose shows the properties of a natural reward rather than a drug reward and is consistent with an heightened palatability of sucrose induced by THC. In view of the incentive-motivational properties of n. accumbens DA, the heightened DA releasing properties of sucrose in the shell induced by THC might contribute, together with the enhanced hedonic taste properties of sucrose, to the property of THC to increase the overall reinforcing properties of food (Gallate et al., 1999; Williams and Kirkham, 1999; Kirkham, 2003; Solinas and Goldberg, 2005).

5. Conclusions

The present study demonstrates that the natural cannabinoid receptor agonist THC markedly increases hedonic reactions to sucrose solution and that these effects are mediated by cannabinoid CB1 receptors. In addition, we show that this THC enables sucrose taste to activate DA transmission in the NAc shell and this effect is sensitive to adaptive regulation after repeated exposure to sucrose, consistently with an increase in palatability.

Altogether these findings reinforce the idea that the endogenous cannabinoid system modulates food palatability and that the cannabinoid system concurs with dopamine systems to signal the occurrence of natural rewards.

Acknowledgments

The research performed was supported by the Intramural Research Program of the National Institute on Drug Abuse, National Institutes of Health, Department of Health and Human Services, the National Research Council of Italy (CNR), Ministero dell'Università e della Ricerca (MURST/MIUR), European Community (EC), and Regione Autonoma della Sardegna (RAS) and the Centre National de la Recherche Scientifique, France.

References

- Abel EL. Cannabis: effects on hunger and thirst. *Behav Biol.* 1975; 15:255–281. [PubMed: 1106391]
- Arnone M, Maruani J, Chaperon F, Thiebot MH, Poncelet M, Soubrie P, Le Fur G. Selective inhibition of sucrose and ethanol intake by SR 141716, an antagonist of central cannabinoid (CB1) receptors. *Psychopharmacology.* 1997; 132:104–106. [PubMed: 9272766]
- Bassareo V, De Luca MA, Di Chiara G. Differential Expression of Motivational Stimulus Properties by Dopamine in Nucleus Accumbens Shell versus Core and Prefrontal Cortex. *J Neurosci.* 2002; 22:4709–4719. [PubMed: 12040078]
- Bassareo V, De Luca MA, Aresu M, Aste A, Ariu T, Di Chiara G. Differential adaptive properties of accumbens shell dopamine responses to ethanol as a drug and as a motivational stimulus. *Eur J Neurosci.* 2003; 17:1465–72. [PubMed: 12713649]
- Bassareo V, De Luca MA, Di Chiara G. Differential impact of pavlovian drug conditioned stimuli on in vivo dopamine transmission in the rat accumbens shell and core and in the prefrontal cortex. *Psychopharmacology.* 2007; 191:689–703. [PubMed: 17072592]
- Berridge KC. Measuring hedonic impact in animals and infants: microstructure of affective taste reactivity patterns. *Neurosci Biobehav Rev.* 2000; 24:173–98. [PubMed: 10714382]
- Berry EM, Mechoulam R. Tetrahydrocannabinol and endocannabinoids in feeding and appetite. *Pharmacol Ther.* 2002; 95:185–190. [PubMed: 12182965]
- Bindra D. Neuropsychological interpretation of the effects of drive and incentive-motivation on general activity and instrumental behavior. *Psychol Rev.* 1968; 75:1–22.
- Chang L, Haning W. Insights from recent positron emission tomographic studies of drug abuse and dependence. *Curr Opin Psychiatry.* 2006; 19:246–252. [PubMed: 16612209]
- Colombo G, Agabio R, Diaz G, Lobina C, Reali R, Gessa GL. Appetite suppression and weight loss after the cannabinoid antagonist SR 141716. *Life Sci.* 1998; 63:PL113–117. [PubMed: 9718088]
- Cota D, Marsicano G, Lutz B, Vicennati V, Stalla GK, Pasquali R, Pagotto U. Endogenous cannabinoid system as a modulator of food intake. *Int J Obes Relat Metab Disord.* 2003; 27:289–301. [PubMed: 12629555]
- De Luca MA, Bimpisidis Z, Bassareo V, Di Chiara G. Influence of morphine sensitization on the responsiveness of mesolimbic and mesocortical dopamine transmission to appetitive and aversive gustatory stimuli. *Psychopharmacology.* 2011; 216(3):345–53. [PubMed: 21340470]
- Di, Chiara G. In-vivo brain dialysis of neurotransmitters. *Trends Pharmacol Sci.* 1990; 11:116–121. [PubMed: 1983340]
- Di Chiara G, Bassareo V, Fenu S, De Luca MA, Spina L, Cadoni C, Acquas E, Carboni E, Valentini V, Lecca D. Dopamine and drug addiction: the nucleus accumbens shell connection. *Neuropharmacology.* 2004; 47:227–41. [PubMed: 15464140]
- Di Marzo V, Matias I. Endocannabinoid control of food intake and energy balance. *Nat Neurosci.* 2005; 8:585–589. [PubMed: 15856067]
- Fillenz M. In vivo neurochemical monitoring and the study of behaviour. *Neurosci Biobehav Rev.* 2005; 29:949–962. [PubMed: 15963566]
- Foltin RW, Fischman MW, Byrne MF. Effects of smoked marijuana on food intake and body weight of humans living in a residential laboratory. *Appetite.* 1988; 11:1–14. [PubMed: 3228283]

- Freedland CS, Poston JS, Porrino LJ. Effects of SR141716A, a central cannabinoid receptor antagonist, on food-maintained responding. *Pharmacol Biochem Behav.* 2000; 67:265–270. [PubMed: 11124390]
- Fride E, Bregman T, Kirkham TC. Endocannabinoids and food intake: newborn suckling and appetite regulation in adulthood. *Exp Biol Med.* 2005; 230:225–34.
- Gallate JE, Saharov T, Mallet PE, McGregor IS. Increased motivation for beer in rats following administration of a cannabinoid CB1 receptor agonist. *Eur J Pharmacol.* 1999; 370:233–240. [PubMed: 10334497]
- Grill HJ, Norgren R. The taste reactivity test. I Mimetic responses to gustatory stimuli in neurologically normal rats. *Brain Res.* 1978; 143:263–279. [PubMed: 630409]
- Hajnal A, Smith GP, Norgren R. Oral sucrose stimulation increases accumbens dopamine in the rat. *Am J Physiol Regul Integr Comp Physiol.* 2004; 286(1):R31–37. [PubMed: 12933362]
- Higgs S, Williams CM, Kirkham TC. Cannabinoid influences on palatability: microstructural analysis of sucrose drinking after delta(9)-tetrahydrocannabinol, anandamide, 2-arachidonoyl glycerol and SR141716. *Psychopharmacology.* 2003; 165:370–377. [PubMed: 12447606]
- Jarrett MM, Limebeer CL, Parker LA. Effect of Delta9-tetrahydrocannabinol on sucrose palatability as measured by the taste reactivity test. *Physiol Behav.* 2005; 86:475–479. [PubMed: 16185725]
- Jarrett MM, Scantlebury J, Parker LA. Effect of delta9-tetrahydrocannabinol on quinine palatability and AM251 on sucrose and quinine palatability using the taste reactivity test. *Physiol Behav.* 2007; 90:425–30. [PubMed: 17118412]
- Kirkham TC. Endogenous cannabinoids: a new target in the treatment of obesity. *Am J Physiol Regul Integr Comp Physiol.* 2003; 284:R343–344. [PubMed: 12529283]
- Lecca D, Cacciapaglia F, Valentini V, Gronli J, Spiga S, Di Chiara G. Preferential increase of extracellular dopamine in the rat nucleus accumbens shell as compared to that in the core during acquisition and maintenance of intravenous nicotine self-administration. *Psychopharmacology.* 2006; 184(3–4):435–46. [PubMed: 16397746]
- Mahler SV, Smith KC, Berridge KC. Endocannabinoid Hedonic Hotspot for Sensory Pleasure: Anandamide in Nucleus Accumbens Shell Enhances ‘Liking’ of a Sweet Reward. *Neuropsychopharmacology.* 2007; 32:2267–2278. [PubMed: 17406653]
- McLaughlin PJ, Winston K, Swezey L, Wisniecki A, Aberman J, Tardif DJ, Betz AJ, Ishiwari K, Makriyannis A, Salamone JD. The cannabinoid CB1 antagonists SR141716A and AM 251 suppress food intake and food-reinforced behavior in a variety of tasks in rats. *Behav Pharmacol.* 2003; 14:583–588. [PubMed: 14665975]
- Miller CC, Murray TF, Freeman KG, Edwards GL. Cannabinoid agonist, CP 55,940, facilitates intake of palatable foods when injected into the hindbrain. *Physiol Behav.* 2004; 80:611–616. [PubMed: 14984793]
- Myers KP, Ferris J, Sclafani A. Flavor preferences conditioned by postingestive effects of nutrients in preweanling rats. *Physiol Behav.* 2005; 84:407–419. [PubMed: 15763578]
- Paxinos, G.; Watson, C. The rat brain in stereotaxic coordinates. 4. Academic Press; an Diego, CA: 1998.
- Piomelli D. The endocannabinoid system: a drug discovery perspective. *Curr Opin Investig Drugs.* 2005; 6:672–679.
- Pontieri FE, Tanda G, Di Chiara G. Intravenous cocaine, morphine, and amphetamine preferentially increase extracellular dopamine in the “shell” as compared with the “core” of the rat nucleus accumbens. *Proc Natl Acad Sci USA.* 1995; 92:12304–12308. [PubMed: 8618890]
- Robinson DL, Venton BJ, Heien ML, Wightman RM. Detecting subsecond dopamine release with fast-scan cyclic voltammetry in vivo. *Clin Chem.* 2003; 49:1763–1773. [PubMed: 14500617]
- Rosenstein D, Oster H. Differential facial responses to four basic tastes in newborns. *Child Dev.* 1988; 59:1555–1568. [PubMed: 3208567]
- Rowland NE, Mukherjee M, Robertson K. Effects of the cannabinoid receptor antagonist SR 141716, alone and in combination with dexfenfluramine or naloxone, on food intake in rats. *Psychopharmacology.* 2001; 159:111–116. [PubMed: 11797078]
- Schultz W. Getting formal with dopamine and reward. *Neuron.* 2002; 36:241–263. [PubMed: 12383780]

- Simiand J, Keane M, Keane PE, Soubrie P. SR 141716, a CB1 cannabinoid receptor antagonist, selectively reduces sweet food intake in marmoset. *Behav Pharmacol.* 1998; 9:179–181. [PubMed: 10065938]
- Solinas M, Goldberg SR. Motivational effects of cannabinoids and opioids on food reinforcement depend on simultaneous activation of cannabinoid and opioid systems. *Neuropsychopharmacology.* 2005; 30:2035–2045. [PubMed: 15812567]
- Solinas M, Goldberg SR, Piomelli D. The endocannabinoid system in brain reward processes. *Br J Pharmacol.* 2008; 154:369–83. [PubMed: 18414385]
- Steiner JE, Glaser D, Hawilo ME, Berridge KC. Comparative expression of hedonic impact: affective reactions to taste by human infants and other primates. *Neurosci Biobehav Rev.* 2001; 25:53–74. [PubMed: 11166078]
- Tanda G, Pontieri FE, Di Chiara G. Cannabinoid and heroin activation of mesolimbic dopamine transmission by a common mu1 opioid receptor mechanism. *Science.* 1997; 276(5321):2048–50. [PubMed: 9197269]
- Thornton-Jones ZD, Vickers SP, Clifton PG. The cannabinoid CB1 receptor antagonist SR141716A reduces appetitive and consummatory responses for food. *Psychopharmacology.* 2004; 179:452–460. [PubMed: 15821957]
- Verty AN, McGregor IS, Mallet PE. Consumption of high carbohydrate, high fat, and normal chow is equally suppressed by a cannabinoid receptor antagonist in non-deprived rats. *Neurosci Lett.* 2004; 354:217–220. [PubMed: 14700735]
- Volkow ND, Fowler JS, Wang GJ. Positron emission tomography and single-photon emission computed tomography in substance abuse research. *Semin Nucl Med.* 2003; 33:114–128. [PubMed: 12756644]
- Williams CM, Kirkham TC. Anandamide induces overeating: mediation by central cannabinoid (CB1) receptors. *Psychopharmacology.* 1999; 143:315–317. [PubMed: 10353436]
- Williams CM, Rogers PJ, Kirkham TC. Hyperphagia in pre-fed rats following oral delta9-THC. *Physiol Behav.* 1998; 65:343–346. [PubMed: 9855485]
- Wise RA. Dopamine and reward: the anhedonia hypothesis 30 years on. *Neurotox Res.* 2008; 14(2–3): 169–83. [PubMed: 19073424]
- Yeomans MR, Gray RW. Opioid peptides and the control of human ingestive behavior. *Neurosci Biobehav Rev.* 2002; 26:713–728. [PubMed: 12479844]

Fig. 1. Effects of THC (0.5 and 1.0 mg/kg i.p.) on behavioral hedonic score after 20% sucrose solution (1 ml, i.o.). Data are expressed as means ($\pm$ SEM) of hedonic score at 30, 60, 120 and 240 minutes after THC administration (panel A) or means of total hedonic score (panel B). * $p < 0.05$ and *** $p < 0.001$ with respect to vehicle, # $p < 0.05$ with respect to THC 0.5, ### $p < 0.001$ with respect to THC 0.5 total hedonic.

Fig. 2.

Behavioral hedonic scores after intraoral administration of water, 5% sucrose and 20% sucrose solutions in rats pretreated with THC (1.0 mg/kg i.p.) or vehicle. Data are expressed as means ($\pm$ SEM) of hedonic score at 30, 60, 120 and 240 minutes after treatment (panel A, B, C) or means of total hedonic scores (panel D). * $p < 0.05$ and *** $p < 0.001$ with respect to vehicle, # $p < 0.0001$ with respect to 5% sucrose or water THC rats, § $p < 0.002$ with respect to water vehicle rats.

Fig. 3. Behavioral aversive scores after quinine HCl (5×10^{-4} M, 1 ml i.o.) or NaCl (saturated, 1 ml i.o.) bitter solutions in rats pretreated with THC (1.0 mg/kg i.p.) or vehicle. Data are expressed as means ($\pm$ SEM) of hedonic score at 30, 60, 120 and 240 minutes after THC administration (panel A, B) or means of total hedonic scores (panel C).

Fig. 4. Blockade of the THC effect on hedonic score by rimonabant (1.0 mg/kg i.p.). Data are expressed as means ($\pm$ SEM) of hedonic score after 20% sucrose administration 30, 60, 120 and 240 minutes after THC treatment (panel A) or means of total hedonic scores (panel B) *** $p < 0.001$ with respect to vehicle, SR-vehicle and SR-THC. Note that total hedonic score of rats pretreated with vehicle or THC (panel B) are the same as in Fig. 1.

Fig. 5. Effect of THC (1 mg/kg i.p.) and of intraoral sucrose 20% administration (1 ml, i.o.) on NAc shell (panel A), NAc core (panel B) and PFCX (panel C) dialysate DA levels. Data are expressed as means ($\pm$ SEM) of change in DA extracellular levels expressed as a percentage of basal values. The arrow indicates the start of THC and sucrose administration. Solid symbols: $p < 0.05$ with respect to basal values; * $p < 0.05$ with respect to THC group.

Fig. 6.

Effect of THC (1 mg/kg i.p.), and blockade of THC's effects by rimonabant (SR, 1 mg/kg i.p., 30 min before THC), on changes in NAc shell dialysate DA produced by 20% sucrose administration (1 ml, i.o.). Data are expressed as means ($\pm$ SEM) of change in DA extracellular levels expressed as a percentage of basal values. The arrow indicates the start of THC and sucrose administration. Solid symbols: $p < 0.05$ with respect to basal values; * $p < 0.05$ with respect to THC group. Note that DA extracellular levels of rats pretreated with vehicle-THC are the same as in Fig. 6 (panel A).

Fig. 7.

Effects of THC (1 mg/kg i.p.) on changes in NAc shell dialysate DA produced by repeated 20% sucrose administration (1 ml, i.o.). Data are expressed as means ($\pm$ SEM) of change in DA extracellular levels expressed as percentage of basal values. The arrow indicates the start of Veh (panel A, B) or THC (panel C, D) and water-sucrose (panel A, C) or sucrose-sucrose (panel B, D) administration. Solid symbols: $p < 0.05$ with respect to basal values; * $p < 0.05$ with respect to panel.