Hyperphagia in Pre-Fed Rats Following Oral $\Delta^9$-THC

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WILLIAMS, C.M., P.J. ROGERS AND T.C. KIRKHAM. Hyperphagia in pre-fed rats following oral $\Delta^9$-THC. PHYSIOI. BEHAV 65(2) 343–346, 1998.—Using a pre-feed paradigm, the effects of orally-administered $\Delta^9$-tetrahydrocannabinol (THC) on low baseline levels of nocturnal feeding were assessed. Following 2-h access to a palatable wet mash diet at dark onset, adult male Lister hooded rats (Charles River) were treated with either sesame seed oil vehicle or $\Delta^9$-tetrahydrocannabinol (0.063, 0.12, 0.25, 0.5, 1.0, or 2.0 mg/kg). One hour later, rats were allowed ad libitum access to standard chow, and intakes were monitored over the subsequent 24 h. Doses of 0.5, 1.0, and 2.0 mg/kg produced substantial hyperphagia during the first hour of testing. Subsequently, rats compensated for their overconsumption so that 24-h intakes were similar in all conditions. The data confirm anecdotal reports of the orexigenic actions of exogenous cannabinoids and suggest a critical role for endogenous cannabinoid systems in the regulation of appetite.

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Cannabinoid Appetite Eating Pre-feed $\Delta^9$-tetrahydrocannabinol

RECENT developments in cannabinoid pharmacology, notably the discovery of cannabinoid receptors within the central nervous system (22) and endogenous ligands, such as anandamide (9), have renewed interest in the behavioral effects of plant-derived cannabinoids. One of the most commonly related effects of marijuana or hashish intoxication in humans is that of increased appetite (2), and hyperphagic actions of cannabinoids can be demonstrated. The following study describes a combination of testing and peripheral administration makes them useful tools in the behavioral characterization of the specific aspects of appetite (hunger, palatability or satiation) mediated by brain cannabinoids. It is important, therefore, to determine the extent to which the ability of exogenous agonists to increase food intake reflects a specific role for endogenous cannabinoid systems in the regulation of appetite and eating behavior, rather than secondary to the induction of abnormal or stereotyped behavior. However, a review of the literature reveals that, while widely acknowledged, cannabinoid-induced overeating is a phenomenon supported by essentially anecdotal accounts. In particular, there is a surprising paucity of reliable experimental data to substantiate orexigenic effects of these substances in animal models, with few experimental advances in 20 years, despite modern progress in cannabinoid pharmacology.

Data from human and animal studies have not been wholly consistent. Although several studies have obtained reliable increases of food consumption in humans with $\Delta^9$-THC (1,17,23), interpretation of some studies is limited by the absence of proper controls and the use of single (sometimes unquantified) doses of cannabinoid. Few animal studies have successfully demonstrated hyperphagic effects of $\Delta^9$-THC (5,13,14). The most common findings have been of either no effect on eating or of intake suppression [e.g., (15,26]. Indeed, the predominance of anorectic effects of $\Delta^9$-THC has lead to their listing in recent textbook accounts as a defining feature of cannabinoid administration in rats (10).

The general failure of earlier animal studies to obtain hyperphagic effects is probably explicable in terms of either inappropriate test situations or the tendency to examine the effects of relatively high doses. For example, there is some evidence for a biphasic dose-response function of $\Delta^9$-THC in relation to eating (13), with higher doses probably producing behavioral effects which are incompatible with the normal expression of feeding. Moreover, when hyperphagic effects of $\Delta^9$-THC have been reported in rat experiments, they have been relatively small in magnitude and have not provided unequivocal confirmation of the marked motivational effects of cannabinoids reported in people.

As a preliminary step in determining the involvement of endogenous cannabinoid systems in the regulation of appetite—and in the absence of straightforward, published reference data, we have begun a series of studies to systematically characterize the feeding-related actions of cannabinoid receptor ligands. A crucial step in this work is to establish the optimal test paradigm with which the hyperphagic effects of cannabinoids can be demonstrated. The following study describes a combination of testing...

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conditions, determined from several pilot experiments, which reliably enable the expression of cannabinoid-induced hyperphagia.

Here we report, for the first time, a comprehensive dose-response and time-course analysis of the effects on eating of orally administered Δ²-THC on spontaneous feeding in rats. Rats, being nocturnal, were tested, unfasted, in the dark phase of the daily cycle during which the bulk of their daily eating normally takes place. Additionally, we used a pre-feed procedure, in which rats were allowed to eat a large meal of palatable food prior to drug administration and testing. This simple measure ensures uniformly low baseline intake levels during the test period and so permits easier observation of drug-induced hyperphagic effects (20). These test conditions allowed us to demonstrate substantial increases in the food intake of pre-satiated rats.

METHOD

Animals

Seven adult, male Lister hooded rats (Charles River), weighing 400–450 g at the start of the experiment, were housed individually under a reversed 12:12 h light:dark cycle (lights off at 1000 hours). All experiments were performed in home cages. Except for 1-h food withdrawal on test days, following drug or vehicle administration, rats had free access to laboratory chow (PCD Mod. C; Special Diet Services, Witham, UK) and tap water.

Drugs

Delta-9-tetrahydrocannabinol (Δ²-THC in ethanol solution; Sigma) was dissolved in a sesame seed oil vehicle and administered orally from a 1-ml syringe (5), at a volume of 1 ml/kg. Fresh solutions were prepared on each test day, 15 min before administration. Each animal received all treatments according to a Latin Square design, with at least 72 h between successive treatments.

Procedure

Drug administration began after habituation of animals to housing conditions and test procedures. Immediately after dark onset at 1000 hours, rats were presented with 30 g of a wet mash diet, consisting of 200 ml of ground chow (rat and mouse expanded ground diet; Special Diet Services) plus 250 ml of tap water. Any remaining food and spillage was removed after 120 min. At 1200 hours, oral doses of either vehicle or Δ²-THC (0.063, 0.12, 0.25, 0.5, 1.0, or 2.0 mg/kg) were administered using a standard 1-ml syringe. Rats were then deprived of food until 1300 hours to allow for drug assimilation. At 1300 hours, pre-weighted amounts of normal laboratory chow were returned to the animals. Subsequent food intake (remaining pellets plus spillage) was measured after 1, 2, 3, 4, 5, and 24 h.

All procedures were in compliance with the requirements of the UK Animals Scientific Procedures Act 1986.

Statistics

Dose-related effects of Δ²-THC on intake were analyzed for each measurement interval using one-way ANOVA. The significance of differences between specific treatment means were assessed using the Newman–Keuls test for multiple comparisons.

RESULTS

Under control conditions, pre-feed provision before dosing was very effective in minimizing test food intake. Rats typically consumed all of the palatable pre-feed mash (30 g) and consumed less than 7 g of chow during the first 5 h of ad lib. access; intake being maintained at a constant, low level throughout (1–2 g/h).

As Fig. 1 illustrates, Δ²-THC produced a clear dose-related increase of chow intake over the first hour of testing, with significant increases induced by 0.5, 1.0 and 2.0 mg/kg [F(6, 36) = 10.61, p < 0.001]. The most marked hyperphagic effect was obtained with the 1.0 mg/kg dose, which produced a greater than four-fold increase in consumption over this interval. Although a more extensive dose-response is required, there was some evidence of a biphasic effect of Δ²-THC on feeding, with 2.0 mg/kg being slightly less effective than 1.0 mg/kg; polynomial contrast analysis revealed a significant cubic trend (p < 0.02). Indeed, after administration of the highest dose, nonspecific behavioral effects of the drug were evident, such as impaired motor coordination. No obvious motoric side effects were apparent at the lower doses.

During hours 2 through 4 of the intake test, animals which received hyperphagic doses of Δ²-THC consumed somewhat less than when treated with the lower, ineffective doses. This depression of eating, although not quite achieving statistical significance [F(6, 36) = 2.34, p = 0.052], was most evident during hour 4 and may represent a compensatory restraint from eating as a response to the previous overconsumption. During hour 5, all except the two highest doses produced similar intakes. At the highest dose, intake remained depressed, while 1 mg/kg-treated animals once again displayed a slight elevation [F(6, 36) = 4.26, p < 0.001] which may reflect recovery of eating following the reduced levels of consumption during the previous 2 h.

Intakes between 5 and 24 h were similar after each dose of Δ²-THC, indicating a complete recovery from the feeding effects of the drug. Total 24 h intake was similar under all conditions, ranging between 26–30 g [F(6, 36) = 1.86, NS].

DISCUSSION

This study has successfully demonstrated that, under relatively naturalistic experimental conditions, substantial hyperphagic effects of peripherally-administered Δ²-THC may be reliably obtained in pre-fed rats. This experiment apparently also represents the first comprehensive dose-response and time-course analysis of exogenous cannabinoid effects on eating reported in this species.

The data indicate that, by this oral route of administration, a relatively narrow range of Δ²-THC doses are capable of inducing overeating, and that this effect apparently occurs in the absence of other non-specific behavioral effects. The highest dose tested (2 mg/kg) seems to represent an upper limit for the analysis of specific Δ²-THC effects on ingestive behavior as substantial motor incoordination was noted which apparently competed with, or impaired the hyperphagic effect of the drug.

It is also noteworthy that the considerable degree of hyperphagia observed in this study is comparable to that induced by central administration of neuropeptide Y (7,8), a peptide which is widely regarded as playing a major role in the control of ingestive behavior. Given the combination of an oral route of administration, substantial first pass hepatic metabolism and the lipophilic nature of the THC, it is likely that a very small proportion of the drug, or its behaviorally active metabolites, would have reached the brain. Thus, it will be of great interest to examine central cannabinoid administration effects on eating to compare its potency relative to other established orexigenic substances. Indeed, comparisons between cannabinoid and neuropeptide Y (NPY) effects may be particularly important, as the over-consumption induced by NPY was recently reported to be attenuated by the central cannabinoid (CB1) receptor antagonist, SR 141716 (3), suggesting that these separate neurochemical systems may interact to regulate eating.

The present data provide no indication of how Δ²-THC increases food intake. It is clearly necessary to obtain a more detailed understanding of the adjustments to normal behavioral sequences which underlie the observed overeating. Given that the animals in the present study were pre-satiated prior to testing, it is feasible
FIG. 1. Nocturnal chow intake in pre-fed rats following oral $\Delta^8$-THC during each measurement interval after food presentation. Note the early hyperphagic effects of the drug. Vehicle or drug administration took place 1 h before start of intake tests. All data points represent the mean intake ($\pm$SEM) of seven rats. Asterisks indicate significant difference from vehicle condition within each interval ($p < 0.05$).
that the observed hyperphagia may result from some inhibition of normal satiation processes, inhibiting intrameal satiety (following the pre-feed) and delaying termination of the test meal. Interestingly, such an effect on satiation has also been proposed for NPY (21), further strengthening the possibility of functional links between cannabinoids and NPY.

Alternatively, the drug may have increased motivation to eat by enhancing the sensory or rewarding properties of the test food. This latter hypothesis is given some indirect support by the finding that Δ⁹-THC will increase intake of palatable sucrose solutions (5). Additionally, it has been reported that a specific CB1 antagonist will reduce ingestion of sweet foods and solutions (3,25). Similar suppressive effects obtained with opioid antagonists have been crucial in implicating endogenous opioid peptides in the mediation of food reward (18,19). Moreover, there is substantial evidence for functional interactions between cannabinoids and opioids, including brain reward systems (24,27). For example, mesolimbic dopamine pathways are activated following peripheral THC administration (6,11,12). This activation, which resembles the effects of opiate receptor agonists on dopamine transmission, can be blocked by opioid antagonists (27), further strengthens the link between cannabinoids and central opioidergic and dopaminergic reward processes.

Detailed observational and meal pattern analyses are clearly required to address these issues, not least to discount the possibility that agonists such as Δ⁹-THC modify eating through actions unrelated to the processes which normally regulate appetite; such as through the generation of nonspecific, stereotyped behaviors. Overall, the magnitude of these hyperphagic effects of Δ⁹-THC, alone, may indicate a pivotal role for endogenous cannabinoid receptors in appetite regulation. If confirmed, advances in the behavioral pharmacology of the cannabinoid agonists and antagonists may ultimately have a significant impact on the development of treatments for conditions such as cachexia and other disorders of eating and body-weight regulation. But our results emphasize the necessity to conduct more extensive analyses of the effects of newly developed cannabinoid receptor agonists and antagonists on feeding. The establishment of a simple experimental paradigm—as reported here—for the reliable demonstration of hyperphagic effects of cannabinoid receptor agonists is thus an important step in this research.

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