Cannabinoids augment the release of neuropeptide Y in the rat hypothalamus

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Abstract

Little is known about the mechanism of action behind the orexigenic activity of cannabinoids. Neuropeptide Y (NPY) is one of the most potent orexigenic factors and is a key mediator in the hypothalamic control of food intake. We examined the effect of cannabinoids on NPY release using a rat hypothalamic explant model. The cannabinoid agonists anandamide (AEA) and CP55,940 both significantly augmented resting and KCl-evoked NPY release. AM251, a cannabinoid receptor antagonist, blocked the augmentation of NPY release elicited by AEA and CP55,940. Additionally, AM251 administered alone, in the absence of exogenous cannabinoid agonists, inhibited NPY release demonstrating the role of endogenous cannabinoids in NPY release. Combined, these findings demonstrate that cannabinoids augment NPY release in the hypothalamus and that this may be a potential mechanism behind the orexigenic activity of cannabinoids.

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1. Introduction

Neuropeptide Y (NPY) is a well established potent orexigenic neuropeptide. The orexigenic activity of NPY has been localized to the hypothalamus, specifically to NPY pathways projecting from the arcuate nucleus (ARC) to the paraventricular nucleus (PVN), lateral hypothalamus (LH), dorsomedial hypothalamus (DMH), and ventromedial hypothalamus (VMH) (Bai et al., 1985; Sahu et al., 1988; Elias et al., 1998). Indeed, injection of NPY into the PVN produces a robust feeding response (Stanley and Leibowitz, 1985).

The appetite stimulating effects of cannabinoids are also well established (Greenberg et al., 1976; Williams and Kirkham, 1999; Hao et al., 2000). Cannabinoids are found exogenously, as in the drug marijuana, as well as endogenously as the arachidonic acid derivative anandamide. Two cannabinoid subtypes have been cloned, CB1, which are found primarily in the brain (Devane et al., 1988; Matsuda et al., 1990), and CB2, which are found primarily in the periphery (Munro et al., 1993). CB1 receptors are found throughout the brain, including the ARC, PVN, LH, and VMH of the hypothalamus (Herkenham et al., 1991; Fernandez-Ruiz et al., 1997).

Cannabinoids are generally thought to function at presynaptic receptors and have been found to inhibit the release of a variety of neurotransmitters (Shen et al., 1996; Gifford et al., 1997; Schlicker et al., 1997; Ameri et al., 1999; Kathmann et al., 1999; Katona et al., 1999; Nakazi et al., 2000). However, cannabinoids have also
been found to enhance the release of dopamine (DA) (Chen et al., 1990a; Chen et al., 1990b). Another study found a biphasic effect of the cannabinoids Δ9-THC and Δ8-THC on the release of norepinephrine (NE) and DA from synaptosomes prepared from striatum and hypothalamus. This study demonstrated inhibition of release at lower concentrations of cannabinoids, and enhancement of release at higher concentrations (Poddar and Dewey, 1980).

NPY is regulated by both orexigenic and anorexigenic factors. Typically, anorexigenic factors such as leptin and alpha melanocyte stimulating hormone (α-MSH) inhibit NPY release and synthesis (Stephens et al., 1995; Wang et al., 1997; Lee and Morris, 1998; King et al., 2000), whereas orexigenic factors such as orexin have been shown to induce Fos expression in NPY neurons (Horvath et al., 1999; Yamanaka et al., 2000). Few studies have examined the interactions between cannabinoids and neuropeptide Y, however, their functional similarities as well as their localization in the same brain structures strongly suggest the existence of such interaction. It is likely that cannabinoids enhance the release of NPY, as cannabinoids are orexigenic in nature, and have been shown to have the ability to enhance transmitter release. The purpose of the present study was to examine the possible interactions between cannabinoids and neuropeptide Y. To assess this possible interaction, we have examined the effect of cannabinoids on the release of NPY using a hypothalamic explant model.

2. Methods

2.1. Hypothalamic explants

All procedures using laboratory animals (rats) were carried out in accordance with National Institute of Health guidelines and were approved by the Institutional Animal Care and Use committee of Saint Louis University Health Sciences Center. Male Sprague-Dawley rats (300–350 g) were anesthetized with a 50 mg/kg intraperitoneal injection of sodium pentobarbital and then euthanized by decapitation. The brain was rapidly removed and placed in a chilled rat brain matrix, where a 4 mm coronal slice containing the relevant areas of the hypothalamus was sectioned out. The explant was taken 1 mm caudally from the point where the anterior commissure was observed to traverse. A 4 mm coronal slice was taken from this point, which was further sectioned 6 mm in the medial-lateral direction and 3 mm in the dorsal-ventral direction. The explant was immediately placed in a small wire mesh basket and lowered into 1 mL of a Krebs-Ringer MOPS buffer, containing: NaCl 118 mM, KCl 4.7 mM, MgSO4 1.2 mM, CaCl2 1.7 mM, KH2PO4 1.2 mM, dextrose 10 mM, NaHCO3 25 mM, and MOPS 23 mM. High KCl buffer used an additional 50 mM of KCl and NaCl was lowered to 68 mM to maintain osmolarity. The vial was then transferred to a water bath, maintained at 37 °C and continuously bubbled with 95/5% O2/CO2.

The explants were then allowed to equilibrate for a period of 30 min, followed by a 60-min treatment with pharmacological agents and, lastly, a 15-min stimulation period. During the stimulation period, the sections were exposed to Krebs–Ringer MOPS buffer containing 50 mM KCl, and sodium adjusted to maintain osmolarity. In experiments using pharmacological agents, these agents were also present in the stimulation buffer.

Four 200 μL samples were then taken from the pharmacologically treated and stimulation periods and immediately placed on ice. The samples were then frozen at −80 °C, until assayed for neuropeptide Y by enzyme immunoassay.

2.2. Drugs

All cannabinoids were purchased from Tocris (St. Louis, MO). CP55,950 and AM251 were prepared in dimethyl sulfoxide (DMSO). Anandamide came as a water soluble emulsion, in a proprietary vehicle called Tocrisolve. Respective vehicles were added in all control experiments.

2.3. Column extraction of NPY

The samples obtained in the brain slice experiments were thawed to room temperature and run over Sep-Collumns packed with 200 mg C18 (Peninsula Laboratories) for extraction of NPY. Briefly, the columns were first washed with 1 mL of acetonitrile followed by three washes of 3 mL 1% trifluoroacetic acid (TFA). The samples were then acidified with 200 μL 1% TFA and loaded onto the columns. The columns were then washed three additional times with 1% TFA, and then eluted with 3 mL of a solution containing 60% acetonitrile and 1% TFA. The eluent was collected, and then vacuum dried overnight in a Speed-Vac (Savant).

2.4. Measurement of NPY

The dried samples obtained by column extraction were then reconstituted with 120 μL of assay buffer and analyzed by enzyme immunoassay (Peninsula Laboratories). The enzyme immunoassay (EIA) is a competition assay measuring the displacement of biotinylated NPY. Briefly, samples were first incubated for 1 h at room temperature with a primary antibody to NPY in a 96 well plate. In addition, secondary antibodies were attached to the wells of the plate. Following this 1 h incubation period, biotinylated NPY was added and
the plate was allowed to set for 2 h. Following this period, the plate was washed five times with assay buffer and streptavidin-horse radish peroxidase (SA-HRP) was added and allowed to incubate for 1 h at room temperature. The plate was then washed five times with assay buffer before the addition of tetramethyl benzidine (TMB), a chromogenic substrate for HRP. Following a 30 min incubation, 2 N HCl was added to terminate the reaction and the plate was read using a microtiter plate reader.

The EIA possesses minimal cross reactivity as indicated by the manufacturer. It detects NPY from rat, human, or porcine origin and has a 3% cross reactivity with human pancreatic polypeptide. The assay shows no cross reactivity for PYY (rat or porcine), VIP (human, rat, or porcine), amylin (human), prepro NPY 68-97 (human), insulin (human), or somatostatin. The EIA is sensitive to 0.04–0.06 ng/mL NPY.

2.5. Statistical analysis

The results were analyzed by one-way ANOVA, followed by post-hoc Newman–Keuls multiple comparison test. Results were considered statistically significant if \( p < 0.05 \).

3. Results

3.1. Cannabinoid agonists increase NPY release

Fig. 1 demonstrates the effect of the cannabinoid agonist CP55,940 on NPY release from hypothalamic explants. CP55,940 (100 nM) significantly increased the release of NPY under resting conditions (Fig. 1a). CP55,940 (10 and 100 nM) also significantly increased KCl-evoked NPY release (Fig. 1b).

We next addressed the effect of the endogenous cannabinoid anandamide on NPY release. Anandamide (AEA) at concentrations of 10, 50, and 100 nM significantly enhanced the resting release of NPY from hypothalamic explants (Fig. 2a). AEA at 50 and 100 nM also augmented KCl-evoked release of NPY (Fig. 2b). These experiments show that NPY release can be modulated by exogenous application of both synthetic and endogenous cannabinoids.

3.2. Cannabinoid antagonists decrease NPY release

To verify that the effect of CP55,940 and AEA were specific and mediated by cannabinoid receptors, we next addressed whether the enhancement of NPY release by cannabinoids could be blocked by the CB1 cannabinoid receptor antagonist AM251. For this experiment, the hypothalamic explants were incubated with both CP55,940 and AM251 (1 \( \mu \)M), for 60 min followed by a 15 min stimulation with KCl. AM251 significantly blocked the CP55,940 enhanced release of NPY in both resting (Fig. 3b) and stimulated (KCl) (Fig. 3b) settings.

The ability of AM251 to block the anandamide enhancement of NPY release was next assessed. Anandamide was administered to the incubation buffer at 100 nM in both the presence and absence of AM251 (1 \( \mu \)M). AM251 significantly blocked the anandamide enhancement of NPY release in both basal (Fig. 4a) and KCl-stimulated (Fig. 4b) conditions.

We then examined the effect of AM251, by itself, in the absence of exogenous agonist. AM251 concentration dependently inhibited the release of basal NPY release (Fig. 5a), and there was a trend to decrease KCl-evoked NPY release (Fig. 5b).
4. Discussion

The effect of cannabinoids on food intake has been well documented. This effect is frequently reported by recreational marijuana users and is documented as far back as 300 A.D. Additionally, endogenous cannabinoids have been shown to have similar orexigenic effects (Williams and Kirkham, 1999). Rimonabant (formally known as SR141716), a cannabinoid receptor antagonist, has been shown to decrease food intake in rats (Colombo et al., 1998) and mice (Di Marzo et al., 2001). Further, mice deficient of the CB1 receptor (the cannabinoid receptor subtype found in brain) eat less than wild-type mice, and rimonabant does not affect food intake in these mice (Di Marzo et al., 2001). The importance of these effects has been further highlighted by the pending approval by the FDA of rimonabant, a cannabinoid receptor antagonist indicated for the reduction of body weight in obese individuals.

Little is currently known about the mechanism behind the orexigenic activity of cannabinoids. There has been some debate as to the role that cannabinoids play in food intake, specifically if the orexigenic activity is homeostatic or hedonic. A homeostatic role indicates the importance of the hypothalamus in mediating the orexigenic activities of cannabinoids, while a hedonic role indicates activation of reward circuitry (for review see: Harrold and Williams, 2003). Direct administration of anandamide into the ventromedial hypothalamus has been shown to increase food intake, providing evidence...
that the orexigenic activity of cannabinoids is at least partly hypothalamic (Jamshidi and Taylor, 2001). Our data presented in this paper supports a role of the hypothalamus in mediating the orexigenic effects of cannabinoids, as cannabinoids were able to influence NPY release in hypothalamic explants.

NPY, as mentioned previously, possesses a potent orexigenic activity. Injection of NPY into the PVN produces a feeding response so robust that the animal eats at its maximum physiologic capability (Stanley et al., 1985). NPY is an important regulator of food intake and its release is inhibited by the anorexigenic hormone leptin (Stephens et al., 1995; Wang et al., 1997; Lee and Morris, 1998; King et al., 2000). Leptin is produced by adipocytes in the periphery, released into the circulation, and acts centrally on the hypothalamus (Zhang et al., 1994; Campfield et al., 1995; Halaas et al., 1995; Pelleymounter et al., 1995; Ahima et al., 1996). One of the primary ways in which leptin exerts its anorexigenic actions is by inhibiting NPY. As expected, the ob/ob mouse (which lacks functional leptin) is obese, while the ob/ob NPY^+/− knockout mouse possesses a leaner phenotype (Erickson et al., 1996).

Clearly, alterations in NPY levels can significantly affect food intake. Here, we show that cannabinoids indeed alter NPY in an explant release model. Although not directly measured in the present study, it is likely that the increase in NPY release caused by the action of cannabinoid agonists results in an increase in food intake in an intact animal. Furthermore, the anorexigenic effect of the pending drug rimonabant, a cannabinoid receptor antagonist, may also be partly due to inhibition of NPY release because we show here that AM251, an analog of rimonabant, was able to significantly decrease NPY release.
Our data represent the first attempt to directly examine the effect of cannabinoids on NPY release. There are a few reports in the literature examining the interactions of NPY and cannabinoids and the resulting effect on food intake. Both NPY and cannabinoids have been shown to increase the intake of sucrose solutions. Sucrose intake was blocked by rimonabant (the CB1 antagonist), indicating the role of endogenous cannabinoids. Further, NPY induced sucrose intake was also blocked by rimonabant (Arnone et al., 1997). The orexigenic effect of NPY has also been shown to be decreased in CB1 knockout mice (Poncelet et al., 2003). These results suggest that CB1 receptors are required for the orexigenic effects of NPY. We did not examine the modulation of cannabinoid release by NPY in the current studies, however these cited studies combined with our present findings suggests the possibility of the presence of a positive feedback loop.

The ability of rimonabant to affect food intake in NPY knockout mice has also been assessed (Di Marzo et al., 2001). Rimonabant was able to reduce food intake in NPY knockout mice, however, it is important to note that NPY knockout mice are of normal body weight, presumably due to compensation by other feeding regulators. It is therefore quite likely that cannabinoids could be affecting other feeding systems in these mice.

It is unclear as to where within the hypothalamus cannabinoids are acting to release NPY. NPY is primarily produced in the arcuate nucleus (ARC), which sends NPY-containing projections to other feeding centers, including the paraventricular nucleus (PVN), ventro- and dorsomedial hypothalamus (VMH and DMH, respectively), and lateral hypothalamus (LH) (Bai et al., 1985; Sahu et al., 1988; Elias et al., 1998). CB1 cannabinoid receptors have been shown to be present in all of these areas (Herkenham et al., 1991; Fernandez-Ruiz et al., 1997), however, their colocalization with NPY neurons has not been demonstrated (Cota et al., 2003).

Based on the present studies, we cannot conclude if the observed effect on NPY release is caused by direct action of cannabinoids on NPY neurons. The explant model maintains the circuitry within the hypothalamus, so it is also possible that cannabinoids are affecting an intermediate factor which, in turn, alters NPY release. The increase in NPY release, therefore, maybe the result of disinhibition, which would fall into agreement with the general inhibitory role of cannabinoids. Further studies are necessary to establish this mechanism in more detail.

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References


