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Local administration of a cannabinoid agonist alters norepinephrine efflux in the rat frontal cortex

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Abstract

Δ^9 -tetrahydrocannabinol, the main psychoactive ingredient in marijuana, activates specific cannabinoid (CB) receptors to exert complex actions on modulatory neurotransmitters involved in attention and cognition. Previous research has demonstrated that systemic administration of the synthetic cannabinoid agonist, WIN 55,212-2, increases norepinephrine efflux in the frontal cortex. The distribution of CB1 receptors on noradrenergic fibers in the frontal cortex suggests this may be one potential site for the regulation of norepinephrine release. In the present study, we first examined the ability of a CB1 antagonist, applied locally in the frontal cortex of adult male Sprague Dawley rats, to block the actions of systemic WIN 55,212-2. Pretreatment with SR 141716A (300 μ M) significantly attenuated the excitatory effects of WIN 55,212-2 (15 mg/kg, i.p.). Next, the impact of direct perfusion of WIN 55,212-2 into the frontal cortex on extracellular norepinephrine efflux was measured. Direct application of WIN 55,212-2 (100 μ M) into the frontal cortex elicited a significant increase in extracellular norepinephrine efflux suggesting that activation of cortical cannabinoid receptors contributes to alterations in norepinephrine levels in this brain region. Finally, local administration of SR 141716A followed by local administration of WIN 55,212-2 revealed a paradoxical inhibition of norepinephrine efflux.

Keywords

WIN 55; 212-2; reverse microdialysis; locus coeruleus

Introduction

Interest in the pharmacotherapeutic properties of cannabinoid ligands has driven a wealth of research focused on the endogenous cannabinoid signaling system. Some evidence suggests that activation of presynaptic CB1 receptors results in inhibition of neurotransmitter release, while others have reported increases in transmitter release following cannabinoid administration [1,38,39]. We have shown that CB1 receptor activation by a synthetic cannabinoid agonist elicits increases in extracellular norepinephrine in the forebrain [30] and that noradrenergic axon terminals express CB1 receptors [29].

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Noradrenergic projections from the locus coeruleus (LC) to the frontal cortex are important for the regulation of attention and cognitive processing [33] and play a key role in vigilance [4]. This focusing of attention is thought to be in part due to the ability of norepinephrine to increase the signal to noise ratio in postsynaptic targets [46]. However, it has also been demonstrated that the effects of norepinephrine on postsynaptic activity follow an inverted U function with optimal responses observed with mid-range norepinephrine levels. At very low or very high concentrations of norepinephrine, postsynaptic effects as well as behavioral performance tend to be disrupted [6,15]. The finding that acute cannabis use impairs the ability to effectively focus attention and reject irrelevant information suggests an interaction of cannabinoids with the coeruleo-cortical noradrenergic pathway with possible negative impact [9,10,37]. Moreover, typical characteristics of chronic cannabis users include an inability to filter out extraneous information, lack of focused attention and failure to habituate to irrelevant stimuli that suggest a dysfunctional gating mechanism or ineffective processing strategies in noradrenergic circuits [37].

Our laboratory has previously shown that systemic administration of WIN 55,212-2 causes an increase in extracellular norepinephrine release in the frontal cortex [30]. We also demonstrated the co-existence of CB1 receptors and dopamine beta hydroxylase in axon terminals in the rat frontal cortex [29]. Following repeated administration of WIN 55,212-2, we showed an increase in tyrosine hydroxylase expression in the LC that was accompanied by an increase in anxiety-like behavior [31]. These findings provide evidence for a functional link between cannabinoid receptors and norepinephrine output. In the present study, we examined the impact of direct CB1 receptor blockade on the effects of systemic cannabinoid agonist-induced norepinephrine efflux. Furthermore, we demonstrated that local application of WIN 55,212-2 is sufficient to elicit increases in extracellular norepinephrine in the frontal cortex. Interestingly, prior administration of a CB1 antagonist followed by local agonist infusion resulted in a decrease in extracellular norepinephrine.

Methods

Subjects

Adult male Sprague-Dawley rats (Harlan Laboratories, Indianapolis, IN) weighing 250–300 g were housed 2–3 per cage on a 12-hour light schedule in a temperature-controlled (20°C) colony room. The Thomas Jefferson University Institutional Animal Care and Use Committee (IACUC) approved the care and use of animals and all studies were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

Microdialysis and high-pressure liquid chromatography

After an acclimation period of approximately one week, rats were anesthetized with isoflurane (1–2% in air) and placed in a stereotaxic apparatus with the skull flat as described in detail in [30]. Vertical concentric microdialysis probes were used. Artificial cerebrospinal fluid (aCSF: 174 mM NaCl, 1.7 mM CaCl₂, 0.9 mM Mg Cl₂, and 4 mM KCl) was continuously perfused through the probe at a rate of 1.5 µl/min by a microliter infusion pump (Harvard Pump '11' VPF Dual Syringe, Harvard Apparatus, Holliston, MA). Rats were allowed to recover overnight. The ability of the cannabinoid receptor antagonist, SR 141716A to block the actions of systemic WIN 55,212-2 on norepinephrine efflux was tested. Approximately 18 hours following surgery, dialysate sample collection began at 20 min. intervals. After collecting samples for at least 2 hours to establish stable baseline levels, the cannabinoid antagonist SR 141716A (300 µM, received as a gift from NIDA) was perfused locally through the dialysis probe directly into the frontal cortex for 60 min (3 samples). At the end of one hour, the perfusion medium was switched back to aCSF. A single sample was collected and then WIN 55,212-2 (15 mg/kg; mesylate salt, Sigma Chemicals, St. Louis, MO) was administered via

i.p. injection and sample collection continued for an additional 3 hours. Control animals received local aCSF through the probe and systemic WIN 55,212-2. SR 141716A was prepared immediately prior to the start of each experiment by first dissolving in 0.4% Tween (Sigma Chemicals) and diluting with aCSF to a concentration of 300 μ M. A liquid switch was used to alternate between aCSF and SR 141716A. Dialysate samples were stored at -80°C for subsequent analysis by HPLC-ED. In a separate experiment, WIN 55,212-2 was infused directly into the frontal cortex via the implanted probe. At the conclusion of the experiment, rats were deeply anesthetized with pentobarbital and 2% pontamine sky blue dye (Alfa Aesar, Ward Hill, MA) was infused through the probe to mark its location. The rats were transcardially perfused with 10% formalin (Fisher Scientific, Pittsburgh, PA), decapitated and the brains removed for subsequent histological verification of probe placement. The data were not included if the placement was outside the infralimbic and prelimbic areas of the frontal cortex.

Dialysate samples (15 μ l) were injected into the HPLC system using an autosampler (ESA Inc, Chelmsford, MA). The detection system consisted of an ESA Coulochem II electrochemical detector with a guard cell and a 5041 enhanced amperometric analytical cell (ESA Inc., Chelmsford, MA) with a glassy carbon in ceramic target electrode, in series. The applied potential of the guard cell was -150 mV and the compounds of interest were quantified at the target electrode set at $+220\text{ mV}$. Peak heights were measured and compared to the peak heights of a 10^{-8} M standard calibrated daily. The detection limit, defined as the sample amount producing a peak height that is twice the height of the background noise, was approximately 0.5 pg of norepinephrine.

The baseline value against which drug effect was compared to was derived from the average of three samples just prior to drug infusion. The neurochemical data are expressed as the mean $\pm$ S.E.M. or absolute value (pg/15 μ l). The overall effect of treatment of WIN-55,212-2 on monoamine efflux in the frontal cortex was analyzed using 2-way analysis of variance (ANOVA) with repeated measures over time ($p < 0.05$). The absolute amount of neurotransmitter measured in dialysates (pg/sample) was used as the dependent variable for assessment of within group effects. Basal values plus the next eight samples post drug injection were used in the analysis. All statistics were performed using JMP software (JMP, Cary, NC).

Results

Effect of SR 141716A on systemic WIN 55-212,2-evoked norepinephrine efflux

Cortical norepinephrine levels were assessed using *in vivo* microdialysis combined with HPLC-ED and these data are shown in Figure 1. Previous experiments from our laboratory have indicated that systemic administration of the cannabinoid agonist WIN 55-212,2 at a dose of 15 mg/kg elicits a 2-fold increase in extracellular norepinephrine in the frontal cortex [30]. To examine whether the effects of WIN 55,212-2 on norepinephrine release were mediated through activation of CB1 receptors located in the frontal cortex, the CB1 receptor antagonist, SR 141716A, was slowly perfused through the dialysis probe into the frontal cortex for a total of 60 min. A single 20 min sample was collected before a systemic WIN 55-212,2 injection was made. Baseline levels of extracellular norepinephrine in the frontal cortex were stable with a mean of $1.53 \pm 0.18\text{ pg/15 } \mu\text{l}$ ($n = 8$). Blockade of CB1 receptors by direct infusion of SR 141716A (300 μ M) into the frontal cortex had no impact on basal levels of norepinephrine but significantly attenuated the increase in extracellular norepinephrine elicited by systemic WIN 55,212-2 (36% vs. 153%). Two way ANOVA with repeated measures showed no significant effect of treatment, a significant effect of time ($F_{\text{time}[7,77]} = 18.5$; $p < .0001$) and a significant interaction ($F_{\text{int}[7,77]} = 2.4$; $p > .05$).

The dose of SR 141716A (300 μ M) that was infused in the frontal cortex in the present study has been shown in other studies to act as an antagonist and have no effects on cAMP levels

when administered alone [45]. In order to verify that SR 141716A did not have any effects on basal norepinephrine release, 300 μ M of the cannabinoid receptor antagonist was infused directly into the frontal cortex for 60 min followed by a systemic injection of saline (Fig. 2). Baseline levels of extracellular norepinephrine in the frontal cortex were stable with a mean of 1.57 ± 0.29 pg/15 μ l (n = 6). Local infusion of SR 141716A followed by systemic vehicle injection did not significantly alter extracellular norepinephrine release in the rat frontal cortex (Fig. 2, gray squares; $F[5,7] = .67$; one way ANOVA with repeated measures).

Effect of local application of WIN 55,212-2 on basal norepinephrine efflux

To verify that local activation of CB1 receptors in the frontal cortex was sufficient to elicit increases in extracellular norepinephrine efflux, WIN 55,212-2 (100 μ M) was administered by reverse microdialysis and cortical norepinephrine efflux following drug exposure was assessed. Local infusion of WIN 55,212-2 caused a significant increase (65%) in the extracellular levels of norepinephrine in the frontal cortex compared to baseline levels (from 1.88 ± 0.24 pg/15 μ l to 3.12 ± 0.42 pg/15 μ l; (Fig. 2, black diamonds; $F[5,8] = 3.03$ ($p < 0.01$) one way ANOVA with repeated measures). An overall ANOVA comparing WIN 55,212-2 and aCSF revealed a significant effect of treatment ($F_{\text{Dose}} [1,11] = 2.80$, $p = 0.12$), time ($F_{\text{Time}} [8,88] = 3.71$, $p < 0.001$) and time by treatment interaction ($F_{\text{Int}} [8,88] = 3.01$, $p < 0.01$). Post hoc analysis showed significantly greater norepinephrine output at 60, 80 and 100 min post injection of WIN 55,212-2 infusion (Dunnett's; $*p < 0.05$). Vehicle treatment did not elicit any change in norepinephrine release (data not shown). These data suggest that local administration of the synthetic cannabinoid agonist WIN 55-212,2 causes an increase in norepinephrine efflux via activation of CB1 receptors in the frontal cortex.

Local WIN 55,212-2 in combination with SR 141716A inhibits norepinephrine efflux

Prior blockade of CB1 receptors with local perfusion of SR 141716A not only prevents the increase in norepinephrine elicited by local WIN 55,212-2 infusion but also reveals an inhibition of norepinephrine efflux (Fig. 2). SR 141716A was perfused through the probe for 60 min followed by perfusion with WIN 55,212-2 for 60 min. A significant decrease in extracellular norepinephrine was observed for approximately 80 minutes followed by a return to baseline levels. Basal extracellular norepinephrine ($1.37 \pm .14$ pg/15 μ l) remained stable during the 60 min of SR141716A perfusion ($1.47 \pm .14$ pg/15 μ l) but fell 44% below baseline following 60 min of WIN 55,212-2 perfusion. This reduction of extracellular norepinephrine was statistically significant determined by one way ANOVA with repeated measures ($F [13, 7] = 3.2$; $p < .01$).

Discussion

The present results extend our previous findings examining cannabinoid modulation of the coeruleo-cortical pathway by showing that (1) local infusion of the cannabinoid receptor agonist WIN 55-212,2 into the frontal cortex stimulates norepinephrine efflux, (2) the enhancement of cortical norepinephrine is mediated via the CB1 receptor as this effect is blocked by pre-treatment with local infusion of SR 141716A into the frontal cortex prior to a systemic cannabinoid injection and (3) local administration of SR 141716A followed by local administration of WIN 55,212-2 reveals a paradoxical inhibition of norepinephrine efflux. These data, combined with our recent study showing localization of CB1 receptors on noradrenergic terminals in the frontal cortex [29], suggests that increases in norepinephrine efflux are mediated, in part, by direct actions of cannabinoid agonists acting on CB1 receptors that are localized to cortical noradrenergic axon terminals. However, given the paradoxical decrease in extracellular norepinephrine when agonist infusion was preceded by an antagonist infusion, we cannot exclude the possibility that norepinephrine efflux is also modulated

indirectly through complex interactions with interneurons or other neurochemically distinct afferents.

Our study does not reveal any tonic activity of endogenous cannabinoids on norepinephrine release in the frontal cortex as administration of the antagonist SR 141716A alone did not elicit any effect on cortical norepinephrine efflux. This finding differs from a study by Tzavara and colleagues who showed that following systemic administration of SR 141716A, norepinephrine levels in the rat anterior hypothalamus and the frontal cortex were increased [41,42]. Discrepancies with our study are likely due to differences in the dose of SR 141716A [42]. In their study, a five-fold higher dose of SR 141716A was used compared to our study. SR 141716A has been reported to act as an inverse agonist [28], thus it is feasible that at the dose used in the Tzavara study, SR 141716A may have been acting as an inverse agonist of the CB1 receptor.

Our findings are in agreement with previously published studies showing that activation of CB1 receptors presynaptically modulates release of neurotransmitters [16,18,19,22,26,34,44]. In some cases, increases in norepinephrine release have been found to occur via a trans-synaptic mechanism whereby activation of the CB1 receptor causes an inhibition of GABA release that target noradrenergic terminals, disinhibiting norepinephrine release (for review, see [36]). Though anatomical evidence for a presynaptic effect occurring in the frontal cortex exists, two alternative hypotheses could also be considered: activation of cortical CB1 receptors may cause an increase in glutamate release onto noradrenergic terminals causing an increase in norepinephrine release, or the inhibition of GABA release onto noradrenergic terminals may also contribute to the overall effect. These hypotheses remain to be tested. Alternatively, additional cannabinoid receptors that are yet to be determined may be involved.

The reason for selecting the frontal cortex as a target of noradrenergic actions are several. The frontal cortex plays an important role in processes related to cognition, mood control, attention and motor performance [17]. This brain region receives a strong modulatory input from monoaminergic transmitters and many affective disorders are thought to reflect disruptions in the regulation of these neurotransmitters [3,23]. Modulation of the LC-frontal cortex pathway via CB1 receptor activation may contribute to changes in attention, cognition and anxiety commonly observed following cannabinoid receptor agonist exposure as this circuit is involved in modulating many of these behaviors [5,7,21,43]. An upregulation of the CB1 receptor in the prefrontal cortex of depressed suicide victims was recently reported [20]. The potential link between chronic cannabis use and affective disorders including anxiety, depression, cognitive impairment, and psychosis is supported by a number of studies [2,8,14,25,32,40]. This alteration in CB1 binding suggests that modulation of the cannabinoid signaling system may prove to be a useful therapeutic target.

Administration of the CB1 antagonist, SR 141716A has revealed an important role for the endogenous cannabinoid system in a variety of behaviors including drug reinforcement, alcohol preference, cocaine craving, arousal and memory functions (see [11] for review). For example, SR 141716A was shown to reduce the perception of the rewarding value of positive reinforcers, attenuate morphine induced place preference and withdrawal [27], reduce alcohol intake at high doses (2, 5 and 10 mg/kg) [12], reduce cocaine craving [13] and suppress food intake [11]. Application of SR 141716A increases arousal [35] and enhances spatial memory in the radial arm maze [24]. Although these data point to potential therapeutic applications, the mechanisms underlying these effects remain unknown. Interactions of the cannabinoid system with other neuromodulatory transmitter systems is well documented and provide an intriguing target for novel therapeutic agents. The present study reports the ability of low doses of SR 141716A to block the actions of a CB1 agonist applied systemically. The dose utilized in these studies is much lower than those previously reported describing an effect of SR 141716A alone

on neurotransmitter output. It is important to note that no effect was seen on norepinephrine efflux when SR 141716A was administered alone in our studies. A somewhat surprising finding was the decrease in norepinephrine observed following direct application of SR 141716A and WIN 55,212-2 into the frontal cortex. However, only one dose was tested and our findings indicate that further studies are warranted to investigate this phenomenon.

Further studies are required to define the impact of chronic cannabinoid administration on noradrenergic function. Importantly, a detailed analysis of the distribution of CB1 receptors in the LC and direct infusion of cannabinoid agonists into the LC is warranted to elucidate the contribution of LC neurons in the modulation of norepinephrine release in the coeruleo-cortical pathway.

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Figure 1.

Local perfusion of SR 141716A, a CB1 antagonist, blocks the increase in extracellular norepinephrine in the frontal cortex evoked by a systemic injection of WIN 55,212-2. SR 141716A was administered via reverse perfusion through the probe for 60 minutes (300 μ M; black bar). WIN 55,212-2 (15 mg/kg, i.p) was injected 20 minutes after the SR infusion ended (gray diamonds). Control animals (black squares) received aCSF through the probe followed by WIN 55,212-2 injection. Black triangles represent animals that received SR 141716A through the probe followed by a systemic vehicle injection.

Figure 2.

Local SR 141716A perfusion has no effect on extracellular NE (gray squares). Local application of WIN 55,212-2 increases extracellular NE (black diamonds). Local perfusion of SR followed by local perfusion of WIN 55,212-2 results in a reduction of extracellular NE (black triangles).