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Systemic effect of cannabinoids on the spontaneous firing rate of locus coeruleus neurons in rats

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Abstract

Previous reports have described modulation of noradrenergic activity by cannabinoid receptors. The aim of the present research was to examine the effect of two synthetic cannabinoid CB_1/CB_2 receptor agonists, *R*-(+)-[2,3-dihydro-5-methyl-3-[(morpholinyl)-methyl]pyrrolol-[1,2,3-de]-1,4-benzoxazinyl]-(1-naphthalenyl) methanone (WIN 55212-2) and (−)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-*trans*-4-(3-hydroxypropyl) cyclohexanol (CP 55940), on the spontaneous activity of locus coeruleus noradrenergic neurons by single-unit extracellular recordings in vivo and in vitro. In anaesthetized rats, intravenous administrations of WIN 55212-2 (31.3–500 μg/kg) or CP 55940 (31.3–500 μg/kg) increased the firing rate of locus coeruleus neurons in a dose-dependent manner. The stimulatory effect of WIN 55212-2 was blocked by pretreatment with the cannabinoid CB_1 receptor antagonist *N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichloro-phenyl)-4-methyl-1 *H*-pyrazole-3-carboxamide (SR 141716A; 2 mg/kg). Paradoxically, local administration of WIN 55212-2 (8.3–31.3 pmol) into the locus coeruleus and intracerebroventricular injections of WIN 55212–2 (10–20 μg) or CP 55940 (20–40 μg) failed to change the spontaneous firing rate of locus coeruleus neurons. Likewise, in rat brain slice preparations perfusion with WIN 55212-2 (10 μM) or CP 55940 (10–30 μM) did not specifically affect the spontaneous firing rate of locus coeruleus cells. Therefore, we conclude that synthetic cannabinoids increase the spontaneous firing activity of noradrenergic neurons in the rat locus coeruleus through cannabinoid CB_1 receptors. This stimulation appears to be indirectly induced via a receptor mechanism probably located at the peripheral level.

Keywords: Noradrenergic neurons; WIN 55212-2; CP 55940; Cannabinoid CB_1 receptors; Single-unit extracellular recordings; Slice; In vivo

1. Introduction

Cannabinoids, the active compounds of marijuana, exert their biological effects by activating specific cannabinoid receptors. Two subtypes of cannabinoid receptors have been characterized and cloned: the cannabinoid CB_1 receptor, which is widely distributed in the central nervous system and some peripheral tissues, and the cannabinoid CB_2 receptor, expressed by immune cells (Howlett et al., 2002). Both receptor subtypes are targeted by selective endogenous brain lipids and exogenous cannabinoid agonists (natural and synthetic) (Pertwee, 1997). In most cases, stimulation of cannabinoid CB_1 receptors leads to an inhibition of synaptic transmission in the central and peripheral nervous systems (Freund et al., 2003; Schlicker and Kathmann, 2001). In the rat brain, CB_1 receptors are found at high levels in the basal ganglia, hippocampus, cerebellum and cerebral cortex, which is consistent with the known effects of cannabinoids on brain functions such as motor activity, memory, perception and antinociception (Herkenham et al., 1991; Moldrich and Wenger, 2000; Tsou et al., 1998).

One of the areas where cannabinoid receptors have been described by autoradiographic techniques is the locus coeruleus (Herkenham et al., 1991). This nucleus, which is the main noradrenergic cell group in the brain, participates in many nervous functions including anxiety, attention, pain and drug addiction (Margalit and Segal, 1979; Nestler and Aghajanian, 1997; Sara and Devauges, 1988; Tanaka et al.,

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2000). A number of studies have shown that cannabinoids modulate the noradrenergic locus coeruleus system. Thus, previous studies have shown that cannabinoids modulate c-Fos expression in the locus coeruleus (Oropeza et al., 2005; Patel and Hillard, 2003) and noradrenaline turnover and extracellular concentration in the projection areas of the locus coeruleus (Jentsch et al., 1997; Oropeza et al., 2005). Moreover, the locus coeruleus appears to have an important role in some of the behavioral signs induced by cannabinoids (Kataoka et al., 1987). Finally, a recent study has shown that cannabinoids modulate *N*-methyl-D-aspartate (NMDA) receptor activity through cannabinoid CB_1 receptors in the locus coeruleus (Mendiguren and Pineda, 2004).

Although these findings imply that cannabinoids affect the activity of noradrenergic system, little is known about the direct action of cannabinoids on the locus coeruleus. For example, immunohistochemical and in situ hybridization studies have suggested a low density of cannabinoid CB_1 receptors in the locus coeruleus (Matsuda et al., 1993; Tsou et al., 1998). Therefore, the aim of this study was to elucidate whether cannabinoid receptors modulate directly the spontaneous activity of locus coeruleus neurons. For this purpose, we assessed in vivo and in vitro the effect of synthetic agonists and antagonists for the cannabinoid CB_1 receptor on the firing rate of noradrenergic neurons in the locus coeruleus.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats weighing 200–300 g were housed under controlled environmental conditions (22 °C, 12:12 h light–dark cycle) with free access to food and water and, prior to in vivo or in vitro assays, they were anaesthetized with chloral hydrate (400 mg/kg, i.p.). All the experiments reported in this manuscript followed the guidelines for care and use of laboratory animals adopted by Spanish Legislation and European Community Council Directive (86/609/ECC). All efforts were made to minimize animal suffering and to reduce the number of animals used.

2.2. Drugs

The following compounds were used in the assays: *R*-(+)-[2,3-dihydro-5-methyl-3-[(morpholinyl)-methyl]pyrrolol[1,2,3-de]-1,4-benzoxazinyl]-(1-naphthalenyl) methanone mesylate salt (WIN 55212-2) (Sigma, St Louis, MO, USA), (−)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-*trans*-4-(3-hydroxypropyl) cyclohexanol (CP 55940) (Tocris Cookson, Bristol, UK) and *N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichloro-phenyl)-4-methyl-1 *H*-pyrazole-3-carboxamide (SR 141716A) (generously provided by Sanofi-Synthélabo Recherche, Montpellier, France). For intravenous administrations drugs were directly dissolved in the final solution consisting of a mixture of 5% cremophor EL, 5% ethanol and 90% saline. For local and intracerebroventricular administrations, drugs were directly dissolved in Dulbecco's buffered saline solution which contained (in mM): NaCl, 136.9; KCl, 2.7; NaH_2PO_4, 8.1; KH_2PO_4, 1.5; $MgCl_2$, 0.5; $CaCl_2$, 0.9 (pH 7.4).

For in vitro studies, drugs were prepared as stock solutions in dimethylsulphoxide (DMSO), stored at −25 °C and on the day of the experiments, diluted in standard artificial cerebrospinal fluid (aCSF) for a final DMSO concentration of 0.1–0.3%. aCSF was composed (in mM) of: NaCl, 129; KCl, 3; NaH_2PO_4 1.25; $MgSO_4$ 2; $CaCl_2$ 2; $NaHCO_3$ 21; and D-glucose 10. In addition, WIN 55212-2 dilutions were prepared in aCSF containing 1 g/l bovine serum albumin to keep cannabinoid in solution and to prevent adsorption to the tubing.

2.3. Electrophysiological procedures

In vivo experiments were performed as previously described (Ruiz-Durántez et al., 2002). After cannulating the trachea, a catheter was inserted in the jugular vein for additional injections of the anaesthetic and drugs, and the rat was placed in a stereotaxic frame with the head oriented at 15° to the horizontal plane (nose down). The body temperature was maintained at 37 °C. A bur hole was drilled in the occipital bone and a recording electrode was situated 3.7 mm posterior to the lamboid fontanel, 1.1 mm lateral to the midline and 5.5–6.5 mm ventral to the cortical surface. The locus coeruleus was initially identified by the typical excitation–inhibition responses to the pressure applied to the contralateral hind paw and the presence just lateral to the locus coeruleus of the mesencephalic nucleus of the 5th nerve. For local applications, a thick-wall pipette with a narrow calibrated inner diameter was broken back, filled with the drug solution and glued adjacent to a recording micropipette. Drug containing solutions were ejected by applying one or more pressure pulses (50–150 ms) generated by a pneumatic pressure device (Picospritzer TM II, General Value Corp) driven by synthetic air. This procedure has been proven to be suitable to evaluate and measure the local effect of active drugs on the firing rate of locus coeruleus neurons (Ruiz-Durántez et al., 2001, 2002). For intracerebroventricular administrations, a 23 gauge steel catheter was inserted into the left lateral ventricle 1.0 mm posterior to bregma and 1.3 mm lateral to the midline and at a depth of 4–5 mm from the skull surface to which it was fixed with dental cement. In previous studies, we have successfully shown a good sensitivity of locus coeruleus noradrenergic cells to active drugs applied by intracerebroventricular techniques (Ruiz-Durántez et al., 2001, 2002). At the end of the experiments, the recording place was verified by histological techniques to be within the locus coeruleus.

In vitro experiments were carried out as previously shown (Pineda and Aghajanian, 1997). Briefly, brains were prepared and 600 μm thick coronal brainstem slices containing the locus coeruleus were incubated at 33 °C in a modified Hass-type interface chamber continuously perfused with aCSF (bubbled with 95% O_2/5% CO_2, final pH: 7.34) at a flow rate of 1.5 ml/min. Drugs were perfused in the bathing medium by switching to the drug-containing solution, which provided an excellent exchange of drugs in the slice, as tested by the effect of a short application of Met-enkephalin. The locus coeruleus nucleus was identified visually in the rostral pons as a dark oval area on the