European Journal of Pharmacology 758 (2015) 153–163

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Neuropharmacology and analgesia

Dissociation between the panicolytic effect of cannabidiol microinjected into the substantia nigra, pars reticulata, and fear-induced antinociception elicited by bicuculline administration in deep layers of the superior colliculus: The role of CB_1-cannabinoid receptor in the ventral mesencephalon

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ARTICLE INFO

Article history:
Received 13 May 2014
Received in revised form
11 March 2015
Accepted 17 March 2015
Available online 2 April 2015

Keywords:
CB_1-cannabinoid receptor-signalled mechanisms
Panic attack-like behaviour
Innate fear-induced antinociception
Superior colliculus
Striatum-nigral disinhibitory/nigro-tectal inhibitory GABAergic pathways
$GABA_A$ receptor

ABSTRACT

Many studies suggest that the substantia nigra, pars reticulata (SNpr), a tegmental mesencephalic structure rich in γ-aminobutyric acid (GABA)- and cannabinoid receptor-containing neurons, is involved in the complex control of defensive responses through the neostriatum-nigral disinhibitory and nigro-tectal inhibitory GABAergic pathways during imminently dangerous situations. The aim of the present work was to investigate the role played by CB_1-cannabinoid receptor of GABAergic pathways terminal boutons in the SNpr or of SNpr-endocannabinoid receptor-containing interneurons on the effect of intra-nigral microinjections of cannabidiol in the activity of nigro-tectal inhibitory pathways. $GABA_A$ receptor blockade in the deep layers of the superior colliculus (dlSC) elicited vigorous defensive behaviour. This explosive escape behaviour was followed by significant antinociception. Cannabidiol microinjection into the SNpr had a clear anti-aversive effect, decreasing the duration of defensive alertness, the frequency and duration of defensive immobility, and the frequency and duration of explosive escape behaviour, expressed by running and jumps, elicited by transitory GABAergic dysfunction in dlSC. However, the innate fear induced-antinociception was not significantly changed. The blockade of CB_1 endocannabinoid receptor in the SNpr decreased the anti-aversive effect of canabidiol based on the frequency and duration of defensive immobility, the frequency of escape expressed by running, and both the frequency and duration of escape expressed by jumps. These findings suggest a CB_1 mediated endocannabinoid signalling in cannabidiol modulation of panic-like defensive behaviour, but not of innate fear-induced antinociception evoked by $GABA_A$ receptor blockade with bicuculline microinjection into the superior colliculus, with a putative activity in nigro-collicular GABAergic pathways.

1. Introduction

The γ-aminobutyric acid-A ($GABA_A$) receptor is involved in the modulation of innate fear-induced behaviour organised by the periaqueductal grey matter (PAG) and deep layers of the superior colliculus (dlSC) (Coimbra and Brandão, 1993; Coimbra et al., 2006). At least part of the neural substrates involved in the control of panic-like responses includes the neostriatum-nigral

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http://dx.doi.org/10.1016/j.ejphar.2015.03.051
0014-2999/

disinhibitory and nigro-collicular inhibitory GABAergic neural pathways (Coimbra and Brandão, 1993; Ribeiro et al., 2005; Castellan-Baldan et al., 2006).

Cannabis sativa has been used for centuries worldwide for the treatment of several diseases (Farquhar-Smith et al., 2000). The main phytocannabinoid components of the *C. sativa* are cannabidiol (CBD) and Δ^9-tetrahydrocannabinol (Δ9-THC). Δ^9-THC is responsible for most of the psychoactive effects of the plant (Zuardi et al., 2006). The first endogenous agonist arachidonoyl ethanolamide (AEA) isolated was named anandamide, from the Sanskrit word "ananda" for "bliss" (Devane et al., 1992). AEA acts on two known cannabinoid receptors: CB_1 and CB_2. CB_1 receptor expression was detected in the central and peripheral nervous systems, especially in the substantia nigra (SN), globus pallidus, cerebellum, hippocampus, cerebral cortex, and ganglion of the dorsal root of spinal nerves (Matsuda et al., 1990). CB_2 receptor expression is found predominantly, but not exclusively, in the cells of the immune system, although CB_2 receptor was also detected in the cerebral cortex, striatum, thalamic nuclei, hippocampus, amygdaloid complex, SN, PAG, paratrochlear nucleus, paralemniscal nucleus, red nucleus, pontine nuclei, inferior colliculus, and parvocellular portion of the medial vestibular nucleus in the rat brain (Gong et al., 2006). The involvement of the endocannabinoid in several physiological and behavioural responses has been widely demonstrated (Crippa et al., 2004; Lisboa et al., 2008).

Peripheral administration of CBD at different doses was recently shown to modulate panic-like defensive behaviour displayed by mice in the presence of wild *Boidae* snakes, even in a potentially safe environment, suggesting a clear panicolytic effect (Uribe-Mariño et al., 2012; Twardowschy et al., 2013). Other researchers showed that CBD at different doses significantly increased the entry ratio (open/total number of entries) in the elevated plus maze test, suggesting an anxiolytic-like effect in rats (Guimarães et al., 1990). Another group of researchers reported, in contrast to the effects seen with Δ^9-THC, that mice treated with CBD spent much time in the open arm of the elevated plus maze, an effect similar to that produced by diazepam, the reference anxiolytic agent (Onaivi et al., 1990).

Additionally, CB_1 and CB_2 receptors appear to modulate chronic pain. In experimental models of chronic pain, there is an increase in the expression of these receptors (Manzanares et al., 2006). For example, models of neuropathic pain stimulate the expression and regulation of CB_1 receptors in structures involved in pain processing, such as the superficial laminas of the dorsal horn of the spinal cord (Lim et al., 2003) and contralateral dorsal thalamic nuclei (Siegling et al., 2001).

The aim of the present study was to investigate the effects of central administration of CBD into the substantia nigra, pars reticulata (SNpr), on $GABA_A$ receptor blockade-induced panic-like responses organised by the dorsal midbrain.

2. Material and methods

2.1. Animals

Male Wistar rats (*Rattus norvegicus*, Rodentia, Muridae), weighing 220–250 g ($n=8$ per group), from the animal facility of University of São Paulo (USP) Campus at Ribeirão Preto were studied. They were housed four per cage in the experimental room for at least 48 h prior to the experiments, with free access to water and food on a 12 h/12 h light/dark cycle (lights on at 7:00 a.m.) at 22–23 °C. All experiments were performed in accordance with the recommendation of the Commission of Ethics in Animal Experimentation of FMRP-USP, which is in accordance with the ethical principles in animal research adopted by the Brazilian Society of Laboratory Animal Sciences (SBCAL), and approved by the FMRP-USP Commission of Ethics in Animal Research on December 15, 2008 (process 204/2008).

2.2. Nociceptive testing

Nociception thresholds were compared in independent groups of rats ($n=8$) using the tail-flick test. Each animal was placed in a restraint apparatus (Insight, Ribeirão Preto, Brazil) with acrylic walls, and its tail was placed on a heating sensor (tail-flick Analgesia Instrument; Insight). A progressive increase in heat was automatically interrupted when the animal removed its tail from the apparatus. The current raised the temperature of the coil (Ni/Cr alloy; 26.04 cm length × 0.02 cm diameter) at a rate of 9 °C per second (Prado and Roberts, 1985) starting at a room temperature of approximately 20 °C. Small current intensity adjustments were performed if necessary in the beginning of the experiment to record baseline and obtain three consecutive tail-flick latencies (TFLs) between 2.5 s and 3.5 s. If the animal did not remove its tail from the heater within 6 s, the apparatus was turned off to prevent skin damage. Three baseline control TFL measurements were taken at 5 min intervals. Tail-flick latencies were also measured for 60 min immediately after the elaboration of escape behaviour.

2.3. Psychopharmacological procedures

2.3.1. Surgery

The animals were anaesthetised with 92 mg/kg ketamine (Ketamina; 0.2 ml of a 10% solution) and 9.2 mg/kg xylazine (Dopaser; 0.1 ml volume) and fixed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA, USA). A stainless steel guide cannula (0.6 mm outside diameter, 0.4 mm inside diameter) was implanted in the midbrain, aimed at the dlSC and SNpr. The upper incisor bar was set 3.3 mm below the interaural line, such that the skull was horizontal between bregma and lambda. The guide cannula was vertically introduced in independent group of rats using the following coordinates, with bregma as the reference: dlSC (anterior/posterior, −6.36 mm; medial/lateral, 1.2 mm; dorsal/ventral, 3.6 mm) and SNpr (anterior/posterior, −6.36 mm; medial/lateral, 2.2 mm; dorsal/ventral, 6.3 mm). The guide cannula was fixed to the skull using acrylic resin and two stainless steel screws. At the end of surgery, each guide cannula was sealed with a stainless steel wire to protect it from obstruction.

2.4. Procedure

Five days after surgery, on the day of the experiment, nociceptive thresholds of the animals were measured ($n=8$) to record the baseline, before the pretreatments.

The rats were then gently wrapped in a cloth and handled to receive the following treatments in the ventral and dorsal midbrain: (a) pretreatment of the SNpr with a microinjection of dimethyl sulfoxide dissolved in physiological saline (10% DMSO)+10% DMSO, followed after 10 min by the treatment of the dlSC with physiological saline; (b) pretreatment of the SNpr with 10% DMSO+cannabidiol (5.0 μg/0.2 μl) dissolved in 10% DMSO, followed after 10 min by the treatment of the dlSC with physiological saline; (c) pretreatment of the SNpr with AM251 (100 pmol/200 nl) dissolved in 10% DMSO, followed after 10 min by the treatment of the dlSC with physiological saline; (d) pretreatment of the SNpr with 10% DMSO+10% DMSO, followed after 10 min by the treatment of the dlSC with bicuculline (40 ng/200 nl); (e) pretreatment of the SNpr with 10% DMSO+cannabidiol (5.0 μg/0.2 μl), followed after 10 min by the treatment of the dlSC with bicuculline (40 ng/200 nl); (f) pretreatment of the SNpr with AM251 (100 pmol/200 nl)+cannabidiol (5.0 μg/0.2 μl), followed after 10 min by the treatment of the dlSC with bicuculline (40 ng/200 nl).