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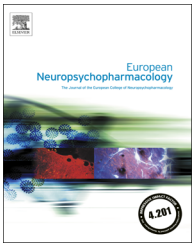

Cannabidiol administration into the bed nucleus of the stria terminalis alters cardiovascular responses induced by acute restraint stress through 5-HT$_{1A}$ receptor

Felipe V. Gomes[b,c,1], Fernando H.F. Alves[b,1], Francisco S. Guimarães[b,c], Fernando M.A. Correa[b], Leonardo B.M. Resstel[b,c], Carlos C. Crestani[a,*]

[a]*Department of Natural Active Principles and Toxicology, School of Pharmaceutical Sciences of Araraquara, São Paulo State University—UNESP, Araraquara, 14801-902, Brazil*
[b]*Department of Pharmacology, School of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto 14090-090, Brazil*
[c]*Center for Interdisciplinary Research on Applied Neurosciences (NAPNA), University of São Paulo, Brazil*

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Abstract
Systemic administration of cannabidiol (CBD) is able to attenuate cardiovascular responses to acute restraint stress through activation of 5-HT$_{1A}$ receptors. Previous results from our group suggest that the bed nucleus of the stria terminalis (BNST) is involved in the antiaversive effects of the CBD. Moreover, it has been proposed that synapses within the BNST influence restraint-evoked cardiovascular changes, in particular by an inhibitory influence on the tachycardiac response associated to restraint stress. Thus, the present work investigated the effects of CBD injected into the BNST on cardiovascular changes induced by acute restraint stress and if these effects would involve the local activation of 5-HT$_{1A}$ receptors. The exposition to restraint stress increased both blood pressure and heart rate (HR). The microinjection of CBD (30 and 60 nmol) into the BNST enhanced the restraint-evoked HR increase, in a dose-dependent manner, without affecting the pressor response. The selective 5-HT$_{1A}$ receptor antagonist WAY100635 by itself did not change the cardiovascular responses to restraint stress, but blocked the effects of CBD. These results showed that CBD microinjected into the BNST enhanced the HR increase associated with acute restraint stress without affecting the blood pressure response. Although these results are not in agreement with those observed after systemic administration of CBD, they are similar to effects observed after reversible inactivation of the BNST. Moreover, similar to the effects observed

*Corresponding author. Tel.: +55 16 3301 6982; fax: +55 16 3301 6980.
E-mail address: cccrestani@yahoo.com.br (C.C. Crestani).
[1]These authors contributed equally to this work.

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after systemic administration, CBD effects in the BNST seem to depend on activation of 5-HT_{1A} receptors.

1. Introduction

Cannabidiol (CBD) is a major component of *Cannabis sativa* that, unlike Δ^9tetrahydrocannabinol (Δ^9-THC), is devoid of typical psychotomimetic effects of cannabis (Mechoulam et al., 2007; Izzo et al., 2009). Numerous pre-clinical and clinical studies have demonstrated that systemic administration of CBD has an antiaversive action and induces antipsychotic, antidepressive and anxiolytic effects (Guimaraes et al., 1990; Zuardi et al., 1993; Zuardi et al., 1995; Zuardi, 2008). In this context, it was observed that systemic treatment with CBD was able to attenuate the acute cardiovascular changes and the delayed anxiogenic effect induced by restraint stress in rats (Resstel et al., 2009). Similar effects were observed after intracisternal administration of CBD (Granjeiro et al., 2011), thus suggesting that these effects are centrally mediated.

The mechanism and brain sites related to effects of CBD are still poorly understood. Although CBD has low affinity for cannabinoids receptors (Thomas et al., 1998), it can block the reuptake of the endogenous cannabinoid anandamide and its metabolism by the enzyme fatty acid amide hydrolase (Bisogno et al., 2001; Mechoulam et al., 2002). In addition, CBD can act as a 5-HT_{1A} receptor agonist (Russo et al., 2005). Indeed, we have recently demonstrated that CBD can attenuate autonomic and behavioral responses to acute restraint stress by facilitating 5-HT_{1A} receptor-mediated neurotransmission (Resstel et al., 2009). Moreover, recent findings have suggested that neuroprotective, antidepressive and anxiolytic effects of CBD seem to be mediated, at least in part, by activation of 5-HT_{1A} receptors (Mishima et al., 2005; Campos and Guimaraes, 2008; Zanelati et al., 2010).

The bed nucleus of the stria terminalis (BNST) is a limbic structure that has a direct influence on autonomic, neuroendocrine and behavioral functions related to stress (Walker et al., 2003; Resstel et al., 2008; Ulrich-Lai and Herman, 2009; Crestani et al., 2010a). In this way, recent findings from a study using reversible inactivation of the BNST suggested an inhibitory influence of this structure on the tachycardiac response evoked by acute restraint stress (Crestani et al., 2009). Moreover, CBD attenuated contextual conditioned fear-induced activation of BNST neurons (Lemos et al., 2010). It was also reported that microinjection of CBD into the BNST induced anxiolytic-like effects and affected baroreflex activity, and these effects were blocked by pretreatment with 5-HT_{1A} receptor antagonist (Alves et al., 2010; Gomes et al., 2011, 2012). Although the above evidence suggest the BNST as an action site of CBD, its involvement in CBD effects on cardiovascular responses to stress is poorly understood.

Acute restraint is an uncontrollable stress situation that produces endocrine and autonomic responses characterized by activation of the hypothalamus–hypophysis–adrenal axis and increases in blood pressure and heart rate (HR) (Choi et al., 2008; Crestani et al., 2009; Busnardo et al., 2010). These responses are mediated by activation of several brain structures, including the BNST (Pacak and Palkovits, 2001; Ulrich-Lai and Herman, 2009). Despite the evidence that CBD could act in the BNST to decrease the behavioral effects of aversive situations, it is still unknown if this drug can modulate the cardiovascular responses to stressful stimuli in this region. To this aim, in the present work we verified if intra-BNST administration of CBD could alter the cardiovascular changes induced by acute restraint stress. We also evaluated whether this effect depends on an interaction with 5-HT_{1A}-mediated neurotransmission.

2. Experimental procedures

2.1. Animals

The experiments were performed with 8-weeks-old male Wistar rats (230–270 g). Animals were housed in groups of four per cage (41 × 33 × 17 cm) in a temperature-controlled room (24 ± 1 °C) under standard laboratory conditions with free access to food and water and a 12 h light/dark cycle (lights on at 06:00 am). Procedures were conducted in conformity with the Brazilian Society of Neuroscience and Behavior guidelines for the care and use of laboratory animals, which are in compliance with international laws and politics. The Institution's Animal Ethics Committee approved the housing conditions and experimental procedures.

2.2. Surgical preparation

Seven days before the experiment the rats were anesthetized with 2,2,2-tribromoethanol (250 mg/kg, i.p.) and fixed in a stereotaxic frame (Stoelting, Wood Dale, Illinois, USA). After scalp anesthesia with 2% lidocaine, the skull was surgically exposed and stainless steel guide cannulae (26 G) were implanted bilaterally into the BNST. Coordinates for cannula implantation into the BNST were: antero-posterior = − 8.6 mm from interaural; lateral = 4.0 mm from the medial suture, vertical = − 5.5 mm from the skull with a lateral inclination of 23° (Paxinos and Watson, 1997). Cannulae were fixed to the skull with dental cement and a metal screw. An obturator inside the guide cannulae prevented obstruction. After surgery, the animals received a polyantibiotic (0.27 g/kg, i.m.; Pentabiotico®, Fort Dodge, Brazil) to prevent infection and a nonsteroidal anti-inflammatory (0.025 g/kg, s.c.; Banamine®, Schering Plough, Brazil) for post-operative analgesia.

One day before the trial, rats were anesthetized with 2,2,2-tribromoethanol (250 mg/kg, i.p.) and a catheter (a 4 cm PE10 segment heat-bound to a 13 cm PE-50 segment, Clay Adams, USA) was inserted into the abdominal aorta through the femoral artery for blood pressure and heart rate recording. The catheter was tunneled under the skin and exteriorized on the animal's dorsum, allowing cardiovascular recordings from conscious animals. After surgery, the nonsteroidal anti-inflammatory flunixine meglumine (Banamine®, Schering Plough, Brazil) was administered for post-operation analgesia.

2.3. Measurement of cardiovascular responses

The arterial cannula was connected to a pressure transducer and the pulsate arterial pressure was recorded using an amplifier