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Effects of intracisternal administration of cannabidiol on the cardiovascular and behavioral responses to acute restraint stress

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ABSTRACT

Systemic administration of cannabidiol (CBD), a non-psychotomimetic compound from *Cannabis sativa*, attenuates the cardiovascular and behavioral responses to restraint stress. Although the brain structures related to CBD effects are not entirely known, they could involve brainstem structures responsible for cardiovascular control. Therefore, to investigate this possibility the present study verified the effects of CBD (15, 30 and 60 nmol) injected into the cisterna magna on the autonomic and behavioral changes induced by acute restraint stress. During exposure to restraint stress (1 h) there was a significant increase in mean arterial pressure (MAP) and heart rate (HR). Also, 24 h later the animals showed a decreased percentage of entries onto the open arms of the elevated plus-maze. These effects were attenuated by CBD (30 nmol). The drug had no effect on MAP and HR baseline values. These results indicate that intracisternal administration of CBD can attenuate autonomic responses to stress. However, since CBD decreased the anxiogenic consequences of restraint stress, it is possible that the drug is also acting on forebrain structures.

1. Introduction

Cannabidiol (CBD) is a component of *Cannabis sativa* which lacks the psychotomimetic effects of its major constituent, Δ^9-tetrahydrocannabinol (Δ^9-THC) (Zuardi, 2008). CBD has shown several potential therapeutic effects (Mechoulam et al., 2002) such as antipsychotic (Zuardi et al., 2006), anticonvulsant (Carlini and Cunha, 1981; Cunha et al., 1980), neuroprotective (Iuvone et al., 2009) and anti-compulsive (Casarotto et al., 2010). Additionally, preclinical and clinical studies indicate that CBD has potential therapeutic activity as an anxiolytic (Bhattacharyya et al., 2010; Crippa et al., 2004; Fusar-Poli et al., 2010; Guimaraes et al., 1990; Moreira et al., 2006). Supporting these studies we have recently observed that systemic administration of CBD attenuates the behavioral and cardiovascular responses induced by aversive situations such as contextual fear conditioning and acute restraint stress (Resstel et al., 2006; Resstel et al., 2009). Acute restraint is an unavoidable stress situation that elicits blood pressure and heart rate (HR) increases (Tavares and Correa, 2006; Tavares et al., 2009). These responses are associated with neuronal activation of forebrain and brainstem structures involved in cardiovascular control (Bhatnagar et al., 1998; Hsu et al., 2007). In addition, restraint stress reduces exploratory activity in both the open-field and open arms of the elevated plus-maze (EPM) 24 h later. These behavioral changes are attenuated by anxiolytic drugs (Kennett et al., 1985; Kennett et al., 1987; Guimarães et al., 1993; Padovan and Guimaraes, 2000).

Anxiolytic effects of CBD have also been observed after direct drug microinjections into the bed nucleus of the stria terminalis (Gomes et al., 2010; Gomes et al., 2011), dorsal periaqueductal gray (Campos and Guimaraes, 2008; Soares et al., 2010) and medial prefrontal cortex (Lemos et al., 2010). Little is known, however, about the possible involvement of central cardiovascular regulatory centers, particularly, in brainstem structures, in CBD anti-stress effects.

Diverse studies have employed the direct administration of drugs into the cisterna magna to investigate a possible involvement of brainstem nuclei on drug-induced autonomic responses (Damaso et al., 2007; Horiuchi et al., 2005; Oliva et al., 2010). Considering that restraint stress induces the activation of several brainstem structures (Pacak and Palkovits, 2001), the aim of the present study was to investigate the effects of intracisternal injection of CBD on the cardiovascular responses induced by restraint stress. In addition, we also investigated the possible effects of CBD on stress behavioral consequences observed 24 h after restraint.

Abbreviations: Δ^9-THC, Δ^9-tetrahydrocannabinol; CBD, cannabidiol; EPM, elevated plus-maze; HR, heart rate; MAP, mean arterial pressure; PAP, pulsate arterial pressure.

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2. Material and methods

2.1. Animals

Male Wistar rats weighing 250–270 g were housed in groups of four per cage (41 × 33 × 17 cm) in a temperature-controlled room (24 ± 1 °C) under standard laboratory conditions, free access to food and water and a 12 h light/dark cycle (lights on at 06:30 A.M.). Experiments were performed during the morning period. 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 (process number: 83/2005).

2.2. Stereotaxic surgery

Seven days before the experiment the rats were anesthetized with 2,2,2-tribromoethanol (250 mg/kg i.p., Sigma-Aldrich, USA) and fixed in a stereotaxic frame (Stoelting, USA). After scalp anesthesia with 2% lidocaine, the skull, the bregma and lambda were surgically exposed and the incisor bar adjusted to a flat skull position. The muscles were retracted from the occipital bone by blunt dissection to allow placement of a vertical guide cannula aimed at the cisterna magna. A small craniotomy was performed from the occipital notch to 1 mm dorsal to the atlanto-occipital membrane using a dental drill. A stainless steel guide cannula (22 G) was implanted aiming at the cisterna magna (antero-posterior = −14.5 mm from bregma; lateral = 0.0 mm from the medial suture, vertical = −7.9 mm from the skull, Paxinos and Watson, 1997). The cannula was fixed to the skull with dental cement and a metal screw. An obturator inside the guide cannula prevented obstruction. After surgery, the animals received a polyantibiotic (Pentabiotico®, Fort Dodge, Brazil) and a nonsteroidal anti-inflammatory (Banamine®, Schering Plough, Brazil).

2.3. Measurement of cardiovascular responses and restraint stress

For mean arterial pressure (MAP) and heart rate (HR) recording the rats were anesthetized with 2,2,2-tribromoethanol (250 mg/kg i.p.). A catheter (a 4 cm PE-10 segment heat-bound to a 13 cm PE-50 segment, Clay Adams, USA) was implanted into the femoral artery. The catheter was tunneled under the skin and exteriorized on the animal's dorsum, allowing cardiovascular recordings from conscious animals.

Cardiovascular responses were evaluated 24 h after surgery. Animals were transferred from the animal room to the experimental room in their home cages where they remained undisturbed for 1 h. Following this adaptation period, the arterial cannula was connected to a pressure transducer and the pulsate arterial pressure (PAP) was recorded using an HP-7754A amplifier (Hewlett Packard, USA) and an acquisition board (MP-100A, Biopac Systems Inc, USA) connected to a personal computer. Both MAP and HR values were derived from PAP recordings and were processed online. Immediately after the intracisternal injections, a 10 min baseline recording was performed followed by a 60 min restraint period in a plastic cylindrical restraint tube (diameter = 6.5 cm, length = 15 cm), ventilated by holes (1 cm diameter) which comprised approximately 20% of the tube surface. The rats returned to their home cages immediately after this restraint period. Each rat was submitted to only a single restraint session to avoid stress tolerance (Guimarães et al., 1993).

2.4. Drugs

CBD (kindly supplied by THC Pharm, Germany) was dissolved in grape seed oil (100%, Campos and Guimaraes, 2008), which was used as vehicle. The pH of grape seed oil and CBD solutions is about 7.0. The solutions were prepared immediately before the tests.

2.5. Intracisternal injections

Seven days after surgery the animals were randomly assigned to one of the treatment groups. Intracisternal injections were performed in non-handled unrestrained rats in accordance with a previous study (Oliva et al., 2010). Intracisternal injections were performed with a thin dental needle (33 G; Small Parts, USA) introduced through the guide cannula until its tip was 0.8 mm below the cannula end, connected to a 10 μL syringe (7001 KH, Hamilton Co., USA) through a PE-10 tubing. The injection needle was carefully inserted into the guide-cannula, and a volume of 1 μL of vehicle or the CBD solution was injected at the rate of 2 μL/min with the help of an infusion pump (Kd Scientific Inc., USA). A polyethylene tubing (PE-10) was interposed between the upper end of the dental needle and the syringe. Successful infusions were confirmed by the displacement of an air bubble inside the polyethylene tubing. At the end of injection, the needle was kept in place for 45 to 60 s to avoid the drug reflux.

2.6. Experimental design

2.6.1. Experiment 1: effects of intracisternal injections of CBD on cardiovascular responses to acute restraint stress

Rats (n = 6/group) received intracisternal injections of vehicle (grape seed oil) or previously reported effective doses of CBD (15, 30 or 60 nmol) (Campos and Guimaraes, 2008; Lemos et al., 2010). Immediately thereafter, a 10 min of baseline cardiovascular recording was performed followed by the 60 min restraint period.

2.6.2. Experiment 2: effects of intracisternal injections of CBD (30 nmol) on the anxiogenic consequences of restraint stress

Rats (n = 7 and 8) were injected into the cisterna magna with either vehicle or CBD (30 nmol) and, 10 min after that, restrained throughout a 60 min period. Thereafter, the rats were housed as described in item 2.1 and, 24 h later, tested in the EPM. Unrestrained control rats were subjected to similar treatments with vehicle or CBD (n = 6 and 7).

2.7. Elevated plus-maze (EPM)

The EPM test was conducted as described by Padovan and Guimaraes (2000). Briefly, the apparatus consisted of two opposite wooden open arms (50 × 10 cm), crossed at right angle by two arms of the same dimensions enclosed by 40-cm high walls with no roof. The maze was set up 50 cm above the floor and had a 1-cm high Plexiglas edge surrounding the open arms so as to prevent the rat fall. The apparatus was located in a sound-attenuated, temperature-controlled (24 ± 1 °C) room lit by a 40 W fluorescent light placed 3 m away from the EPM. Rat behaviors were filmed throughout 5 min and analyzed off-line with the aid of Any-Maze software (Stolelting, USA). At the end of trials, the maze was cleaned up with an alcohol solution.

2.8. Histology

At the end of the experiments the rats were anesthetized with urethane (1.25 g/kg i.p., Sigma-Aldrich, USA) and injected into the cisterna magna with 1 μL of 1% Evan's Blue dye. After that, the chest was opened, the descending aorta occluded, the right atrium severed and the brain perfused with 10% formalin through the left ventricle. The brain of rats was removed and examined for staining.

2.9. Statistical analysis

MAP and HR values were continuously recorded for 10 min before and 60 min during the exposure to restraint stress. Data were expressed as means ± SEM. of MAP or HR changes (respectively ΔMAP and ΔHR) sampled at 2.5 min intervals. Points sampled during the 10 min before restraint were used as control baseline value. MAP and HR changes were

analyzed using two-way ANOVA with treatment as independent factor and time as repeated measurement. Moreover, one-way ANOVA followed by Bonferroni's *post-hoc* test was used to compare the effect of the treatments on the restraint-evoked MAP and HR maximum responses.

The percentage of entries and time spent in the open arms (100 × open/(open + enclosed)) during the 5-min sessions in the EPM were calculated for each animal. These results were analyzed by two-way ANOVA using condition (restraint or no restraint) and treatment (CBD or vehicle) as main factors. *Post-hoc* analysis was performed using the Bonferroni test. Differences were considered significant at $P<0.05$ level.

3. Results

3.1. Experiment 1: effects of intracisternal injections of CBD on cardiovascular responses to restraint stress

Groups did not differ in either the MAP or HR baseline levels before restraint stress (Table 1).

Acute restraint induced a marked and sustained increase in both the ΔHR ($F_{27,560}=23.94$, $P<0.001$) and ΔMAP ($F_{27,560}=18.08$, $P<0.001$). However, although the treatment effects differed significantly (ΔMAP: $F_{3,560}=55.85$, $P<0.001$ and ΔHR: $F_{3,560}=55.75$, $P<0.001$), there were no drug-treatment significant interactions among groups. Rats treated with 30 nmol CBD showed ΔMAP (6.95 ± 3.49) and ΔHR (32.74 ± 6.50) responses to restraint stress significantly smaller (ΔMAP: $F_{3,20}=4.66$, $P<0.05$ and ΔHR: $F_{3,20}=4.80$, $P<0.05$) as compared to vehicle-treated rats (ΔMAP: 17.10 ± 1.87 and ΔHR: 60.34 ± 5.49) (Fig. 1B and D). Remaining doses were ineffective.

Typical experimental recordings showing the effects of intracisternal injections of CBD (30 nmol) on cardiovascular responses observed during acute restraint can be seen in Fig. 2.

3.2. Experiment 2: effects of intracisternal injections of CBD (30 nmol) on the anxiogenic consequences of restraint stress

EPM performance differed significantly among groups ($F_{3,24}=4.98$, $P<0.01$). Vehicle-treated rats showed a significant reduction in the percentage of open arms entries 24 h after the stress (Bonferroni's test, $P<0.05$; Fig. 3). CBD-treated stressed group did not differ from either the CBD- or the vehicle-treated non-stressed groups. There was no effect on the percentage of time spent in the open arms ($F_{3,24}=1.91$, $P>0.05$). Additionally, no effects on number of enclosed arms entries were found ($F_{3,24}=0.53$, $P>0.05$).

4. Discussion

Our results showed that acute exposure to restraint stress induced a marked and sustained increase in MAP and HR. The stressed animals also presented a decrease in the percentage of open arms entries in the EPM 24 h after restraint. Even if a similar effect in the percentage of time spent in these arms did not reach significance level, the lack of any change in the number of enclosed arm entries points to a specific stress-induced reduction in open arm exploration, which is usually interpreted as an anxiogenic effect (File, 1992). These results agree with previous studies that have investigated the autonomic and behavioral consequences of restraint stress (Barron and Van Loon, 1989; Irvine et al., 1997; McDougall et al., 2000; Guimarães et al., 1993; Kennett et al., 1985; Kennett et al., 1987; Padovan and Guimaraes, 2000). The cardiovascular and behavioral effects of restraint stress were attenuated by intracisternal administration of CBD. Thus, it is not possible to rule out the possibility that the attenuated cardiovascular response to restraint after CBD treatment may be related to the reduced aversive response.

Confirming previous reports (McQueen et al., 2004; Resstel et al., 2006; Resstel et al., 2009), CBD did not induce any significant change on MAP and HR baseline values. This suggests that CBD may act specifically on stress-activated cardiovascular pathways. Similar results have been observed after systemic administration of this compound (Resstel et al. 2009). Moreover, as described in other studies (Campos and Guimaraes, 2008; Guimaraes et al., 1990; Lemos et al., 2010; Zanelati et al., 2010), CBD had non-linear effects on cardiovascular parameters. These curves are commonly observed with cannabinoids (Viveros et al., 2005) and their mechanisms could reflect the complex pharmacology of these compounds involving, for example, the activation of TRPV1 receptors at higher concentrations (Campos and Guimarães, 2009).

Acute restraint stress induces significant neuronal activation in important medullary areas involved in autonomic control, including the nucleus of the solitary tract and ventrolateral medulla (Goebel et al., 2009). Several studies have employed intra-cisterna magna administration of drugs to evaluate a possible involvement of the brainstem nuclei on autonomic responses (Damaso et al., 2007; Horiuchi et al., 2005; Oliva et al., 2010). Thus, Damaso et al. (2007), using a larger volume (5 μl) than that of the present study, described that the solution injected would spread from the dorsal surface, laterally to the ventral surface of the brainstem. Moreover, Horiuchi et al. (2005) observed that, following intracisternal injection of 5 μl of dye, the rostral solution spreading reaches the pons. It is conceivably, therefore, that in our study CBD could be acting in brainstem regions such as the nucleus of the tractus solitary and the rostral ventrolateral medulla, two important medullary areas involved in autonomic control that can play a role in stress-induced cardiovascular responses (Korte et al., 1992; Mayorov and Head, 2003). Besides these regions, several others areas of the central nervous system have also been involved in the cardiovascular responses associated with stress. They include the septal lateral area (Kanaya et al., 2003; Kubo et al., 2002); anterior hypothalamic area (Krout et al., 2005; Kubo et al., 2001); paraventricular nucleus of the hypothalamus (Busnardo et al., 2010; Tavares et al., 2009), medial prefrontal cortex (Tavares and Correa, 2006); bed nucleus of stria terminalis (Crestani et al., 2009) and the medial amygdala (Fortaleza et al., 2009; Kubo et al., 2004), which are directly connected to brainstem regions that integrate autonomic and neuroendocrine responses during stressful situations (Pacak and Palkovits, 2001).

Although CBD has been shown to change neural activity of limbic areas in humans (Crippa et al., 2004), only recently the brain sites of its antiaversive effects have started to be investigated in laboratory animals. CBD injected into the bed nucleus of the stria terminalis, for example, attenuates behavior and cardiovascular responses induced by contextual fear conditioning (Gomes et al., 2010), causes anxiolytic-like effects in the EPM and Vogel conflict test (Gomes et al., 2011) and modulates the parasympathetic component of baroreflex (Alves et al., 2010). Moreover, similar to the bed nucleus of the stria terminalis, intra-dorsolateral periaqueductal gray and prelimbic medial prefrontal cortex administration of CBD induces anxiolytic-like effects (Campos and Guimaraes, 2008; Lemos et al., 2010).

Diffusion of CBD to these rostral brain regions cannot be ruled out as an explanation to the present results. Actually, Proescholdt et al. (2000), using quantitative *in vivo* autoradiography, showed labeling in the hypothalamus and brainstem 5 and 10 min after intracistenal injections

Table 1
Basal values of the MAP and HR in vehicle and CBD treated rats.

Group	MAP (mm Hg)	HR (bpm)
Vehicle	97 ± 4	364 ± 7
CBD 15 nmol	98 ± 2	363 ± 7
CBD 30 nmol	100 ± 4	368 ± 8
CBD 60 nmol	101 ± 2	349 ± 8
	$F_{3,20}=0.31$	$F_{3,20}=1.24$

The values in the table represent the means ± SEM, One-way ANOVA (n = 6/group). CBD, cannabidiol; HR, heart rate; MAP, mean arterial pressure.

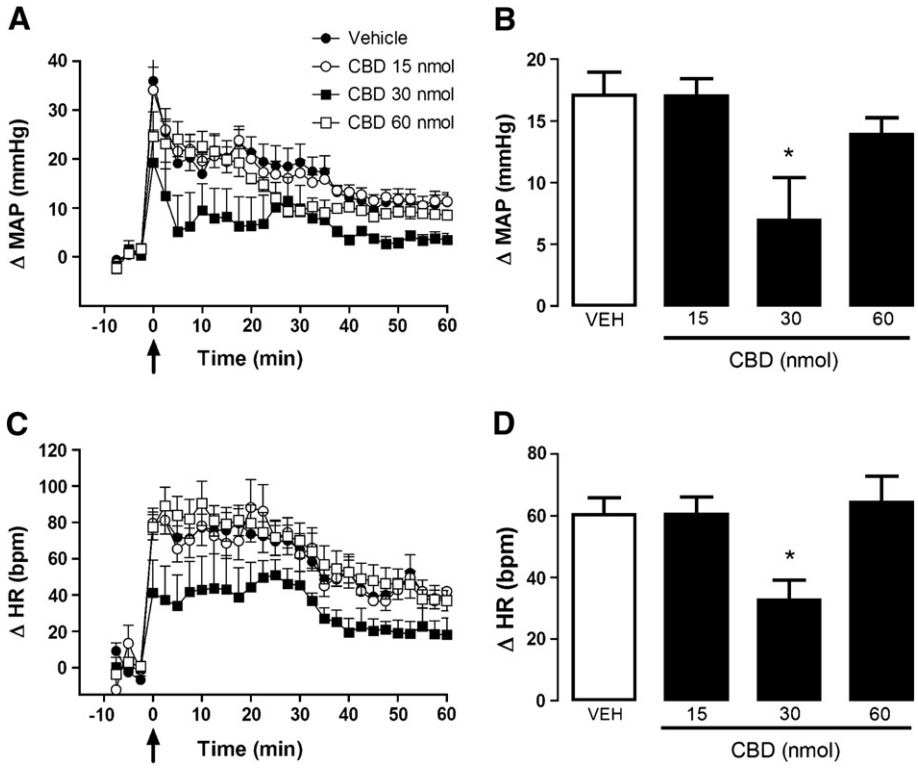

Fig. 1. A) Time-course of mean arterial pressure changes (ΔMAP) during restraint in rats treated with CBD (15, 30 or 60 nmol) or vehicle (1 μL). B) Restraint-evoked changes (time 0 to 60 min) in ΔMAP after intracisternal treatment with CBD (15, 30 or 60 nmol) or vehicle. C) Time-course of heart rate changes (ΔHR) during restraint in rats treated with CBD (15, 30 or 60 nmol) or vehicle. D) Restraint-evoked changes (time 0 to 60 min) in ΔHR after intracisternal treatment with CBD (15, 30 or 60 nmol) or vehicle (n = 6/group). The animals received intracisternal injection 10 min before the beginning of the restraint. The arrows indicate the beginning of the restraint period. Data shown represent the means ± SEM. *P<0.05, compared to vehicle group; ANOVA followed by Bonferroni's *post hoc* test.

of the tracer. Moreover, behavioral effects of intracisternal administration of drugs have been reported by several studies (Makino et al., 2000; Martinez et al., 1997; Montkowski et al., 1997; Shestakova et al., 2009). Nevertheless, previous studies investigating intra-cerebral administration of CBD into the dorsolateral periaqueductal gray (Campos and Guimaraes, 2008) and the bed nucleus of the stria terminalis (Gomes et al., 2010; Gomes et al., 2011) on anxiety models have found anxiolytic effects of a 30 nmol dose, with lower doses being ineffective. Since the injected volumes (0.2 μL) were much smaller than that of the present study (1 μL) and the behavioral tests were also performed 10 min after drug administration, it is unlikely that in our work an effective concentration of CBD could have reached these midbrain and forebrain structures at the moment of the cardiovascular measurements. Studies with restraint models, however, have shown that pharmacological

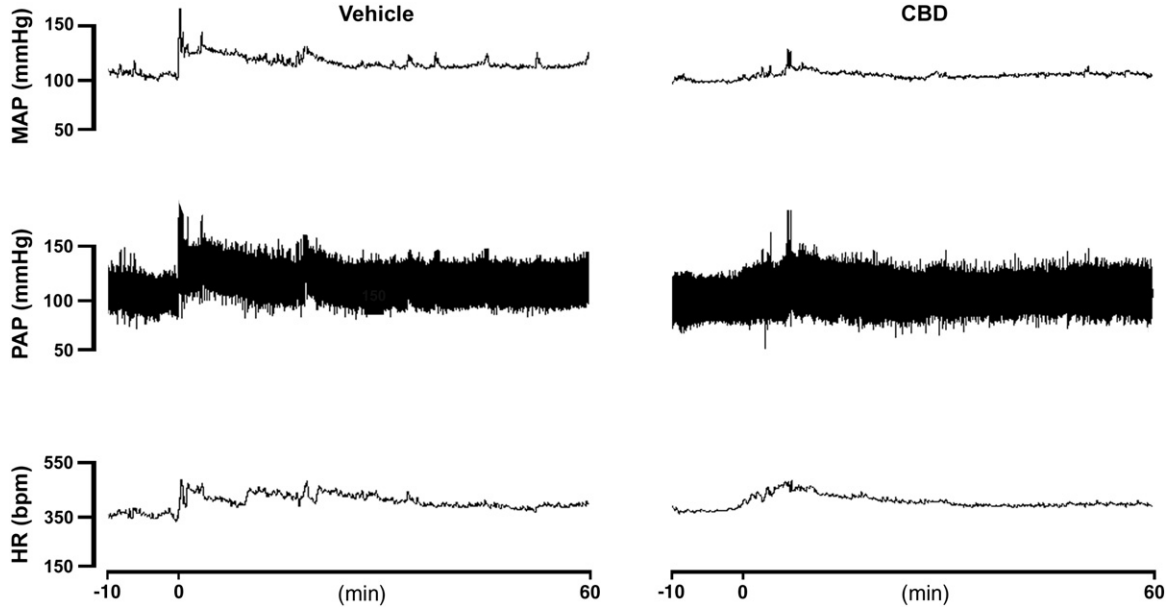

Fig. 2. Mean arterial pressure (MAP), pulsate arterial pressure (PAP) and heart rate (HR) individual recordings showing the cardiovascular changes evoked by acute restraint in animals treated with vehicle (1 μL) and CBD (30 nmol). The restraint period started at time 0. The animals received intracisternal injection 10 min before the beginning of the restraint.

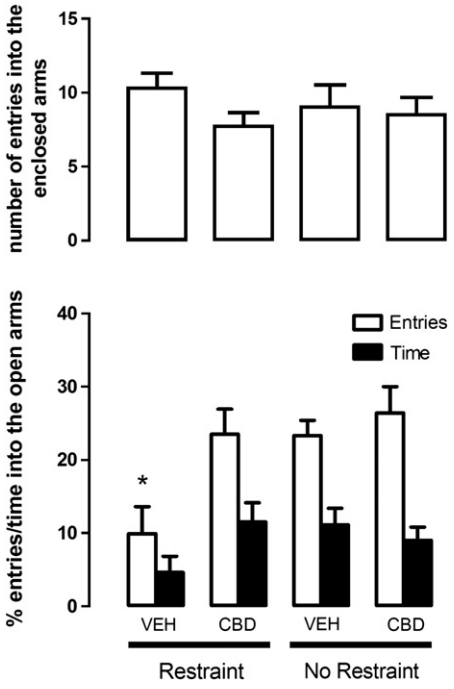

Fig. 3. Effects in the elevated plus-maze (EPM) of intracisternal injection of vehicle (1 μL) or CBD (30 nmol) 10 min before a 60 min restraint period. Non-stressed groups (no restraint) that received vehicle or CBD (30 nmol) were used as control (n = 6–8/group). The EPM test was performed 24 h after the restraint period. The upper panel shows the number of entries into the enclosed arms whereas the lower panel shows the percentage of entries (white columns) and the time spent (black columns) in the open arms. Data represent the mean ± SEM. *P<0.05, compared to others groups; ANOVA followed by Bonferroni's *post hoc* test.

interventions after stress are able to prevent its delayed (24 h) anxiogenic consequence (Guimarães et al., 1993). Since CBD was administered 10 min before the restraint session, the possibility that its behavioral effects in this study depend on CBD action at the end or after the stress period remains to be investigated.

The mechanism of action of CBD is not fully understood. It has a low affinity for cannabinoid receptors (Petitet et al., 1998; Thomas et al., 1998) but can block the reuptake of endocannabinoid anandamide and/or its metabolism by the enzyme, fatty acid amide hydrolase (Bisogno et al., 2001). Moreover, CBD may possess agonistic properties at 5-HT_{1A} receptors (Russo et al., 2005). CBD has also been found to activate TRPV1 receptors (Bisogno et al., 2001), enhance adenosine signaling through inhibition of uptake (Carrier et al., 2006) and allosterically modulate 5-HT_3 (Yang et al., 2010) and μ- and δ-opioid receptors (Kathmann et al., 2006). The involvement of these mechanisms in the effects of intracisternal administration of CBD remains to be tested.

In conclusion, the present study shows that intracisternal administration of CBD attenuates the behavioral and cardiovascular responses induced by acute restraint stress. Additional studies are needed to investigate the specific brain regions involved in these effects.

Conflict of interest

The authors declare no conflicts of interest.

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