# RESEARCH PAPER

# 5-HT$_{1A}$ receptors are involved in the cannabidiol-induced attenuation of behavioural and cardiovascular responses to acute restraint stress in rats

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**Background and purpose:** Cannabidiol (CBD) is a non-psychotomimetic compound from *Cannabis sativa* which induces anxiolytic- and antipsychotic-like effects in rodents. These effects could be mediated by facilitation of the endocannabinoid system or by the activation of 5-HT$_{1A}$ receptors. As either of these mechanisms could promote adaptation to inescapable stress, the aim of the present work was to test the hypothesis that CBD would attenuate the autonomic and behavioural consequences of restraint stress (RS). We also investigated if the responses to CBD depended on activation of 5-HT$_{1A}$ receptors.

**Experimental approach:** Male Wistar rats received i.p. injections of vehicle or CBD (1, 10 or 20 mg kg$^{-1}$) and 30 min later were submitted to 60 min of restraint where their cardiovascular responses were recorded. The protocol of the second experiment was similar to the first one except that animals received i.p. injections of the 5-HT$_{1A}$ receptor antagonist WAY100635 (0.1 mg kg$^{-1}$) before CBD treatment and exposure to restraint. 24 h later they were also tested in the elevated plus-maze (EPM), an animal model of anxiety.

**Key results:** Exposure to RS increased blood pressure and heart rate and induced an anxiogenic response in the EPM 24 h later. These effects were attenuated by CBD. WAY100635 by itself did not change the cardiovascular and anxiogenic response to RS, but blocked the effects of CBD.

**Conclusion and implications:** The results suggest that CBD can attenuate acute autonomic responses to stress and its delayed emotional consequences by facilitating 5-HT$_{1A}$ receptor-mediated neurotransmission.
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**Keywords:** cannabinoids; cardiovascular system; elevated plus-maze; 5-HT$_{1A}$ receptor

**Abbreviations:** CBD, cannabidiol; EPM, elevated plus-maze; HR, heart rate; MAP, mean arterial pressure; THC, $\Delta^9$-tetrahydrocannabinol

## Introduction

Marijuana (from *Cannabis sativa*) is one of the most widely abused drugs in the world. In humans, it elicits subjective changes that include euphoria, heightened sensitivity to external stimuli and relaxation (Martin *et al.*, 1991; Compton *et al.*, 1992; Johns, 2001). The major constituent of cannabis is $\Delta^9$-tetrahydrocannabinol (THC) and this is thought to be the main ingredient responsible for its psychoactive properties (Mechoulam, 1970; Mechoulam *et al.*, 1970; Ilan *et al.*, 2005). The discovery of specific binding sites for THC led to the discovery of the cannabinoid receptors (Devane *et al.*, 1988; Matsuda *et al.*, 1990; Munro *et al.*, 1993) and, so far, two sub-types of the cannabinoid receptor have been identified, CB$_1$ and CB$_2$ (Pertwee, 2005; nomenclature follows Alexander *et al.*, 2008). The activation of CB$_1$ receptors by THC is thought to account for most of the central effects of cannabis (Huestis *et al.*, 2001). Anandamide and 2-arachidonoyl glycerol, referred to as endocannabinoids, are the major endogenous agonists of the CB$_1$ receptor (Di Marzo *et al.*, 1998; Piomelli, 2003).

Cannabidiol (CBD), another cannabinoid generally found in relatively high concentrations in cannabis, exhibits a somewhat different pharmacology compared with THC (Mechoulam et al., 2002). CBD attenuates the psychotomimetic and anxiogenic effects of THC in humans (Karniol et al., 1974; Zuardi et al., 1982). Moreover, systemic administration of CBD induced antipsychotic (Zuardi et al., 1991; Zuardi et al., 2006) and anxiolytic-like effects (Guimaraes et al., 1990; Resstel et al., 2006).

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 anandamide (Bisogno et al., 2001) and its metabolism by the enzyme, fatty acid amide hydrolase (FAAH), (Watanabe et al., 1998; Di Marzo et al., 1999; Mechoulam and Hanus, 2002; Mechoulam et al., 2002). Moreover, CBD may possess agonistic properties at $5$-$HT_{1A}$ receptors (Russo et al., 2005). Although there are contradictory results, several studies indicate that activation of these receptors can induce anxiolytic-like effects and mediate adaptation to stress (Blier and de Montigny, 1994; Blier and Ward, 2003; Joca et al., 2003; Joca et al., 2007).

Acute restraint is an uncontrollable stress situation that produces endocrine and autonomic responses characterized by increases in glucocorticoids levels, blood pressure and heart rate (HR) (Tavares and Correa, 2006; Hsu et al., 2007). These responses are accompanied by activation of several brain structures (Pacak and Palkovits, 2001). In addition to physiological responses, animals submitted to restraint also develop behavioural changes reflected, for example, in reduced exploratory activity in an open field 24 h after stress (Kennett et al., 1985a; 1987; Mechiel Korte and De Boer, 2003), increased immobility in a forced swimming test (Sevgi et al., 2006) and reduced exploration of the open arms of an elevated plus-maze (EPM) (Guimaraes et al., 1993; Padovan and Guimaraes, 2000). These stress-induced behavioural changes can be attenuated by systemic or intracerebral administration of anxiolytic and antidepressant drugs (Kennett et al., 1985a; 1987; Guimaraes et al., 1993; Padovan and Guimaraes, 2000; Mechiel Korte and De Boer, 2003). A possible effect of CBD on these changes, however, has not yet been investigated. Therefore, in the present work we tested the hypothesis that systemic administration of CBD would attenuate the acute physiological changes and the behavioural consequences of restraint stress. We also evaluated the involvement of $5$-$HT_{1A}$ receptors in the effects of CBD in this model.

## Methods

### Animals
The Institution's Animal Ethics Committee approved housing conditions and experimental procedures for animals. Male Wistar rats weighing 230–250 g were used. The animals were provided by our local Animal farm facility. After arriving at the Animal Care Unit of the Department of Pharmacology, School of Medicine of Ribeirão Preto, University of Sao Paulo, the animals were kept in groups of four per cage for 48 h. After this adaptation period they are housed individually in plastic cages with free access to food and water under a 12 h light/dark cycle (lights on at 06:30 h) for a further 24 h period before being subjected to the surgical procedure (see below).

### Surgical preparation
Twenty-four hours before being submitted to restraint stress, animals had a polyethylene catheter implanted into the femoral artery under anaesthesia (tribromoethanol, 250 mg kg$^{-1}$ i.p.), for the recording of arterial blood pressure and HR. The catheter was exposed on the dorsum of the animals and attached to the skin, allowing arterial pressure recordings from conscious animals.

### Acute restraint
*Experiment 1* In the morning period (between 7 a.m. and 12 a.m.), the animals were transferred to the experimental room in their home box. Mean arterial pressure (MAP) and HR were recorded using an HP-7754A amplifier (Hewlett Packard, Palo Alto, CA, USA) connected to a signal acquisition board (Biopac M-100, Goleta, CA, USA) and computer processed. After a few minutes of baseline recording, rats received a single i.p. injection of one of the following drugs: vehicle or CBD (1, 10 or 20 mg kg$^{-1}$). Thirty minutes later they were submitted to a 60 min restraint period in a small plastic cylindrical restraining tube (diameter = 6.5 cm and length = 15 cm). After the restraint period the animals returned to their cages. Each animal was submitted to only one session of restraint to prevent the development of stress tolerance (Guimaraes et al., 1993).

*Experiment 2* Based on the results obtained in the first experiment, we chose the dose of 10 mg kg$^{-1}$ of CBD to use in this second study. The protocol was similar to the first experiment except that before the restraint period the animals received, first, an i.p. injection of vehicle or WAY (0.1 mg kg$^{-1}$) followed, 30 min later, by a second injection of vehicle or CBD (10 mg kg$^{-1}$). As in experiment 1, 30 min after the last injection the animals were restrained for 60 min. One day later they were tested in the EPM. We also had a general control group of unrestrained animals treated with the saline+CBD vehicle that were tested 24 h later in the EPM.

### The EPM test
The EPM test was conducted as described before (Padovan and Guimaraes, 2000). Briefly, the apparatus consisted of two opposite open arms (50 × 10 cm) crossed at a right angle by two arms of the same dimensions enclosed by 40 cm high walls with no roof. The maze was located 50 cm above the floor. Rodents naturally avoid the open arms of the EPM and anxiolytic compounds typically increase the exploration of these arms without changing the number of enclosed-arm entries (Pellow et al., 1985; Carobrez and Bertoglio, 2005). The EPM was cleaned and dried before each session and the Ethovision software (Version 1.9, Noldus, Netherlands) was employed for behavioural analysis.

### Data analysis
Mean arterial pressure and HR values were continuously recorded for 10 min before the 60 min restraint stress period.

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**Table 1** Basal values of the MAP and HR in vehicle (control), CBD and WAY100635 treated rats

| Group | | MAP (mmHg) | HR (bpm) |
|---|---|---|---|
| Control | $n = 6$ | $105 \pm 2$ | $338 \pm 13$ |
| CBD 1 mg | $n = 5$ | $103 \pm 3$ | $363 \pm 14$ |
| CBD 10 mg | $n = 5$ | $100 \pm 3$ | $349 \pm 11$ |
| CBD 20 mg | $n = 5$ | $97 \pm 3$ | $369 \pm 9$ |
| WAY100635 | $n = 5$ | $99 \pm 2$ | $371 \pm 14$ |
| | | $F_{4,25} = 1.5$, $P > 0.05$ | $F_{4,25} = 1.3$, $P > 0.05$ |

The values in the table represent the means $\pm$ SE. One-way ANOVA. CBD, cannabidiol; HR, heart rate; MAP, mean arterial pressure.

Data were expressed as means $\pm$ SEM of MAP or HR changes (respectively $\Delta$ MAP and $\Delta$ HR) sampled at 5 min intervals. Points sampled during the 10 min before restraint were used as control baseline value. MAP and HR changes were analysed using two-way ANOVA with treatment as independent factor and time as repeated measurement factor. When interaction between the factors was observed, groups were compared using one-way ANOVA followed by Bonferroni's *post hoc* test.

The per cent of entries (100 × open/total entries) and time spent in the open arms (100 × open/open + enclosed) of the EPM were calculated for each rat. These data, together with the number of enclosed arm entries, were analysed by one-way ANOVA followed by Bonferroni's *post hoc* test. Values of $P < 0.05$ were taken as showing statistically significant differences between means.

*Drugs*
The following drugs were used: CBD (THC Pharma, Frankfurt, Germany): 1, 10 or 20 mg kg$^{-1}$, suspended in polyoxyethylenesorbitan monooleate (Tween 80) 2%-saline (Resstel *et al.*, 2006), WAY100635 (WAY, Sigma, St. Louis, MO, USA): 0.1 mg kg$^{-1}$ dissolved in saline (Kaster *et al.*, 2005) and tribromoethanol (Aldrich, St. Louis, MO, USA). The solutions were prepared immediately before use and injected intraperitoneally in a volume of 1 mL kg$^{-1}$. The appropriate vehicles were used in each experiment.

## Results

*Experiment 1*
*Effects of CBD on cardiovascular responses to acute restraint* Systemic injection of CBD (1, 10 and 20 mg kg$^{-1}$) did not affect baseline blood pressure ($F_{4,25} = 1.2$, $P > 0.05$) or HR ($F_{4,25} = 0.9$, $P > 0.05$) values when compared with vehicle control ($n = 6$, Table 1). As represented in Figure 1, acute restraint induced a marked and sustained increase of HR and MAP during the 60 min test. There were significant effects of treatment (MAP: $F_{3,240} = 93.5$, $P < 0.001$; HR: $F_{3,240} = 123$, $P < 0.001$), time (MAP: $F_{14,240} = 83.6$, $P < 0.001$; HR: $F_{14,240} = 27.9$, $P < 0.001$) and treatment versus time interaction (MAP: $F_{42,240} = 5.1$, $P < 0.01$; HR: $F_{42,240} = 7.9$, $P < 0.01$).

Cannabidiol decreased the stress-induced cardiovascular responses at doses of 10 and 20 mg kg$^{-1}$ (HR, $F_{3,16} = 19.9$,
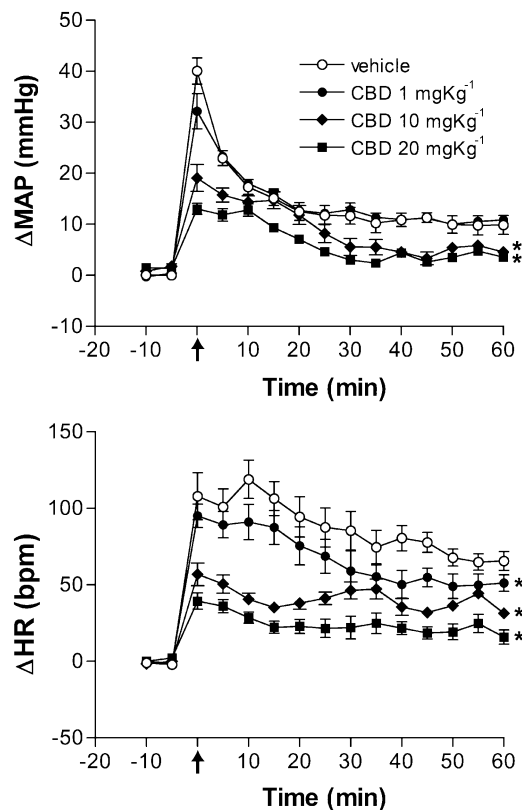


**Figure 1** Effects of different doses of cannabidiol (CBD, 1, 10 or 20 mg kg$^{-1}$, $n = 5$ per group) or vehicle (Tween 80 2%-saline, 1 mL kg$^{-1}$, $n = 6$) on changes in mean arterial pressure ($\Delta$MAP) and heart rate ($\Delta$HR) of animals submitted to 60 min of restraint stress. The arrow indicates the beginning of the restraint period. Data shown represent the means $\pm$ SEM. *$P < 0.05$, compared with vehicle group; ANOVA followed by Bonferroni's *post hoc* test.

$P < 0.001$, MAP, $F_{3,16} = 14.6$, $P < 0.001$). The dose of 1 mg kg$^{-1}$ was also able to attenuate HR responses ($P < 0.05$). A dose-dependency was demonstrated by nonlinear regression analysis and showed a significant correlation between doses and attenuation of the increased MAP ($r^2 = 0.82$, df = 13, $P < 0.01$) and HR ($r^2 = 0.73$, df = 13, $P < 0.01$) (Fig. 2).

*Experiment 2a*
*Effects of WAY100635 on CBD effects on acute cardiovascular responses to restraint* WAY ($n = 5$) did not affect baseline values of blood pressure ($F_{4,25} = 1.2$, $P > 0.05$) or HR ($F_{4,25} = 0.9$, $P > 0.05$) compared with CBD (10 mg kg$^{-1}$, $n = 6$) and vehicle ($n = 6$, Table 1).

There were significant effects of restraint (MAP: $F_{3,270} = 35.95$, $P < 0.001$; HR: $F_{3,270} = 104.3$, $P < 0.001$), time (MAP: $F_{14,270} = 59.66$, $P < 0.001$; HR: $F_{14,270} = 66.4$, $P < 0.001$) and treatment versus time interaction (MAP: $F_{42,270} = 2.1$, $P < 0.01$; HR: $F_{42,270} = 1.9$, $P < 0.01$) (Fig. 3).

The decrease of the cardiovascular responses by systemic administration of CBD (10 mg kg$^{-1}$) was prevented by WAY (MAP: $F_{1,135} = 139.5$, $P < 0.001$; HR: $F_{1,135} = 290.3$, $P < 0.001$) (Fig. 3). The latter drug, by itself, had no effect on cardiovascular responses to restraint (MAP: $F_{1,135} = 1.3$, $P > 0.05$; HR: $F_{1,135} = 1.1$, $P > 0.05$) (Fig. 3).
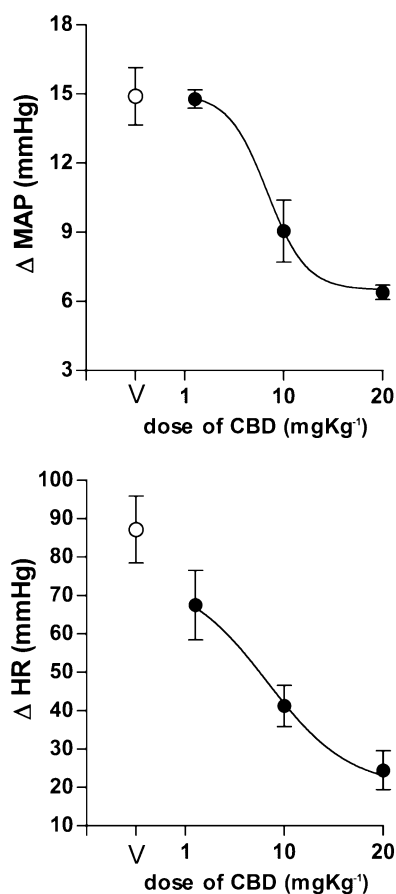
**Figure 2** Mean arterial pressure (ΔMAP) and heart rate (ΔHR) changes in response to the injection of increasing doses of cannabidiol (CBD, 1, 10, 20 mg kg$^{-1}$, $n = 5$/group) in rats. V: vehicle (Tween 80 2%-saline, 1 mL kg$^{-1}$, $n = 6$). Dose-effect curves were generated by nonlinear regression analysis. Data shown represent the means ± SEM of the variation of MAP and HR during the 60 min of acute restraint.



**Figure 3** Effects of pre-treatment with of saline (Sal) or WAY100635 (WAY, 0.1 mg kg$^{-1}$) followed by second injection of vehicle (Veh, Tween 80 2%-saline) or cannabidiol (CBD, 10 mg kg$^{-1}$) immediately before a 60 min restraint period on increase in the mean arterial pressure (ΔMAP) and heart rate (ΔHR) induced by restraint stress. The arrow indicates the beginning of the restraint period. Data shown represent the mean ± SEM of five to six animals. *$P < 0.05$, compared with vehicle group; ANOVA followed by Bonferroni's *post hoc* test.

Typical experimental recordings showing the effects of CBD and WAY on cardiovascular responses observed during acute restraint can be seen in Figure 4.

*Experiment 2b*
*Effects of CBD and WAY100635 on delayed behavioural consequences in the EPM induced by restraint* Acute restraint induced a significant decrease in the percentage of open arm entries ($F_{3,25} = 7.72$, $P < 0.001$) compared with unrestrained controls ($n = 6$), when tested 24 h after stress (Fig. 5). CBD administration in restrained rats ($n = 6$) increased the percentage of open arm entries ($P < 0.001$, Fig. 5) compared with controls (vehicle-treated restrained animals, $n = 6$). This effect was prevented by pre-administration of WAY ($P < 0.001$, $n = 6$). No effect was found in animals treated only with WAY ($P > 0.05$, $n = 6$) (Fig. 5). There was also no effect on the percentage time spent in the open arms and in the number of enclosed arm entries ($P > 0.05$).

## Discussion

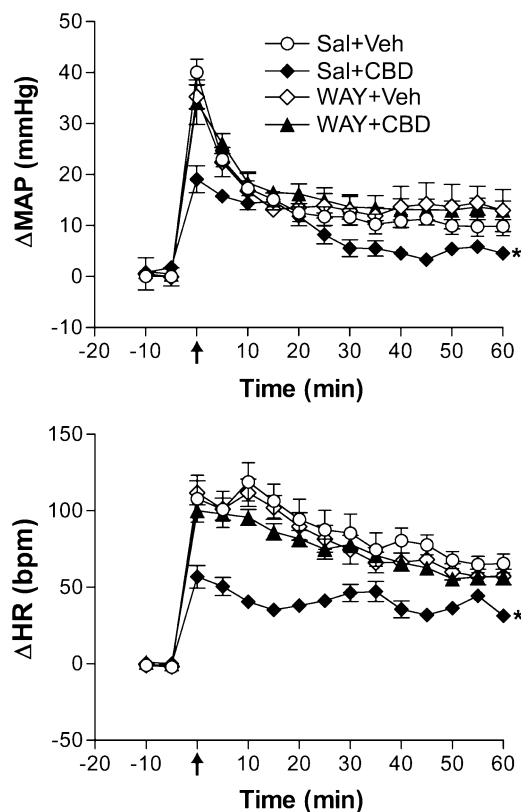The present results showed that (i) CBD reduced the pressor and tachycardic responses to restraint stress, in a dose-

dependent manner; (ii) CBD attenuated the increased anxiety behaviour caused by a previous exposure to restraint; and (iii) effects of CBD on cardiovascular and behavioural responses to restraint can be blocked by WAY100635, a 5-HT$_{1A}$ receptor antagonist.

Acute exposure to restraint stress has been shown to evoke several physiological and behavioural changes (Tavares and Correa, 2006; Hsu *et al.*, 2007) and, as expected, in the present study animals exhibited significant increases in MAP and HR during restraint. Moreover, they showed decreased exploration of the open arms of the EPM 24 h after restraint stress. These delayed behavioural consequences of acute restraint stress have been described in several models, including the open field and EPM (Kennett *et al.*, 1985a; Guimaraes *et al.*, 1993; Padovan and Guimaraes, 2000; Mechiel Korte and De Boer, 2003), and are sensitive to systemic and intra-cerebral injection of anxiolytic and antidepressant drugs (Kennett *et al.*, 1985a; Kennett *et al.*, 1987; Guimaraes *et al.*, 1993; Padovan and Guimaraes, 2000; Mechiel Korte and De Boer, 2003).

Cannabidiol did not induce any significant change in basal MAP and HR which agrees with the reported lack of significant cardiovascular effects of this drug (McQueen *et al.*, 2004;
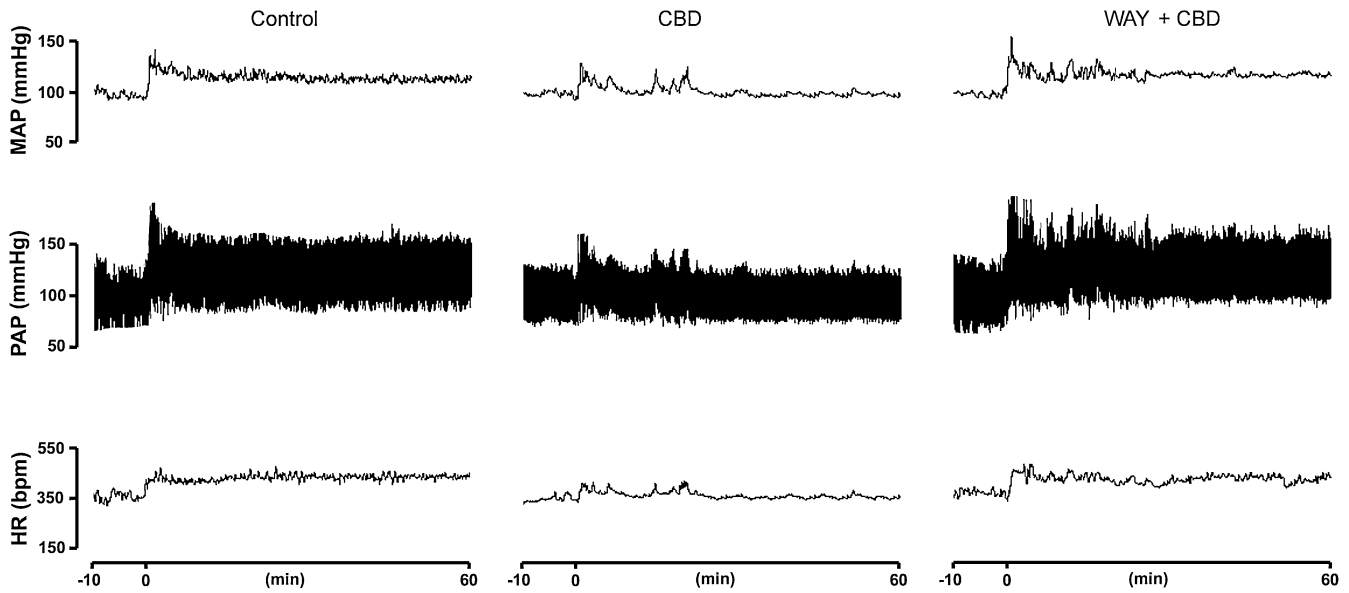
**Figure 4** Mean arterial pressure (MAP), pulsatile arterial pressure (PAP) and heart rate (HR) individual recordings showing the cardiovascular changes evoked by acute restraint in animals treated with vehicle (control, Tween 80 2%-saline), cannabidiol (CBD) or cannabidiol after WAY100635 (WAY+CBD). The restraint period started at time 0.
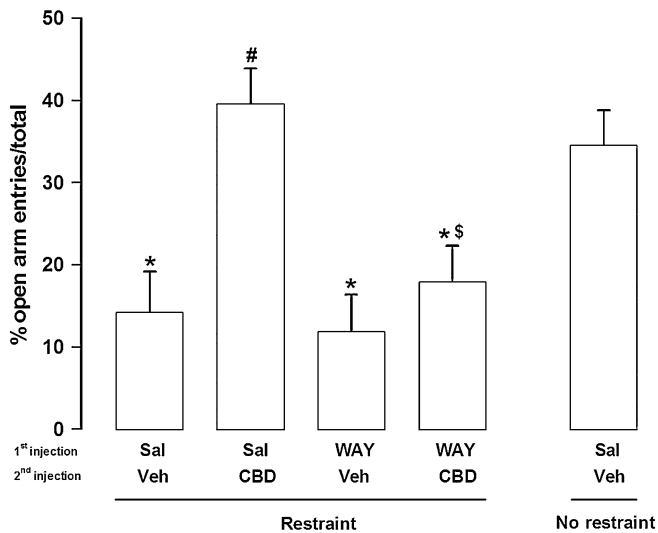


**Figure 5** Effects in the elevated plus-maze (EPM) of a first systemic injection of saline (Sal) or WAY100635 (WAY, 0.1 mg kg$^{-1}$) followed by a second injection of vehicle (Veh, Tween 80 2%-saline) or cannabidiol (CBD, 10 mg kg$^{-1}$) immediately before a 60 min restraint period ($n = 6$ per group). A non-stressed group (no-restraint) that received i.p. injections of saline followed by vehicle was used as general control. The EPM test was performed 24 h after the restraint period. Data represent the mean ($\pm$SEM) per cent of open arm entries. *$P < 0.05$, compared with control group; #$P < 0.05$, compared with restraint-vehicle group; $$P < 0.05$, compared with restraint-CBD group; ANOVA followed by Bonferroni's *post hoc* test.

Resstel *et al.*, 2006). At low doses, it also does not interfere with memory and learning processes (Lichtman *et al.*, 1995; Fadda *et al.*, 2004; 2006). It is unlikely, therefore, that the attenuation of the cardiovascular and delayed behavioural responses to restraint depend on direct cardiovascular effects or memory impairment induced by the drug, but rather on an attenuation of the emotional response to stress. In agreement

with this proposal, acute administration of CBD has been shown to induce anxiolytic-like effects in several animal models, including the EPM, Vogel conflict test and contextual fear conditioning (Guimaraes *et al.*, 1990; Onaivi *et al.*, 1990; Moreira *et al.*, 2006; Resstel *et al.*, 2006). The effective doses of CBD in these previous studies were, in general, similar to ours. In the study by Guimaraes *et al.* (1990), however, CBD produced an inverted U-shaped dose-response curve, with the highest dose (20 mg kg$^{-1}$) being ineffective. The reasons for this difference are unknown, but may depend on the model used (Calabrese, 2008). As the EPM test is based on exploratory activity, it could be more prone to interference of non-specific drug effects that affects this parameter (Calabrese, 2008). In addition, at least for classical anxiolytics such as diazepam, the EPM is more sensitive than other models such as the Vogel conflict test (see Calabrese, 2008).

The mechanisms of the anxiolytic and anti-stress effects of CBD are not clear. It has a low affinity for cannabinoid receptors (Petitet *et al.*, 1998; Thomas *et al.*, 1998) although, under certain circumstances, it may act as an antagonist of CB$_1$- and CB$_2$-receptor agonists (Pertwee *et al.*, 2002). CBD could also block the reuptake and metabolism of anandamide, facilitating endocannabinoid-mediated neurotransmission (Watanabe *et al.*, 1998; Di Marzo *et al.*, 1999; Bisogno *et al.*, 2001; Mechoulam *et al.*, 2002). However, several effects of CBD have been shown to be independent of the endocannabinoid system (Hayakawa *et al.*, 2007). More recently, Russo *et al.* (2005) reported that CBD can displace the 5-HT agonist [$^3$H]8-OH-DPAT from cloned human 5-HT$_{1A}$ receptors expressed in Chinese hamster ovary cultured cells. Moreover, using signal transduction studies, this work also showed that CBD can act as an agonist at these receptors. The observation that CBD has agonistic properties at 5-HT$_{1A}$ receptors has been supported by *in vivo* studies where the neuroprotective and anti-oxidative effects induced by CBD were blocked by pre-treatment with the 5-HT$_{1A}$ antagonist WAY100135 (Mishima

*et al.*, 2005; Hayakawa *et al.*, 2007). Our results corroborate these findings, by showing that the stress-attenuating effects induced by CBD were prevented by systemic pretreatment with WAY100635, a selective antagonist at these receptors. In agreement with our results, a recent study from our laboratory showed that CBD interacts with 5-HT$_{1A}$ receptors in the dorsolateral periaqueductal gray to produce anxiolytic-like effects in the EPM (Campos and Guimaraes, 2008).

5-HT$_{1A}$ receptors are widely distributed in the brain, especially in structures traditionally related to stress and anxiety, such as the raphé nuclei, hippocampus, prefrontal cortex, amygdala and hypothalamus (Chalmers and Watson, 1991). Although the role of 5-HT in anxiety is still a matter of intense debate (Millan, 2003), several pieces of evidence suggest that activation of 5-HT$_{1A}$ receptors facilitates adaptation to stress (Kostowski *et al.*, 1992; Dekeyne *et al.*, 2000; Tsuji *et al.*, 2000; Blier and Ward, 2003; Joca *et al.*, 2003; Kagamiishi *et al.*, 2003; Rioja *et al.*, 2004; Joca *et al.*, 2007). For example, pre-administration of a 5-HT$_{1A}$ agonist before an acute immobilization period blocked the stress-induced anxiogenic effect observed in an elevated T-maze test performed 24 h later (Rioja *et al.*, 2004). Similar results were reported by Tsuji *et al.* (2000), who observed that administration of 5-HT$_{1A}$ receptor agonists before restraint stress attenuated, in a dose-dependent manner, the development of stress-induced anxiogenic effect observed 24 h later in the hole-board test of anxiety. Data from 5-HT$_{1A}$ receptor knockout mice give further support to this hypothesis, as these animals display anxiogenic-like behaviour in different paradigms (Zhuang *et al.*, 1999; Tsetsenis *et al.*, 2007). It is proposed that the lack of this receptor would promote a bias in the processing of threatening cues which could render the animal more susceptible to the development of behavioural consequences of stress (Tsetsenis *et al.*, 2007). On the other hand, facilitation of this neurotransmission would mediate adaptation to stress (Graeff *et al.*, 1996). This suggestion is supported by results showing that behavioural adaptation to stress is accompanied by sensitization of 5-HT$_{1A}$-mediated neurotransmission (Kennett *et al.*, 1985b; 1987; Samad and Haleem, 2007) and that an increased expression of this receptor in the brain is associated with reduced anxiety-like behaviour (Kusserow *et al.*, 2004). Therefore, these studies support the possibility that activation of 5-HT$_{1A}$ receptors protects animals against various emotional changes caused by stressful stimuli, perhaps by facilitating mechanisms involved in the ability to cope with the stressful situation.

The exact mechanism through which 5-HT$_{1A}$ agonists induce their anxiolytic activity remains unclear. 5-HT$_{1A}$ receptors are located presynaptically (somatodendritic autoreceptors) in 5-hydroxytryptaminergic cell bodies in the raphé nuclei of the brain stem and postsynaptically, predominantly in limbic structures such as the hippocampus and the hypothalamus (Verge *et al.*, 1985; 1986; Chalmers and Watson, 1991). It is still controversial whether the anxiolytic-like effects induced by acute systemic administration of 5-HT$_{1A}$ agonists are due to the activation of the pre- or the post-synaptic receptors (File *et al.*, 1996; Lopez-Rubalcava, 1996). Moreover, depending on the structure where post-synaptic 5-HT$_{1A}$ receptors are located, their activation may lead to anxiolytic or anxiogenic-like effects (Graeff *et al.*, 1996; Zan-

grossi *et al.*, 2001). Therefore, the exact mechanism of action of 5-HT1A agonists as anxiolytic compounds is complex and still warrants further investigation.

In conclusion, the present findings indicate that CBD, by activating 5-HT$_{1A}$ receptors, can attenuate physiological and behavioural responses to restraint stress. This finding raises the possibility that CBD could be useful for treating psychiatric disorders thought to involve impairment of stress-coping mechanisms, such as depression and post-traumatic stress disorder.

## Acknowledgements

## Conflicts of interest

The authors state no conflict of interest.

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