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Cannabidiol, a constituent of *Cannabis sativa*, modulates sleep in rats

Eric Murillo-Rodríguez[a,*], Diana Millán-Aldaco[a], Marcela Palomero-Rivero[a], Raphael Mechoulam[b], René Drucker-Colín[a]

[a] *Depto de Neurociencias, Instituto de Fisiología Celular, Ciudad Universitaria, Circuito Interior, Universidad Nacional Autónoma de México, México DF, CP 04510, Mexico*

[b] *Department of Medicinal Chemistry and Natural Products, School of Pharmacy, Hebrew University, Jerusalem, Israel*

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Abstract **Δ^9-tetrahydrocannabinol (Δ^9-THC) and cannabidiol (CBD) are two major constituents of *Cannabis sativa*. Δ^9-THC modulates sleep, but no clear evidence on the role of CBD is available. In order to determine the effects of CBD on sleep, it was administered intracerebroventricular (icv) in a dose of 10 μg/5 μl at the beginning of either the lights-on or the lights-off period. We found that CBD administered during the lights-on period increased wakefulness (W) and decreased rapid eye movement sleep (REMS). No changes on sleep were observed during the dark phase. Icv injections of CBD (10 μg/5 μl) induced an enhancement of *c*-Fos expression in waking-related brain areas such as hypothalamus and dorsal raphe nucleus (DRD). Microdialysis in unanesthetized rats was carried out to characterize the effects of icv administration of CBD (10 μg/5 μl) on extracellular levels of dopamine (DA) within the nucleus accumbens. CBD induced an increase in DA release. Finally, in order to test if the waking properties of CBD could be blocked by the sleep-inducing endocannabinoid anandamide (ANA), animals received ANA (10 μg/2.5 μl, icv) followed 15 min later by CBD (10 μg/2.5 μl). Results showed that the waking properties of CBD were not blocked by ANA. In conclusion, we found that CBD modulates waking via activation of neurons in the hypothalamus and DRD. Both regions are apparently involved in the generation of alertness. Also, CBD increases DA levels as measured by microdialysis and HPLC procedures. Since CBD induces alertness, it might be of therapeutic value in sleep disorders such as excessive somnolence.**

Keywords: Anandamide; Cannabinoid receptor; Insomnia; Dopamine

1. Introduction

Cannabis sativa preparations (marijuana, hashish, bhang and others) are the most widely used illicit drugs in the world [1]. Δ^9-tetrahydrocannabinol (Δ^9-THC) and cannabidiol (CBD) are two major constituents of marijuana [2,3]. Δ^9-THC is a psychoactive compound and produces stereotypical behaviours [4]. The cannabinoid receptor CB_1 is thought to be responsible for the majority of the effects in the central nervous system (CNS) elicited by Δ^9-THC, since it binds and activates this receptor [5].

CBD does not bind to CB_1 and is not psychoactive. The chemistry of CBD has been well explored [6–8] and some of its CNS pharmacological properties have been examined [9], including its anticonvulsant, sedative and anxiolytic effects [10–12]. For instance, Guimaraes and co-workers found that CBD (2.5–10 mg/kg) induced an anxiolytic-like effect evaluated by the elevated plus maze assay whereas Lastres-Becker et al. have reported that CBD displays neuroprotection against 6-hydroxydopamine toxicity [13,14].

While it is well established that Δ^9-THC increases sleep [15–17], contradictory results on the effects of CBD have been published. For example, Monti et al. reported a reduction of sleep by systemic administration of CBD [18]. On the other hand, Carlini and Cunha showed that CBD improved sleep in insomniacs [19]. Recently, Nicholson et al. found that 15 mg of CBD administered to young adults increased wakefulness (W) during sleeping time [20].

The mechanism of sleep modulation by CBD remains unclear. Presumably, it could include changes in dopamine (DA) levels, as the nigrostriatal dopaminergic system has been pointed out as an important element in the manifestations of the cannabinoid-induced behavioural alterations. Indeed the extracellular levels of DA are enhanced following Δ^9-THC administration [21–23]. The effects of CBD on catecholamine levels are not known.

As indicated above although CBD does not bind to the CB_1 cannabinoid receptor [24,25], it elicits numerous CNS-associated effects, including the sleep–wake cycle. In view of the divergent effects reported so far we decided to reinvestigate this phenomenon using techniques different from those reported previously. First we determined the pharmacological effects of unilateral intracerebroventricular (icv) administration of CBD on sleep in rats. Then we analysed the effects of CBD on *c*-Fos immunoreactivity followed by measurement of DA extracellular levels collected from nucleus accumbens (AcbC) using microdialysis and HPLC. Finally, we looked into the

*Corresponding author. Fax: +52 55 5622 5607.
E-mail address: emurillo@ifc.unam.mx (E. Murillo-Rodríguez).

Abbreviations: ANA, anandamide; ACSF, artificial cerebrospinal fluid; Δ^9-THC, Δ^9-tetrahydrocannabinol; CBD, cannabidiol; DA, dopamine; DMH, dorsomedial hypothalamic nucleus; DRD, dorsal raphe nucleus; FAAH, fatty acid amide hydrolase; HVA, homovanillic acid; icv, intracerebroventricular; MPO, medial preoptic nucleus; NA, noradrenaline; AcbC, nucleus accumbens; OEA, oleoylethanolamide; REMS, rapid eye movement sleep; 5-HT, serotonin; SWS, slow wave sleep; W, wakefulness; 5-HIAA, 5-hydroxy-indoleacetic acid; L-DOPA, 3,4-dihydroxy-L-phenylalanine

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possibility that anandamide (ANA) could block the alertness induced by CBD as we have previously reported that the endocannabinoid ANA increases sleep [26–28].

2. Materials and methods

2.1. Animals

Male wistar rats ($n = 50$; 250–300 g) were housed at constant temperature (21 ± 1 °C) and under a controlled light–dark cycle (lights-on: 07:00–19:00 h). Food and water were provided *ad libitum*.

2.2. Surgery, EEG/EMG electrodes and icv cannulae

Under deep anesthesia (acepromazine [0.75 mg/kg], xylazine [2.5 mg/kg], and ketamine [22 mg/kg, ip]) all animals ($n = 12$) were implanted for sleep studies with EEG and EMG electrodes and a cannula (23 gauge) was placed into one lateral ventricle ($A = -0.8$; $L = -1.6$; $H = -3.6$; [29]). All electrodes and cannulae were placed and secured onto the skull using dental cement. These procedures have been reported previously by our group [26,27]. After surgeries, all animals were placed into the sleep-recording chambers for habituation.

2.3. Surgery, microdialysis guide-cannulae

A different group of rats was used for microdialysis study ($n = 14$). A guide-cannula (IC guide. BioAnalytical Systems [BAS], West Lafayette, IN, USA) was placed stereotaxically into the Accumbens nucleus, core (AcbC; target coordinates: $A = +1.2$; $L = 2.0$; $H = -7.0$; [29]). The guide-cannulae was then fixed onto the skull with a thin layer of dental cement. After surgery, each animal was placed into the Microdialysis Bowl to habituate to the experimental conditions. All animals were allowed to recover for at least 7 days after all surgeries.

2.4. Administrations

CBD was prepared in our lab as previously reported [25] and ANA was purchased from Sigma (USA). All compounds were dissolved in vehicle (PEG/saline; 5:95 v/v). In order to test the effects of CBD on the sleep during the lights-on or lights-off period, rats were injected icv either with vehicle or CBD (10 μg/5 μl) at 07:00 h or at 19:00 h.

For the combination experiment in which we examined the effect of ANA on the effects produced by CBD, additional groups of rats were used. Control group ($n = 6$) received vehicle. The following groups received either ANA (10 μg/5 μl; $n = 6$) or CBD (10 μg/5 μl; $n = 6$). The last group ($n = 6$) received ANA (10 μg/2.5 μl) and 15 min later CBD (10 μg/2.5 μl). Injections were done at 07:00 h.

All icv administrations were done slowly over 1 μl/min with the injector left in the target for an additional 15 s to ensure extrusion from the tip and to minimize distribution of treatments upwards on the cannulae. After all injections, the cannula was withdrawn and the stylet was replaced. Right after microinjections, animals were attached to the sleep-recording system.

2.5. Analyses of sleep recordings

The EEG/EMG data recordings were scored manually and epochs for W, slow wave sleep (SWS) and rapid eye movement sleep (REMS) were measured as described previously [26,27]. The analysis was restricted to 4 h after injections since pharmacokinetic studies show that CBD is rapidly absorbed. On iv administration, CBD has a terminal half life of 9 h [30].

2.6. Microdialysis sampling procedures

One week after surgery and a day before the experiment, a microdialysis probe (1 mm of length; polyacrylonitrile, MWCO = 30 000 Da; 340 μm OD; BAS) was inserted through the guide cannula into the target structure at 7:00 h and the tissue were allowed to stabilize for 24 h. During this period artificial cerebrospinal fluid (ACSF, composition: NaCl (147 mM), KCl (3 mM), CaCl (1.2 mM), MgCl (1.0 mM), pH 7.2) was perfused through a FEP Teflon Tubing (0.65 mm OD × 0.12 mm ID) continuously using a 2.5 ml gastight syringe. All procedures have been reported previously [28]. A syringe pump (CMA/100) controlled the speed of perfusion of the ACSF (flow rate: 1 μl/min). Since we had found changes in sleep after CBD injection during the lights-on period, we administered the treatments at the beginning of the lights-on period and right after this, samples were collected during 4 h.

2.7. Analysis of DA

Immediately after collection samples were injected into a HPLC (BAS) for DA analysis. Briefly, the mobile phase consisted of monochloroacetic acid (0.1 M), sodium octylsulfate (223 mM) and disodium ethylenediaminetetraacetate dihydrate (0.5 mM) with a flow rate of 80 μl/min. Separation was achieved by a BAS microbore column (biophase octyl, 5 μm, 250 × 4.6 mm; BAS). Electrochemical detection was performed via BAS 4C detector. Chromatographic data was recorded on a PC computer and peak heights of DA in microdialysis samples were compared to standards. DA peaks were initially identified by running samples (also 10 μl) that contained different concentrations of DA. All details of this procedure were reported by our group previously [31].

2.8. Immunohistochemical study

At the end of the experiments, animals received either vehicle or CBD (10 μg/5 μl, icv). The treatments were administered at the beginning of the lights-on period and the animals were sacrificed 1-h post-injection with a lethal dose of pentobarbital. They were perfused transcardially with 0.9% saline solution followed by paraformaldehyde and then followed by 20% sucrose–0.1 M PBS for 48 h. The brains were cut (frozen sections, 30 μm, coronal) and collected in 1:5 serial orders. Immunohistochemistry for *c*-Fos was done as described in detail by our group [32]. In addition to the *c*-Fos study, sections were lightly counterstained with Neutral Red to allow better visualization of the cannula track. All studies were conducted in accordance with the principles and procedures described in the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.9. Statistical analysis

The data are presented as means and standard errors. Student *t* test was used to compare control and CBD groups in the first two experiments and a P-value < 0.05 was considered statistically significant. In the following experiments, statistical analysis was carried out by one-way analysis of variance (ANOVA). When a significant P-value was found among the groups, the post-hoc Sheffé test was used to asses differences in two group comparisons (STATVIEW).

3. Results

3.1. Effects of CBD in sleep

We injected CBD ($n = 6$; 10 μg/5 μl) or vehicle ($n = 6$) during the normal sleeping period (07:00–19:00 h, lights-on) of rats. CBD markedly increased the total time spent in W (DF = 10; $t = -2.267$; $P < 0.05$; Fig. 1A). We also found that CBD induced a significant diminution of REM sleep (DF = 10; $t = 3.756$; $P < 0.05$), whereas no statistical changes were observed in SWS. As shown in Fig. 1B, CBD enhanced W about 1 h after drug administration (DF = 10; $t = -2.507$; $P < 0.05$) as well as during the last hour (DF = 10; $t = -3.808$; $P < 0.005$). The effects of CBD on SWS hour by hour are displayed in Fig. 1C. We found that CBD also modified this sleep stage during the second hour (DF = 10; $t = -2.672$; $P < 0.05$) and the fourth hour (DF = 10; $t = 2.126$; $P < 0.05$). Analysis of REMS hour by hour after administration of CBD is shown in Fig. 1D. CBD inhibited the time spent in REMS across 4 h of sleep recordings; however, statistical difference was found only at the fourth hour (DF = 10; $t = 3.709$; $P < 0.005$).

On the other hand, CBD injected ($n = 6$) at the beginning of the lights-off period did not modify the total time of W (DF = 10; $t = -0.546$; $P > 0.59$), SWS (DF = 10; $t = 0.212$; $P > 0.83$) and REMS (DF = 10; $t = 1.25$; $P > 0.23$) compared

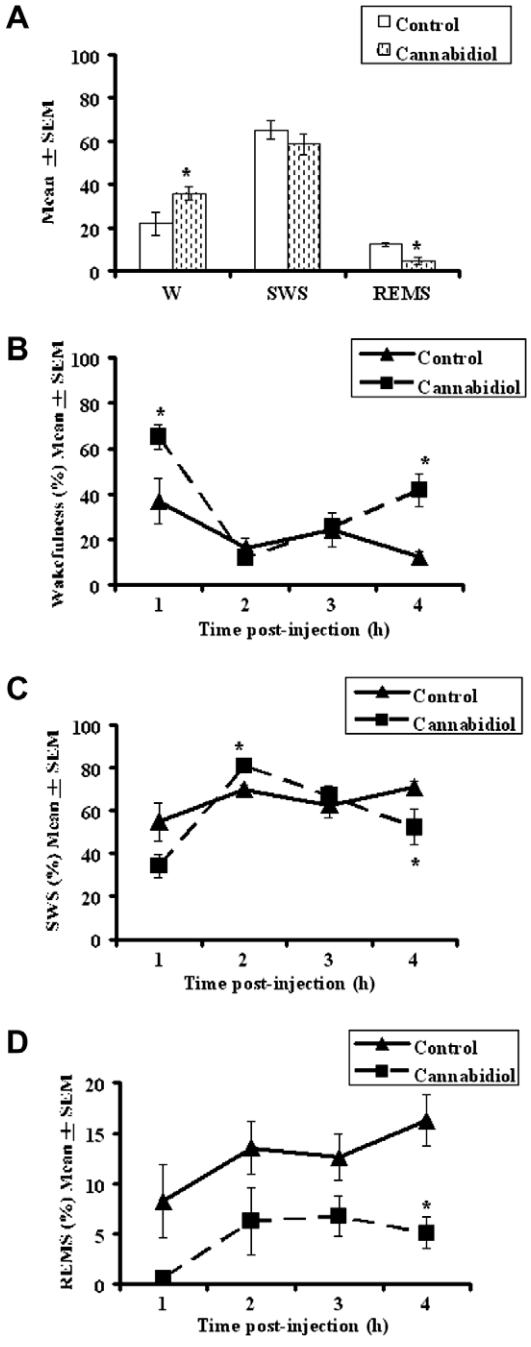

Fig. 1. The icv administrations of either vehicle or CBD (10 μg/5 μl) were done at the start of the lights-on period, and right after injections, the sleep was recorded during 4 h. The effects of CBD on the total time of W, SWS and REMS are shown in panel A. Hour-by-hour group averages of W (B); SWS (C) and REMS (D) after icv injection of either vehicle or CBD (means ± S.E.M. of total time of recording (%); ∗ vs control, $P < 0.05$).

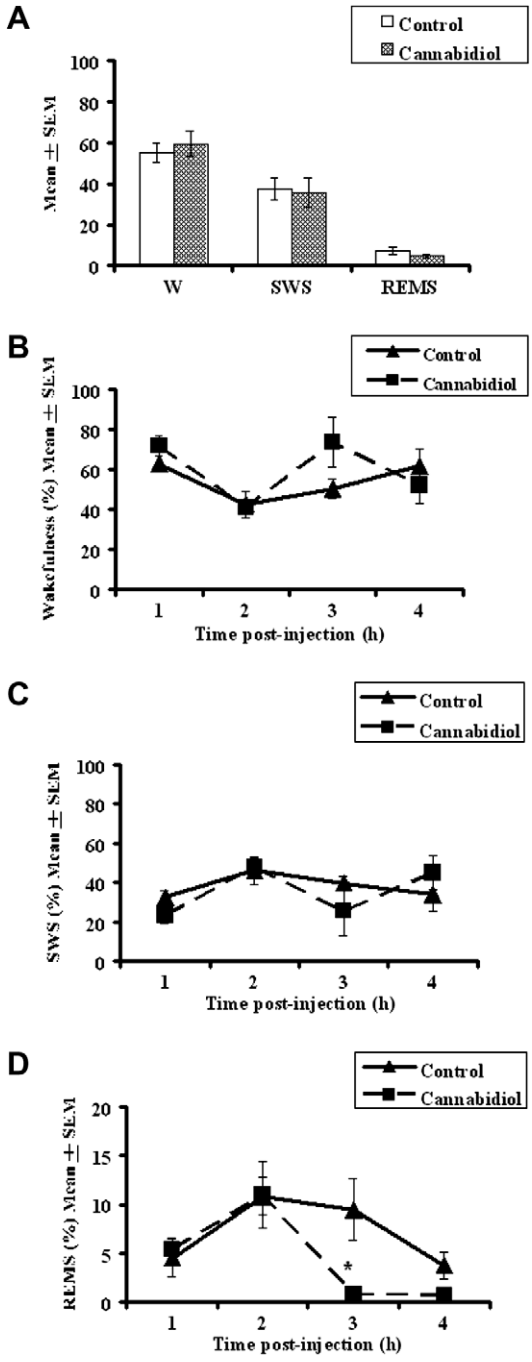

Fig. 2. The icv injection of CBD (10 μg/5 μl) at the start of the lights-off period did not modify the total time (4 h of sleep recordings) of W, SWS and REMS as shown in panel A. The time course of W (B), SWS (C) and REMS (D) show no significant statistical differences (means ± S.E.M. of total time of recording [%]; ∗ vs control, $P < 0.05$).

to control group ($n = 6$; Fig. 2A). Analysis hour by hour of the pharmacodynamic effects of CBD on sleep stages are shown in Fig. 2B–D, respectively, showing no significant changes.

3.2. c-Fos expression after CBD injection

Treatment with CBD (10 μg/5 μl) consistently increased the pattern of neuronal activation marked by Fos expression in brain regions, such as lateral hypothalamus and dorsal raphe nucleus (DRD). Some of the most striking changes in *c*-Fos expression were found within specific hypothalamic areas implicated in the alertness control (Fig. 3). Neurons of hypothalamus are active during W [33], and because all animals were mainly awake, it was not surprising that *c*-Fos-immunoreactivity hypothalamic neurons were activated in rats that received CBD. Following unilateral icv injection of CBD (10 μg/5 μl), labelled *c*-Fos cells were observed in hypothalamic areas,

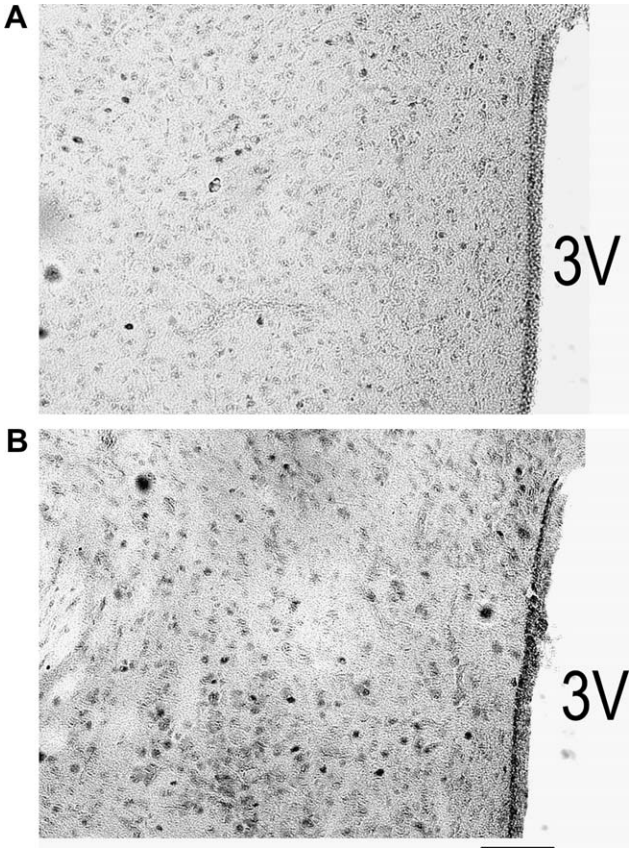

Fig. 3. Immunohistochemical staining for *c*-Fos in the hypothalamus of the rat. Panel A shows the expression of *c*-Fos from a control rat (vehicle). Panel B shows the *c*-Fos immunostaining obtained from a CBD-treated animal (10 μg/5 μl icv). The treatments were administered at the beginning of the lights-on period and the animals were sacrificed 1 h post-injection. Abbreviations: 3V, 3rd ventricle. Scale bar: 100 μm.

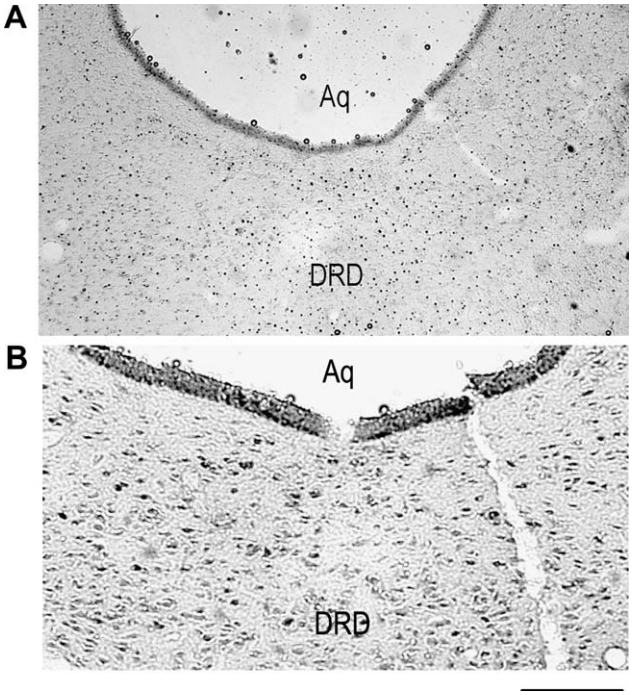

Fig. 4. Immunohistochemical staining for *c*-Fos in the DRD of the rat. Panel A shows the expression of *c*-Fos from a control rat (vehicle). Panel B shows the immunostaining obtained from a CBD-treated animal (10 μg/5 μl icv). The treatments were administered at the start of the lights-on period and the animals were sacrificed 1 h post-injection. Abbreviations: Aq, aqueduct (Sylvius); DRD, dorsal raphe nucleus, dorsal part. Scale bar: 100 μm.

mainly in the medial preoptic nucleus (MPO) and dorsomedial hypothalamic nucleus (DMH). Therefore, we found that CBD induced an increase in *c*-FOS expression in MPO compared with the control group (Fig. 3).

On the other hand, in the brainstem, only neurons in the DRD showed significant labelling pattern of Fos-immunoreactivity after treatment with CBD (10 μg/5 μl). Representative examples of *c*-Fos immunoreactivity are shown in Fig. 4. All icv injections were made unilaterally. No differences among control and CBD group were evident in other brainstem nuclei, including the pedunculopontino, laterodorsal tegmental nuclei and locus coeruleus.

3.3. Effects of CBD on DA levels

Fig. 5 identifies the location of the cannula in the AcbC nucleus from where the DA was measured. The variation in location of the cannula across the rats was as follows: anterior–posterior was Bregma +1.70 mm to Bregma 0.70 mm; lateral was 1.5–2.0 mm and dorsal–ventral was 7.5–7.9 mm.

Results showed that levels of noradrenaline (NA) were significant increased compared with a control group ($n = 7$) in the first hour (DF = 12; $t = -2.844$; $P < 0.01$; Fig. 6A) and in the second hour (DF = 12; $t = -2.137$; $P < 0.005$; Fig. 6A) post-injection of CBD (10 μg/5 μl; $n = 7$). After injection of CBD, epinephrine levels were also enhanced compared with levels in control rats in the first hour (DF = 12; $t = 12.006$; $P < 0.0001$; Fig. 6B) as well as in the second hour (DF = 12; $t = -5.387$; $P < 0.0002$; Fig. 6B).

CBD also induced enhancement in DA levels one hour after injection (DF = 12; $t = -3.290$; $P < 0.05$; Fig. 6C) and this effect remained in the second hour (DF = 12; $t = -2.149$; $P < 0.05$; Fig. 6C) whereas 3,4-dihydroxy-L-phenylalanine (L-DOPA) extracellular levels decreased one hour after injection of CBD (DF = 12; $t = 2.799$; $P < 0.01$; Fig. 6D) as well as in the second hour (DF = 12; $t = 2.281$; $P < 0.05$; Fig. 6D).

Lastly, serotonin (5-HT) was increased 1 h post-CBD administration (DF = 12; $t = -16.741$; $P < 0.0001$; Fig. 6E) as well as 2 h post-CBD injection (DF = 12; $t = -19.058$; $P < 0.0001$; Fig. 6E). Levels of 5-hydroxy-indoleacetic acid (5-HIAA) were decreased 1 h after administration of CBD (DF = 12; $t = 6.074$; $P < 0.0001$; Fig. 6F). Fig. 6G shows that no effect was found on extracellular levels of homovanillic acid (HVA) after CBD injection.

3.4. Effects of the sleep-inducing endocannabinoid ANA on the waking-inducing properties of CBD

As found in our previous experiments [26–28], ANA substantially decreased waking and increased total sleep time. Administration of ANA 15 min before CBD did not block either the wake-inducing effect caused by CBD or the diminution in SWS ($F = 22.472$; DF = 20; $P < 0.0001$; Fig. 7). Despite the evident sleep-inducing effect caused by ANA, the enhancement in W as well as the diminution in total sleep time was still present with the administration of CBD after 4 h of sleep recordings.

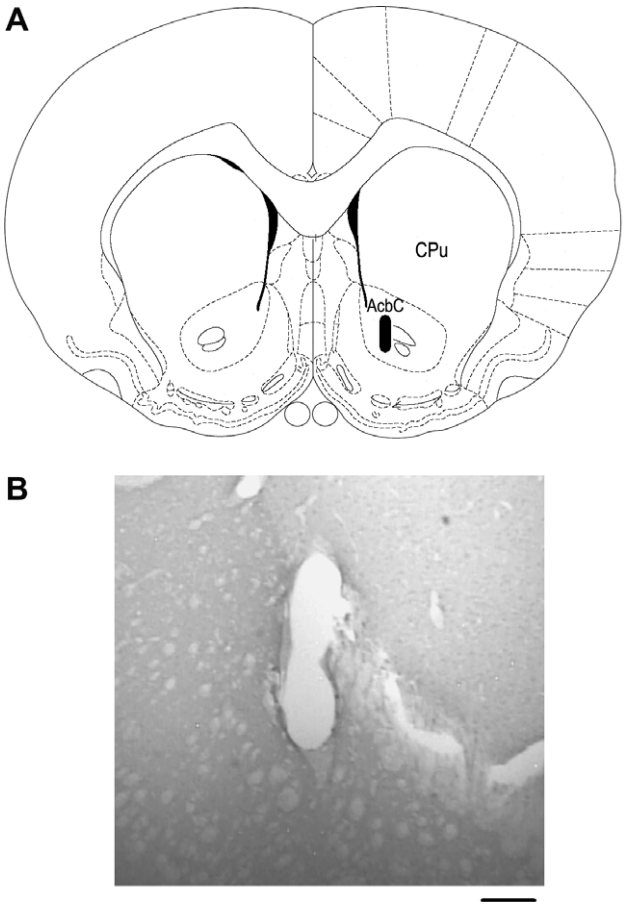

Fig. 5. Histological verification of the microdialysis probe position. Panel A shows the schematic representation of the localization of the microdialysis probe in the AcbC represented by the black bar. Stereotaxic coordinates, drawings and abbreviations were taken from the Paxinos and Watson [29] atlas. Microphotography of the track of the microdialysis probe in the AcbC is shown in Panel B. Abbreviations: AcbC: nucleus accumbens, core; CPu, caudate putamen. Scale bar: 100 μm.

4. Discussion

The experiments described in this report were designed to throw light on the pharmacological effects of CBD on sleep patterns of rats. Several physiological parameters were considered, such as the effects of CBD on the sleep–wake cycle, DA formation, *c*-Fos expression and interaction with ANA on sleep.

Under our conditions, icv administrations of CBD (10 μg/5 μl) during the lights-on period increased waking, but decreased REM sleep in rats (Fig. 1A). The alertness was observed already after the first hour post-injection (Fig. 1B). Injection of CBD during the lights-off period did not modify the sleep wake-cycle (Fig. 2A).

We also found that CBD increased *c*-Fos expression as reported by others [34]. The induction of Fos protein encoded by the immediate early gene *c*-fos, is often used as a marker of neural activation. The present results demonstrate that CBD induced changes in the Fos-immunoreactive cells in some waking-related brain areas, including hypothalamic nuclei (Fig. 3) as well as DRD (Fig. 4). The hypothalamic nuclei have been associated with waking [35–37]. For example, the DMH contains neurons that are specifically active during W [38].

As shown in Fig. 4, a population of neurons in the DRD exhibited enhanced *c*-Fos-immunoreactivity after CBD microinjection compared to controls. The DRD firing activity is higher in the waking state and decreases during sleep, being virtually absent in REM sleep [39–41]. These findings emphasize the hypothesis that one of the neurochemical mechanisms underlying the vigilance-promoting action of CBD could be related to its ability to enhance the serotoninergic transmission. It has been reported recently that CBD displays 5-HT agonists [42], suggesting with this a potential activation of 5-HT receptors via CBD.

Catecholamine levels collected from AcbC were monitored during 4 h post-injection of CBD (10 μg/5 μl, icv). We found an increase in the levels of NA, epinephrine, DA and 5-HT (Fig. 6A–C and E, respectively). On the other hand, the extracellular levels of L-DOPA and 5-HIAA were decreased (Fig. 6D and F, respectively).

The enhancement in DA levels in our study is supported by the findings of McPartland and Russo [43]. We believe that waking induced by CBD may be associated with the increase in the release of DA since it is known that lesions of DA cell groups induce a reduction in arousal in rats [44] as well as in Parkinson's disease patients [45,46]. The regulation of the sleep–wake cycle in mammals' includes neuromodulators such as DA; for instance, administration of DA receptor agonists [47] or DA uptake inhibitors [48] induces an increase in waking. The role of DA on alertness has been also tested in invertebrates including *Drosophila* [49]. On the other hand, the relative changes in the concentrations of DA have been demonstrated by Lena et al. [50]. Using the microdialysis technique, the authors reported that extracellular levels of DA were elevated during waking [50]. Finally, the electrophysiological activity of DA neurons is higher during alertness as suggested by Lu et al. [51]. The evidence mentioned above indicates that indeed DA plays an active role in the promotion of waking [52]. In our study, we found that CBD increased W and enhanced the extracellular levels of DA collected from AcbC, finding which is supported by McPartland and Russo [43]. A possible mechanism of this effect remains to be elicited. However, we cannot rule out some hypothesis, including that CBD might be acting at different receptors. For instance, it has been suggested that CBD may bind to the vanilloid receptor [24] or by modulating Ca^{2+} influx. Recently, Drysdale et al. reported that CBD elevated intracellular Ca^{2+} concentrations in hippocampal cultures [53]. Thus, the present results provide scope for speculation on the role of CBD on the mechanisms of release of DA.

While AcbC is a good target for DA sampling it may not be a good option for 5-HT sampling. However data obtained from microdialysis study suggests that the increase in NA, epinephrine and 5-HT levels could be an additional distant target for CBD effects.

While Δ^9-THC and CBD cause some similar actions (albeit possibly based on different mechanisms) such as anti-inflammatory [54] and antiemetic effects [55], here we show a dichotomy in their effects on sleep patterns. Δ^9-THC increases sleep [15–17], whereas CBD induces an opposite effect. Nicholson et al. have reported that CBD increases waking in humans [20].

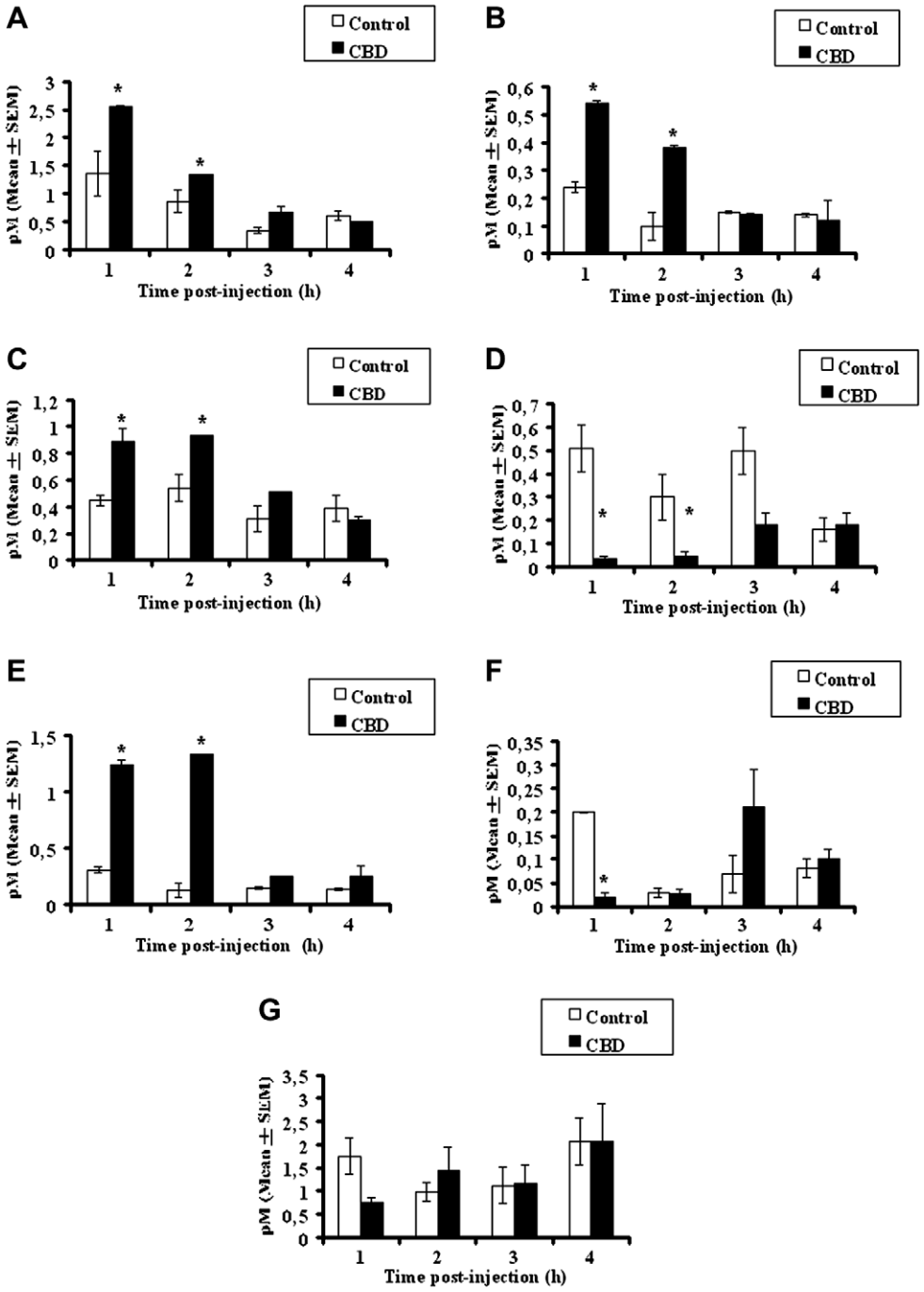

Fig. 6. Extracellular levels of catecholamines measured in the AcbC during a 4-h period after the administration of either vehicle or cannabidiol (CBD, 10 μg/5 μl icv). Noradrenaline (A), epinephrine (B), dopamine (C), L-DOPA (D), 5-HT (E), 5-HIAA (F) and HVA (G). The treatments were administered at the beginning of the lights-on period and right after this, samples were collected. Each point represents the means ± S.E.M. of pM (* vs control, $P < 0.05$).

Little is known about the molecular mechanism of CBD actions. Contrary to Δ^9-THC, it has very little affinity for the known cannabinoid receptors, CB_1 and CB_2 [24]. We assume that the effects of CBD found in our study might be through action on a receptor not yet described. An alternative explanation involves the fatty acid amide hydrolase (FAAH) (the intracellular enzyme that catalyzes the hydrolysis of endogenous cannabinoids ligand, ANA [56] and the anorexic lipid oleoylethanolamide (OEA) [57]). In the last experiment of our study we hypothesized an enhanced effect of ANA on sleep following CBD injection. Surprisingly, CBD increased waking even in presence of ANA. We do believe that CBD might be inhibiting FAAH; this could lead an increase in endogenous ANA levels in the brain. However, the use of the URB597 (a FAAH inhibitor) enhances OEA levels in the brain in higher rates than ANA as reported by Fegley et al. [58]. In our lab, we have tested the physiological properties of URB597 as well as OEA on sleep–wake cycle and have found a significant in-

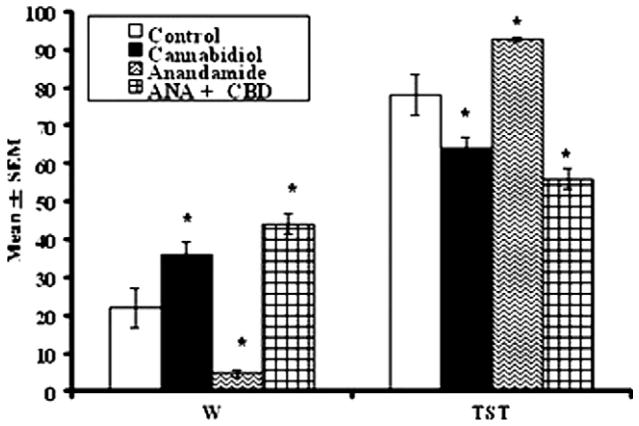

Fig. 7. Effects of icv administrations of either vehicle, cannabidiol (CBD, 10 μg/5 μl), anandamide (ANA; 10 μg/5 μl), ANA (10 μg/2.5 μl) and 15 min later CBD (10 μg/2.5 μl; ANA + CBD) on the total time of W and total sleep time (TST). The treatments were administered at the beginning of the lights-on period and right after this, sleep was recorded during 4 h. Each point represents the means ± S.E.M. of total time of recording (%; ∗ vs control, $P < 0.05$).

crease in waking. The mechanism of this observation is likewise unknown. It is highly possible that CBD inhibits FAAH activity and this leads to an increase in the levels of OEA, which facilitates waking.

Our study suggests that CBD induces alertness via activation of neurons in the hypothalamus and DRD. Additionally, levels of DA, NA, epinephrine as well as 5-HT were enhanced after CBD injections. These effects can be induced by a novel cellular pathway involved in the alertness caused by CBD. It seems that the enzymatic process involved in the formation of catecholamines might be under the influence of CBD. Likely the component enzymes in the pathway could be inhibited or stimulated by CBD. For example, we found that CBD increased DA but decreased levels of L-DOPA, meaning that the activity of tyrosine hydroxylase could be under inhibition whereas DOPA decarboxylase may be stimulated. Complementary experiments testing the role of CBD on the biosynthesis of catecholamines would provide us a better understanding of the phenomena.

CBD has been tested lately as a therapeutical agent [14,59–64]. The present results indicate an additional potential therapeutic benefit of this compound such as excessive somnolence. Early studies have indicated that sleep may be modulated by cannabinoids [15–17] or cannabis extracts. In most experiments with extracts however the levels of CBD were not measured. Here we describe the waking properties of CBD. It might be considered to treat sleep disorders such as excessive somnolence. This common sleep disturbance is defined as trouble falling asleep or staying asleep. It can cause sleepiness or fatigue during the day, may affect mood and result in trouble focusing on tasks. According to National Sleep Foundation results from the 2005 *Sleep in America* poll indicate that 60% of America's adults who drive or have a license report that, within the past year, they have driven a car or motor vehicle when feeling drowsy. We now could consider the use of CBD, the non-psychoactive constituent of *C. sativa*, to treat sonmolence.

In summary, administration of CBD to rats produced waking in association with activation of hypothalamic and DRD, two brain regions implicated in the control of alertness. Extracellular levels of NA, epinephrine, DA, and 5-HT were enhanced after CBD injections. The wake-inducing properties of CBD were not blocked by the sleep-inducing endocannabinoid ANA. Activation of specific arousal regions may underlie the W produced by CBD in people with somnolence. Future studies will be needed to address the question of whether vanilloid receptors, Ca^{2+} influx or the interactions between CBD and CB_1-signalling pathways contribute to the alertness and DA release actions of CBD.

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