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The role of endogenous cannabinoids in the hypothalamo-pituitary-adrenal axis regulation: in vivo and in vitro studies in CB1 receptor knockout mice

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Abstract

Exogenous cannabinoids affect multiple hormonal systems including the hypothalamo-pituitary-adrenocortical (HPA) axis. These data suggest that endogenous cannabinoids are also involved in the HPA control; however, the mechanisms underlying this control are poorly understood. We assessed the role of endogenous cannabinoids in the regulation of the HPA-axis by studying CB1 receptor knockout (KO) and wild type (WT) mice. Basal and novelty stress-induced plasma levels of adrenocorticotropin (ACTH) and corticosterone were higher in CB1-KO than in WT mice. We investigated the involvement of the pituitary in the hormonal effects of CB1 gene disruption by studying the in vitro release of ACTH from anterior pituitary fragments using a perifusion system. Both the basal and corticotropin releasing hormone (CRH)-induced ACTH secretion were similar in CB1-KO and WT mice. The synthetic glucocorticoid, dexamethasone suppressed the CRH-induced ACTH secretion in both genotypes; thus, the negative feedback of ACTH secretion was not affected by CB1 gene disruption. The cannabinoid agonist, WIN 55,212–2 had no effects on basal and CRH-stimulated ACTH secretion by anterior pituitary slices. In our hands, the disruption of the CB1 gene lead to HPA axis hyperactivity, but the pituitary seems not to be involved in this effect. Our data are consistent with the assumption that endogenous cannabinoids inhibit the HPA-axis via centrally located CB1 receptors, however the understanding of the exact underlying mechanism needs further investigation.

Keywords: CB1; Cannabinoid; CB1-KO; WIN 55,212–2; ACTH; Corticosterone; HPA-axis; Stress; Pituitary; Perifusion

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Introduction

Various preparations of *Cannabis sativa* e.g. marijuana and hashis were used for medicinal and recreational purposes for at least 4,000 years. The cloning of the cannabinoid receptors CB1: (Matsuda et al., 1990) CB2: (Munro et al., 1993) and the identification of endogenous cannabinoid ligands (anandamide: (Devane et al., 1992); 2-arachidonoylglycerol: (Mechoulam et al., 1995)) prompted a large interest in the functions of the endogenous cannabinoid system.

The CB1 cannabinoid receptor is expressed in various regions of the brain (Herkenham et al., 1991) and was shown to play important roles in locomotion, pain perception, memory, feeding, anxiety, etc. (Porter and Felder, 2001). In addition to their neural and behavioral effects, exogenous cannabinoids affect the production of various hormones, including the gonadal steroids, growth hormone, prolactin, thyroid hormones and glucocorticoids (Brown and Dobs, 2002). There are a series of studies suggesting that endogenous cannabinoids also play a role in the control of the hypothalamo-pituitary-adrenal axis (HPA).

The CB1 receptor is expressed in the hypothalamus and pituitary of rats (Herkenham et al., 1991) and CB1 receptors were found on the anterior lobe of the human pituitary gland (Pagotto et al., 2001). In Cushing's adenomas, WIN 55,212–2 (known as CB1 agonist) applied simultaneously with CRH resulted in a synergistic effect on ACTH production (Pagotto et al., 2001). In rat anterior pituitary cultures, the cannabinoid agonist THC elevated the levels of cAMP, which is an important second messenger in the CRH-induced ACTH release (Rodriguez de Fonseca et al., 1999). The CB1 cannabinoid receptor antagonist, AM251 blocked the negative feed-back of glucocorticoids on hypothalamic CRH secretion (Di et al., 2003). Taken together, these data suggest an important role of the endogenous cannabinoid system in the control of the HPA-axis. However, the mechanisms underlying such effects are poorly known at present. E.g. intracerebroventricular (i.c.v.) application of the cannabinoid agonist anandamide stimulated the release of ACTH in rats (Weidenfeld et al., 1994). The ACTH secretory effect of anandamide was described even in that CB1 receptor knockout (KO) and wild type (WT) mice we used in the present study, suggesting the existence of a further and unknown cannabinoid receptor in the hypothalamus (Wenger et al., 2003). In contrast, CB1 receptors were shown to promote the negative feedback of glucocorticoids on the HPA-axis (Di et al., 2003). In addition, it is not known whether cannabinoids affect the HPA axis centrally or at the level of the pituitary. Based on the available literature, one can hypothesize that CB1 receptors located in both the hypothalamus and pituitary play a role in the control of the HPA-axis, but evidence is lacking.

In the present study we aimed to further investigate the role of endogenous cannabinoids in the regulation of the HPA-axis. For this purpose we compared the hormonal stress reaction of the CB1-KO mice to that of its WT control in the novelty stress paradigm, and also some in vitro ACTH secretory qualities of the adenohypophyses of the two genotypes.

Materials and methods

Animals

The parents of the CB1-KO and WT mice used in this study were produced in IRIBHN, Université libre de Bruxelles, and were characterized earlier (Ledent et al., 1999). The process of production involved

heterozygote breeding for 14 generations on a CD1 (Charles River, France) outbred background, with selection for the mutant CB1 gene at each generation. The experimental CB1-KO and wild type mice were male offspring of these genotyped mice, were two-three months old, and weighed 30–35 g. Food and water were available ad libitum. Temperature and humidity were 23 ± 2 °C and 60 ± 10%, respectively. To avoid any stressful stimuli subjects were kept in individual semitransparent plastic cages (36 × 20 × 10 cm) at least 2 weeks prior to experimentation. Experiments were conducted in the light phase between 10.00 and 13.00 h (lights on: 06:00–18:00 hours). Experiments were approved by the Animal Welfare Committee of the Institute.

Novelty stress

Mice housed individually were placed into an empty plastic cage (36 × 40 × 20 cm, without bedding material) for 10 min. Before placing the subjects into the novel environment cages were cleaned with 1% acetic acid and dried to eliminate olfactory cues. During the stress session, cages remained uncovered, but animals had no visual contact with each other or the experimenter. Control animals were left in their individual home-cage until blood sampling. After 10 min, control and stressed mice (n = 14–15) were quickly decapitated in a random order, and trunk blood was collected on ice-cold tubes containing 20 μl 20% K_2-EDTA anticoagulant. Blood was centrifuged and plasma samples were stored at −20 °C until assayed.

The assessment of pituitary ACTH secretion

Pituitary ACTH secretion was assessed by a slightly modified version of the method of Ács et al. (Acs et al., 1987). Mice were decapitated between 08.45 and 09.30 h. The pituitary was sampled and the posterior lobe was discarded. The anterior pituitary was immediately transferred into Dulbecco's modified Eagle's medium (Sigma-Aldrich, Budapest, Hungary) containing 2.5 g bovine serum albumin/l (Fraction V, Calbiochem, La Jolla) (DMEM). The anterior pituitary was subsequently minced with a razor blade into approximately 0.5 mm or smaller segments. The segments were transferred on preswollen Sephadex G-10 slurry (Pharmacia Fine Chemicals, Uppsala, Sweden). The columns were connected to multichannel peristaltic pump (GILSON, France) using 16 mm internal diameter plastic tubing. Column effluent was collected every 5 min on ice using a Foxy 200 fraction collector (ISCO. Inc, Nebrasca, USA) modified to collect 8 channels simultaneously. Transit time from the medium reservoir to the fraction collector was 150 s. Media, columns and tubing were maintained at 37 °C. Column effluent was stored at −20 °C until assayed. Eight columns were used in parallel.

1. *Preparatory perifusion*. The column volume was adjusted to 0.4 ml and the segments were perifused with DMEM for 1 h at approximately 100 μl/min flow rate. During the next hour, segments were perifused at 200 μl/min to allow the basal ACTH release to stabilize.
2. *Basal secretion of ACTH*. After the second hour of the preparatory perifusion ACTH release was measured under basal conditions for 15 min. Anterior pituitary segments from WT and CB1-KO mice were assessed in parallel, with a random assignment to columns (n = 19–26).
3. *CRH-induced increases in ACTH secretion*. After measuring basal secretion, the anterior pituitary segments were stimulated with 0.05 nM CRH for 5 min (n = 19–26).

4. *The response of ACTH secretion to the synthetic glucocorticoid dexamethasone*. 35 min after the first CRH stimulus (see above), half of the anterior pituitary segments were perifused with DMEM containing 30 nM dexamethasone for 30 min. The other half of the segments was perifused with a medium containing the vehicle of dexamethasone in similar concentration. For the last 5 min, 0.05 nM CRH was added to the medium in both cases (1 h after the first stimulus; n = 5–7).
5. *The response of ACTH secretion to the cannabinoid agonist WIN 55,212–2*. This study was performed in a different set of anterior pituitary segments that underwent the phases described at items 1–3. 35 min after the first CRH stimulus (see item 3), half of the anterior pituitary segments were perifused with DMEM containing 1 μM WIN 55,212–2 for 30 min. The other half of the segments was perifused with a medium containing the vehicle of WIN 55,212–2 in similar concentration. For the last 5 min, 0.05 nM CRH was added to the medium in both cases (n = 4–6).
6. *Checking the time consistency of ACTH secretion and the late dexamethasone effect*. Anterior pituitary segments were stimulated additional times with 0.05 nM CRH. The treatments were applied 1 h apart (n = 45).

The schedule of perifusion and treatments is shown in Table 1.

Drugs and doses

CRH and dexamethasone (Sigma-Aldrich, Budapest, Hungary) were dissolved in 0.01 N HCl and 98% ethanol, respectively (10 mM). WIN 55,212–2 (Tocris, UK) was dissolved in a few drops of dimethyl sulfoxide. All drugs were diluted in DMEM to the final concentration.

The dose of CRH applied here increased ACTH secretion significantly and reliably in earlier experiments (Antoni and Dayanithi, 1990). The synthetic glucocorticoid, dexamethasone in 10–100 nM dose suppressed CRH-induced ACTH secretion by 25–30% in earlier experiments (Widmaier and Dallman, 1984). 1 μM WIN 55,212–2 was shown earlier to have significant effects on CRH-, VIP- or TRH-induced hormone release (Ho et al., 2000; Pagotto et al., 2001).

Table 1
The schedule of the in vitro assessment of ACTH secretion in anterior pituitaries

Period	Treatment	Duration (min)
Preparatory perifusion 1	DMEM*	60
Preparatory perifusion 2	DMEM	60
Basal ACTH release	DMEM	15
CRH1	DMEM + CRH	5
Recovery	DMEM	35
Pre-treatment	DMEM + WIN, DEX or vehicle	25 + 5 (overlap with CRH2)
CRH2	DMEM + CRH	5
Recovery	DMEM	55
CRH3	DMEM + CRH	5
Recovery	DMEM	55

DMEM = Dulbecco's modified Eagle's medium with 2.5 g/l bovine serum albumine; CRH = 0.05 nM corticotropin releasing hormone; WIN = 1 μM WIN 55,212–2; DEX = 30 nM dexamethasone. * flow rate: 100 μl/min (flow rate was increased to 200 μl/min for the rest of the experiment); WT and CB1-KO mice were assessed in parallel and were randomly assigned to columns.

Hormone measurements

ACTH and corticosterone were assessed by specific and direct radioimmunoassays as described previously (Zelena et al., 2003). In brief, ACTH and corticosterone antibodies were raised in rabbit in our laboratory. Tracers were iodinated by the chloramine-T method and 50 μl or 10 μl plasma aliquots were assayed in the ACTH or corticosterone RIA, respectively. In the perifusion studies 25 μl aliquots of medium were assayed after appropriate dilution by RIA buffer.

Data analysis and statistics

ACTH and corticosterone plasma levels were shown as mean ± S.E.M. and were analyzed by two-factor ANOVA (Factor 1 = genotype, Factor 2 = stress exposure). Before analysis, data underwent logarithmic transformation to ensure the homogeneity of variances.

ACTH secretion was characterized as described earlier (Antoni and Dayanithi, 1990; Dayanithi and Antoni, 1989). In brief, basal ACTH secretion was expressed as the average ACTH concentration of the three effluent fractions obtained previous to treatments (each fraction was collected over 5 min). CRH-induced ACTH secretion was expressed as percent change compared with basal values. Basal release was measured before each CRH stimulus, and was used as comparison for the respective CRH-induced increase, to compare the decline in basal level. 0.05 nM CRH increased the ACTH secretion in three consecutive fraction, therefore the net CRH-induced ACTH release/15 min was calculated according to the following formula: (X + Y + Z) / 3(B), where X, Y and Z are the ACTH concentrations of effluent fractions 1, 2 and 3 (respectively) obtained after the CRH stimulus, whereas B is the basal release. There was substantial variation in the net release of ACTH induced by various stimuli between columns in different experiments, which made it necessary to standardize the data. Net ACTH release is expressed as percentage of the net ACTH release elicited by 0.05 nM CRH, which was applied to all columns (Antoni and Dayanithi, 1990). Basal and CRH-induced ACTH secretion was assessed by one-factor ANOVA (CB1-KO vs. WT). The effects of dexamethasone and WIN 55212–2 on basal and CRH-induced secretion were assessed by two-factor ANOVA (Factor 1 = genotype, Factor 2 = treatment). The time consistency of ACTH secretion was assessed by repeated measure ANOVA (CB1-KO vs. WT).

Results

Plasma hormone concentrations

ACTH and corticosterone levels were higher in CB1-KO than in WT mice under both resting and stressful conditions (n = 14–15, Fig. 1). Both genotype and stress exposure affected significantly the plasma levels of ACTH ($F_{stress}(1,54) = 27.38$, $p = 0.0003$, $F_{genotype}(1,54) = 15.17$, $p = 0.0003$) and corticosterone ($F_{stress}(1,54) = 215.77$, $p = 0.0000$; $F_{genotype}(1,54) = 5.78$, $p = 0.02$). Significant interaction between stress and genotype was not estimated (ACTH: $F_{interaction}(1,54) = 0.02$, $p = 0.88$; corticosterone: $F_{interaction}$ $(1,54) = 1.32$, $p = 0.26$).

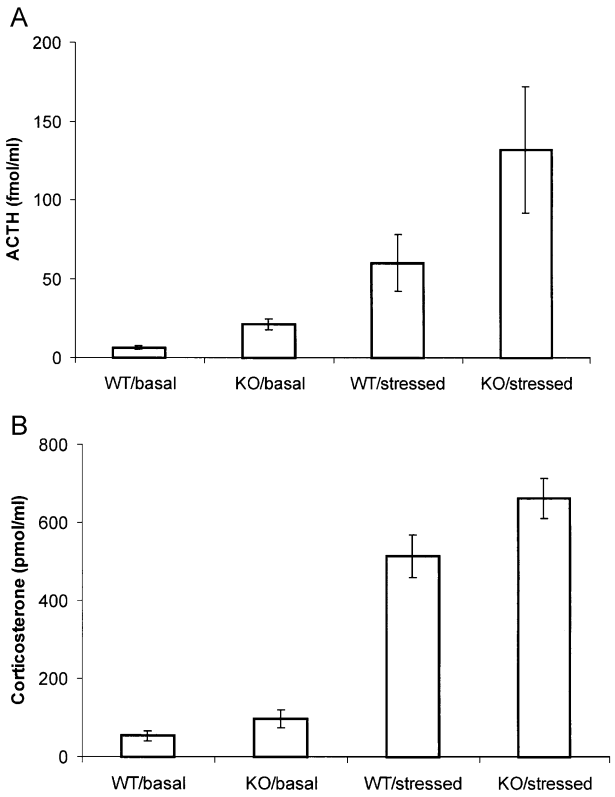

Fig. 1. Plasma ACTH (fmol/ml; A) and corticosterone (pmol/ml; B) levels in wild-type (WT) and CB1 receptor knockout (KO) mice in resting (basal) and novelty stress (stressed) conditions (n = 14–15). Novelty stress was applied for 10 min and animals were immediately sacrificed. Two way ANOVA yielded significant effects of both genotype and stress but not significant interaction of these factors. (ACTH: F*stress*(1,54) = 27.38, p = 0.0003, F*genotype*(1,54) = 15.17, p = 0.0003, F*interaction*(1,54) = 0.02, p = 0.88; Corticosterone: F*stress*(1,54) = 215.77, p = 0.0000; F*genotype*(1,54) = 5.78, p = 0.02, F*interaction* (1,54) = 1.32, p = 0.26).

The consistency of basal and stimulated ACTH secretion

In vehicle treated controls (i.e. in the mice treated with the vehicles of dexamethasone and WIN 55,212–2 before the second CRH pulse) the time affected basal ACTH secretion as measured before each CRH pulses (Fig. 2; F(2,78) = 72.35; p = 0.000), therefore we used the ACTH secretion before each stimulus as the basal value for that pulse. Neither significant effect of genotype (F(1,39) = 0.0017; p = 0.967), nor significant interaction between genotype and treatment (F(2,78) = 0.288; p = 0.75) was found. No significant effect was seen on consecutive CRH-stimulated net ACTH releases in either genotype (F*genotype*(1,17) = 0.002; p = 0.966; F*time*(2,34) = 1.55; p = 0.23; F*interaction*(2,34) = 0.64; p = 0.535).

Basal ACTH release of anterior pituitary lobes

The basal ACTH release of untreated anterior pituitary lobes was 184.3 ± 13 and 183.5 ± 12 fmol/ml in WT and CB1-KO mice (n = 19–26), respectively. The difference was statistically not significant (F(1,43) = 0.0021; p = 0.96).

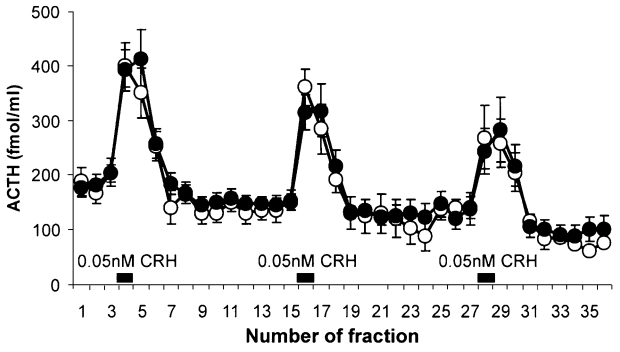

Fig. 2. ACTH release (fmol/ml) from wild type (open circle) and CB1 receptor knockout (filled circle) mice (n = 9–13). Fractions were collected in every 5 min for 3 h and 0.05 nM CRH was applied for each column for 5 min in every hour altogether 3 times (filled rectangle).

CRH-stimulated ACTH release

The CRH-induced increase in ACTH secretion was similar in the two genotypes. ACTH release increased after CRH stimulation by 190.9 ± 8% and 192.9 ± 5% compared with basal levels in WT and CB1-KO mice, respectively (n = 19–26; F*genotype*(1,43) = 0.049; p = 0.83; the analysis was done on the first, untreated CRH-induced net ACTH release).

The effects of dexamethasone on ACTH secretion

The synthetic glucocorticoid dexamethasone (as compared with vehicle treatment) suppressed the effect of CRH on ACTH secretion (during its application (2.CRH): F(1,20) = 4.13, p = 0.055); 1 h after its administration (3.CRH): F(1,16) = 23.9, p = 0.0001), but this effect did not depend on genotype

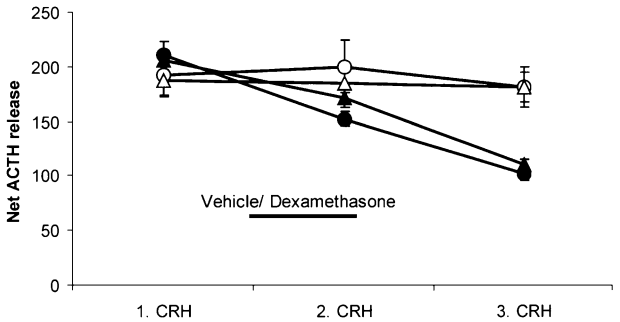

Fig. 3. Effect of dexamethasone on the CRH stimulated net ACTH release of wild-type and CB1 receptor knockout anterior pituitary gland in percent of the first CRH stimulus (n = 5–7). On each column 0.05 nM CRH was applied once in every hour for 5 min, altogether 3 times. Dexamethason was applied to the column from 25 min before the second stimulus to the end of the second stimulus. Dexamethason significantly inhibited the CRH-induced ACTH elevation already at the end of the 30 min treatment (F(1,20) = 4.13, p = 0.055). This effect lasted at least 1 hours after finishing the treatment (F(1,16) = 23.9, p = 0.0001). There was no significant difference between wild-type and CB1 receptor knockout animals (F(1,20) = 0.017, p = 0.9). DMEM = solvent, DEX = dexamethasone, open circle = pituitary of wild type mice + solvent, filled circle = pituitary of wild type mice + DEX, open triangle = pituitary of CB1 receptor knockout mice + solvent, filled triangle = pituitary of CB1 receptor knockout mice + DEX.

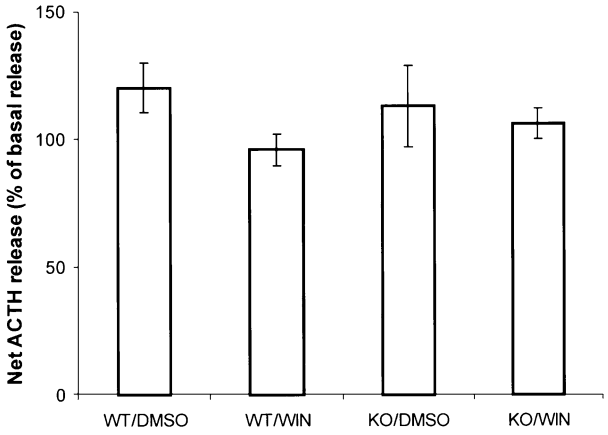

Fig. 4. Effect of WIN 55,212–2 on the basal net ACTH release of wild-type and CB1 receptor knockout anterior pituitary glands (n = 4–6). No significant change could be detected (F*genotype*(1,15) = 0.01, p = 0.9; F*treatment*(1,15) = 0.19, p = 0.7; F*interaction*(1,15) = 1.3, p = 0.27). WT = pituitary of wild type mice, KO = pituitary of CB1 receptor knockout mice, WIN = WIN 55,212–2 CB1 receptor agonist, DMSO = Dimethyl Sulfoxide solvent.

(2.CRH: F(1,20) = 0.017, p = 0.9; 3.CRH: F(1,16) = 0.07, p = 0.79) and there was no interaction either (2.CRH: F(1,20) = 1.28, p = 0.27; 3.CRH: F(1,16) = 0.08, p = 0.77; Fig. 3; n = 5–7).

The effects of WIN 55,212–2

The CB1 receptor antagonist WIN 55,212–2 failed to affect unstimulated ACTH release in WT and CB1-KO mice (Fig. 4). (F*genotype*(1,15) = 0.01, p = 0.9; F*treatment*(1,15) = 0.19, p = 0.7; F*interaction*(1,15) = 1.3, p = 0.27).

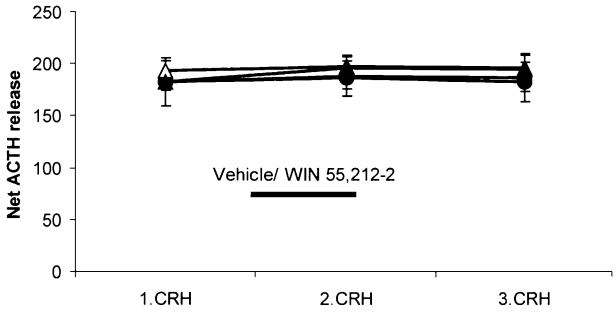

Fig. 5. Effect of WIN 55,212–2 on the CRH stimulated net ACTH release of wild-type and CB1 receptor knockout anterior pituitary glands as percent of response to first CRH stimulus (n = 4–6). On each column 0.05 nM CRH was applied once in every hour for 5 min, altogether 3 times. WIN 55,212–2 was applied to the column from 25 min before the second stimulus till the end of the second stimulus. Neither the WIN 55,212–2 treatment nor the genotype has significant effect at any timepoint studied (F*genotype*(1,17) = 0.57, p = 0.46; F*treatment*(1,17) = 0.023, p = 0.88; F*interaction*(1,17) = 0.000, p = 0.99). DMSO = Dimethyl Sulfoxide solvent, WIN = WIN 55,212–2, open circle = pituitary of wild type mice + solvent, filled circle = pituitary of wild type mice + WIN, open triangle = pituitary of CB1 receptor knockout mice + solvent, filled triangle = pituitary of CB1 receptor knockout mice + WIN.

WIN 55,212–2 had no effect on CRH-stimulated ACTH release (n = 4–6; Fig. 5) (second CRH stimulus, F*genotype*(1,17) = 0.57, p = 0.46; F*treatment*(1,17) = 0.023, p = 0.88; F*interaction*(1,17) = 0.000, p = 0.99).

Discussion

As compared with WTs, CB1-KO mice showed higher plasma levels of ACTH and corticosterone under both basal and stressful conditions. However, the in vitro ACTH release by the anterior pituitary of WT and CB1-KO mice was similar under both basal conditions and after CRH stimulation. In the further in vitro experiments the synthetic glucocorticoid dexamethasone suppressed the CRH-induced ACTH secretion in both genotypes; thus, the negative feedback of ACTH secretion was not affected by CB1 gene disruption. The cannabinoid agonist WIN 55,212–2 did not affect the in vitro ACTH secretion in either genotype, suggesting that pituitary CB1 receptors are probably not involved in the regulation of the HPA-axis in mice.

Early studies showed that peripherally administered THC stimulates ACTH secretion (Puder et al., 1982). Later studies showed that the cannabinoid receptors are expressed at the level of both the hypothalamus and pituitary (Herkenham et al., 1991), suggesting that the effects of cannabinoids on the HPA-axis are direct (i.e. mediated by their receptors), and not necessarily related to their sedative or cognitive effects.

The central mediation of cannabinoid effects on HPA-axis is supported by several studies. E.g., i.c.v. administered anandamide stimulated the release of ACTH in rats (Weidenfeld et al., 1994). In the CB1-KO mice anandamide could stimulate the in vivo ACTH secretion less effectively than in the WT control, suggesting the involvement of CB1 receptors in the HPA axis regulation (Wenger et al., 2003). However the authors discuss the observed ACTH elevation in CB1 KO mice as a sign of the presence of unknown cannabinoid receptor. Moreover, WIN 55,212–2, known as CB1 agonist, effectively attenuated the glutamate release from hippocampal synaptosomes of the CB1-KO animal (Kofalvi et al., 2003), indicating a CB1 receptor-independent function of this drug. The known CB1 and the unidentified CBx receptors apparently share common activities in the brain, as e.g. regulating the hypophyseotroph CRH-erg cells.

Somewhat contradictory findings were obtained by (Di et al., 2003), who showed that the cannabinoid antagonists AM251 and AM281 blocked the negative feedback of glucocorticoids on CRH release in hypothalamic slice preparations. In the same preparations, the cannabinoid agonist WIN 55,212–2 mimicked the CRH suppressing effects of glucocorticoids. The discrepancy between the effects of i.c.v. and those of hypothalamically administered cannabinoids is unknown at present, but both studies suggest that centrally located cannabinoid receptors are involved in the control of the HPA axis, and glucocorticoids may be involved in the process. One can hypothesize that endocannabinoid signalling in different brain structures plays different roles in the control of HPA axis.

In our experiment, CB1-KO mice showed significantly elevated basal and stress-induced levels of plasma ACTH and corticosterone. In an other laboratory and in a differently designed experiment, no difference was observed in the basal hormonal levels of the two genotypes, however one can see a more or less consistent higher ACTH concentration in the KO genotype vs. WT after vehicle administration (Wenger et al., 2003). Mice in our study were housed individually to avoid inter-male aggression and sacrificing was very quickly performed. So, in our stress experiment, CB1-KO mice showed

significantly elevated plasma ACTH and corticosterone levels, which may be explained by the findings of Di et al. (Di et al., 2003), who supposed that CB1 receptors promote the negative feedback of glucocorticoids on the HPA-axis.

Substantial amount of CB1 receptors was found in the anterior pituitary of humans, and some receptors were located on the corticotroph cells (Pagotto et al., 2001). This finding raises the possibility that cannabinoids affect the function of the HPA-axis by a direct action on the pituitary corticotroph cells. In our study, however, in vitro ACTH secretion by anterior pituitaries was affected neither by CB1 gene disruption nor by the cannabinoid agonist WIN 55,212–2 in a pharmacologically appropriate dose. In in vitro experiments with mouse tissue or isolated organs 1.0 μM WIN 55,212–2 were usually sufficient to exert its pharmacological effects (Nicholson et al., 2003; Pertwee et al., 1995; Burkey et al., 1997), however some authors used different doses (Kofalvi et al., 2003). Especially in the WT control of our study the effective dose of WIN 55,212–2 was 1.0 μM on the CB1 receptor mediated electric activity of the hippocampal principal cells (Hajos et al., 2000).

In addition, in our in vitro experiments the stimulatory and suppressive effects of CRH and dexamethasone remained intact in CB1-KO mice. These data suggest that the pituitary in mice does not mediate the effects of cannabinoids on HPA-axis function. The lack of effect may be explained by species differences. Noteworthy, no data are available on the CB1 expression of corticotrophs in mice. Alternatively, the effects of cannabinoids on corticotroph cells are minimal, and the CB1 receptors of these cells are unable to substantially affect ACTH secretion. On the other hand, the lack of the effect of WIN 55,212–2 on the ACTH secretion we measured may also indicate that the regulation of the secretory activity of corticotrophs, at least in mice, does not involves that unknown CBx cannabinoid receptors that was hypothesized by a number of investigators (Wenger et al., 2003; Breivogel et al., 2001). One can hypothesize that the main role of pituitary CB1 receptors is to control other hormonal systems. E.g., it was shown that cannabinoids have marked effects on GH and prolactin release (Pagotto et al., 2001; Rodriguez de Fonseca et al., 1999).

Taken together, our data seem to support the in vitro finding that CB1 cannabinoid receptors are involved in the glucocorticoid feedback inhibition at the level of the paraventricular nucleus (Di et al., 2003), however in vivo this regulatory effect of CB1 receptors may have only partial importance. Especially in the CB1-KO mice, the compensatory role of the uncharacterized cannabinoid receptors may further complicate the scenario. Additional investigations are needed to clarify the exact underlying mechanisms.

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