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Highlights

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Monoacylglycerol lipase inhibition-induced changes in plasma corticosterone levels, anxiety and locomotor activity in male CD1 mice

Mano Aliczki, Dora Zelena, Eva Mikics, Zoltan K. Varga, Otto Pinter, Nikoletta Venczkone Bakos, Janos Varga, Jozsef Haller *

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- We assessed the effects of MAGL inhibition on corticosterone and behavior in mice.
- MAGL blockade increased basal but not stress-induced corticosterone levels.
- MAGL inhibition had locomotoion enhancing and anxiolytic effects.
- Anxiolysis was partly dependent on corticosterone effects.

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Supplementary data

Q2 Supplementary Fig. 1

Q3 Supplementary Fig. 2

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ABSTRACT

The hypothalamus–pituitary–adrenal-axis is strongly controlled by the endocannabinoid system. The specific impact of enhanced 2-arachidonoylglycerol signaling on corticosterone plasma levels, however, was not investigated so far. Here we studied the effects of the recently developed monoacylglycerol lipase inhibitor JZL184 on basal and stress-induced corticosterone levels in male CD1 mice, and found that this compound dramatically increased basal levels without affecting stress responses. Since acute changes in corticosterone levels can affect behavior, JZL184 was administered concurrently with the corticosterone synthesis inhibitor metyrapone, to investigate whether the previously shown behavioral effects of JZL184 are dependent on corticosterone. We found that in the elevated plus-maze, the effects of JZL184 on "classical" anxiety-related measures were abolished by corticosterone synthesis blockade. By contrast, effects on the "ethological" measures of anxiety (i.e. risk assessment) were not affected by metyrapone. In the open-field, the locomotion-enhancing effects of the compound were not changed either. These findings show that monoacylglycerol lipase inhibition dramatically increases basal levels of corticosterone. This endocrine effect partly affects the anxiolytic, but not the locomotion-enhancing effects of monoacylglycerol lipase blockade.

Introduction

There is an increasing amount of information suggesting that the activity of the hypothalamus–pituitary–adrenal axis (HPA-axis)—a crucial element in maintaining homeostasis under stress—is partly regulated by the endocannabinoid system. In laboratory models, cannabinoids seem to alter HPA-axis activity in a bidirectional manner. It was consistently shown, that basal levels of corticosterone are increased by treatments with phytocannabinoids (e.g. Δ^9-tetrahydrocannabinol, cannabidiol or cannabinol), endocannabinoids (e.g. anandamide (AEA)) and synthetic cannabinoids (e.g. WIN55,212-2, HU210 or CP55,940) (Barna et al., 2009; Johnson et al., 1978; Martin-Calderon et al., 1998; Romero et al., 2002; Weidenfeld et al., 1994; Zuardi et al., 1984). Disparate data suggest that enhancement of endocannabinoid activity *via* the blockade of AEA degrading enzyme fatty acid amide hydrolase (FAAH) by the selective inhibitor URB597 also result in elevated basal corticosterone levels (Saber-Tehrani et al., 2010), however, these findings were not replicated (Hill et al., 2010; Kerr et al., 2012) and the effect of increased AEA levels on corticosterone was shown not to be mediated by signaling *via* the CB_1 cannabinoid receptor (CB_1R) (Wenger et al., 2003). In contrast with the effects of cannabinoids on basal HPA-function, increased endocannabinoid activity *via* treatment with CB_1R agonists or inhibition of FAAH activity seem to dampen the activation of the HPA-axis in acute stress (Ganon-Elazar and Akirav, 2009; Hill et al., 2009, 2010; Patel et al., 2004). While there is a large amount of information available on the effects of CB_1R agonists and FAAH blockade on corticosterone levels under basal or stressful conditions, similar effects resulting from the blockade of monoacylglycerol lipase (MAGL), the enzyme hydrolyzing 2-arachidonoylglycerol (2-AG), the other main endocannabinoid, are still to be studied. Recently, behavioral effects of MAGL inhibition were reported to depend on the stressfulness of the testing environment (Aliczki et al., 2012; Sciolino et al., 2011), which can suggest that MAGL blockade can alter HPA-axis function.

Endocannabinoids affect both brain areas involved in emotional behavior (e.g. the prefrontal cortex, amygdala and hippocampus; (Rubino et al., 2008a; Zarrindast et al., 2008) and the HPA-axis (at all levels, the hypothalamus, hypophysis, and adrenal cortex; (Cota et al., 2007; Di et al., 2003, 2005; Pagotto et al., 2001). It is likely that the ultimate effects of endocannabinoid action result from an interaction between the neural and endocrine effects, as glucocorticoids are also powerful modulators of behavior (Mikics et al., 2004).

In the present study, we assessed the effects of JZL184-induced MAGL blockade on basal and stress-induced activity of the HPA-axis by the measurements of corticosterone levels. The findings showed that JZL184 treatment increases basal levels of plasma corticosterone,

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therefore we studied whether the behavioral effects of MAGL inhibition that we reported earlier (Aliczki et al., 2012) depended on corticosterone-synthesis. To study this issue, we inhibited corticosterone-synthesis with the steroid 11β-hydroxylase inhibitor metyrapone.

Material and methods

Subjects

Subjects were two month-old male CD1 (Charles River laboratories, Budapest, Hungary) mice weighting 30–35 g. They were kept under a light/dark cycle of 12 h with the lights on at 0700 h. Food and water were available *ad libitum*, temperature and humidity were kept at 23 ± 2 °C and 60 ± 10%, respectively. In contrast to rats that are highly social, individual housing is not stressful in the mouse, which is a solitary species (Arndt et al., 2009; Benton and Brain, 1981; Capanna et al., 1984). Moreover, mice establish strong dominance hierarchies (Capanna et al., 1984; Poshivalov, 1980), which may have constituted a confounding factor in this study. Therefore, animals were housed individually for 2 weeks before experimentation. Mice were experimentally naïve, had no drug history, and were used in one experiment only.

Experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and were reviewed and approved by the Animal Welfare Committee of the Institute of Experimental Medicine.

Drugs

The MAGL inhibitor JZL184 (Cayman Chemical, Ann Arbor, MI) was dissolved in 0.2 ml dimethylsulfoxide (DMSO) and was diluted to the final volume with saline containing 0.4% methylcellulose. It was injected intraperitoneally in doses 0 (Vehicle), 8 and 16 mg/kg body weight, respectively, in a volume of 10 ml/kg body weight. JZL184 doses were selected based on earlier studies (Aliczki et al., 2012; Long et al., 2009a; Sciolino et al., 2011). The corticosterone synthesis blocker metyrapone (2-Methyl-1,2-di-3-pyridyl-1-propanone) (Sigma Aldrich, Saint Louis, MO) was dissolved in saline containing 5% Tween 80 and administered in doses 0 (Vehicle) and 30 mg/kg intraperitoneally in a volume of 5 ml/kg body weight. The selection of the metyrapone dose was based on preliminary experiments (see Supplementary data).

Behavioral tests

All behavioral tests were conducted in the early light phase of the day in a separate quiet testing room under approximately 400 lx light intensity, which was similar to that employed in the maintenance rooms. Behavioral tests were video recorded with a Sony DCR-SR75 digital camcorder and later analyzed with the H77 computer based event recorder software (Jozsef Haller, Institute of Experimental Medicine, Budapest, Hungary).

In the forced swimming procedure, mice were placed in a glass cylinder (40 cm high, 14 cm diameter) filled with 35 °C temperature water for 6 min. Water was changed and cylinders were cleaned and between subjects. Immediately after swimming, blood was sampled to assess the effects of JZL184 on stress responses. To avoid confounds from locomotor behavior, behavior was also analyzed. We scored time spent with floating (subject do not show movement except the ones needed to keep the head over the surface of water), struggling (vigorous limb movement, forelimbs break the surface of water, subjects attempts to climb up on the inner wall of the cylinder) and swimming (coordinated movement, involving movements with all four limbs, limbs do not break the surface of water). We mention that we did not pre-expose animals to forced swimming, i.e. no "behavioral despair" was studied and, in addition a single treatment was employed. Because of these large differences from the "behavioral despair" paradigm developed by Porsolt et al. (1977), the behavior of subjects was not necessarily indicative of depression-like states. The test was used exclusively to stress the subjects.

The open-field was a white non-transparent plastic box of 45 × 45 × 25 cm (height). Subjects were placed in one of the corners of the open-field and were allowed to explore it for 5 min. The apparatus was covered with a transparent Plexiglas lid during testing and was cleaned with tap water and paper towel between subjects. Locomotor activity was scored by counting the crossings of the lines that divided the open-field into 16 equal squares. Exploration in the central area (i.e. the 4 squares in the center of the apparatus) was also scored as a measure of anxiety-like behavior in the open-field. The grid was drawn on the video screen; thus, it was invisible to subjects.

The elevated plus-maze was made of black-painted aluminum. It consisted of two open arms (30 × 7 cm) and two closed arms (30 × 7 cm with 30 cm high walls) that were connected by a central platform (7 × 7 cm). The plus-maze was elevated to 70 cm from the floor. Subjects were placed on the central platform facing one of the open arms and were allowed to explore the apparatus for 5 min. The apparatus was cleaned with tap water and paper towel between tests. The number of entries into the closed arms was considered as a measure of locomotor activity, whereas time spent in open arms was used as an indicator of anxiety (Pellow et al., 1985). Subjects were considered to enter a compartment when all four legs crossed the lines separating the compartments. Risk-assessment activities were also analyzed as "ethological" measures of anxiety (Cole and Rodgers, 1993). Particularly, we scored the frequency and duration of head-dipping (HD; exploratory movement of head/shoulders over the side of the maze) and stretched attend posture (SAP; exploratory posture in which the body is stretched forward then retracted to the original position without any forward locomotion). HDs and SAPs were differentiated based according to their occurrence in different parts of the maze. As risk assessment from protected areas (i.e. from the closed arms or central platform) were shown to correlate negatively with open arm exploration (Cole and Rodgers, 1993; Cruz et al., 1994; Fernandez Espejo, 1997), protected SAPs and HDs were studied here as ethological indicators of anxiety-like behavior, similar to many earlier publications (Cruz et al., 1994; Navarro et al., 2006; Rodgers et al., 1992; Wall et al., 2003).

Blood sampling and corticosterone measurement

For pre-stress corticosterone measurements blood was sampled into EDTA-containing glass capillaries by tail incision 40, 120, and 240 min after pharmacological treatment. The effects of injections *per se* were investigated in a separate study, where we compared plasma corticosterone in undisturbed and vehicle-injected mice. We found that vehicle injections 40 min before blood sampling caused no significant changes in plasma corticosterone (see Supplementary data). In addition, plasma corticosterone levels were normal and similar in vehicle-treated groups at all time-points. Therefore pre-stress values were considered to reflect basal corticosterone levels. Stress levels were measured from trunk blood sampled on EDTA-containing plastic tubes after the forced swimming test. In the study that evaluated the efficacy of metyrapone on abolishing the effects of JZL184 on corticosterone production, blood was sampled by decapitation. After sampling, blood was centrifuged at 4 °C, the blood plasma was separated, and stored at −20 °C till analysis. Plasma corticosterone was measured by radioimmunoassay as described earlier (Toth et al., 2011). The corticosterone antiserum was raised in rabbits against corticosterone-carboximethyloxime BSA. ^{125}I-labelled corticosterone-carboximethyloxime-tyrosine-methyl esther was used as tracer. The interference with plasma transcortin was eliminated by inactivating transcortin at low pH. The sensitivity of the assay was 1 pmol/ml. Intra- and inter-coefficient of variation was 10 and 25%, respectively.

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Table 1
The experimental design of *Experiments 1* and *2*.

	Treatment	After lag time (40, 120, 240 min)	Immediately after blood sampling	Immediately after forced swimming
Experiment 1	0 (vehicle) 8 mg/kg 16 mg/kg	Blood sampling from tail (baseline)	Forced swimming (6 min)	Blood sampling from trunk (stress-induced)
	Treatment	40 min after treatment	Immediately after open-field test	Immediately after elevated plus-maze test
Experiment 2	0 (vehicle) 8 mg/kg 16 mg/kg	Open field test (5 min)	Elevated plus-maze test (5 min)	Blood sampling from trunk (stress-induced)

Experimental design

Two experiments were conducted to assess the effects of JZL184 on baseline and stress-induced plasma corticosterone levels, locomotor activity and anxiety -like behavior in the early light phase of the day. The experimental design was shown in Table 1.

In *Experiment 1* we studied the effects of JZL184 on basal and stress-induced plasma corticosterone levels. Subjects received JZL184 (0 (Vehicle), 8 and 16 mg/kg, respectively) in a random order and blood-samples were collected from the tail veins 40, 120 and 240 min later (*Experiments 1a*, *b* and *c*, respectively). After blood-sampling, mice were exposed to forced swimming for 6 min then decapitated and trunk blood was collected for evaluating stress-induced corticosterone levels. Experiments were performed in several series balanced over experimental groups. Blood samples for baseline and stress-induced corticosterone measurements were collected from the same subject at particular time points, but different animals were used for different time-points; sample sizes were 6–9 per group.

In *Experiment 2*, we studied whether metyrapone-induced inhibition of corticosterone-synthesis affected the behavioral effects of MAGL blockade that we reported earlier. In a preliminary experiment, we selected 30 mg/kg as the dose of metyrapone to be employed in *Experiment 2*. This dose decreased basal corticosterone levels but did not affect locomotor activity (see Supplementary data). In *Experiment 2a*, we investigated if corticosterone-synthesis blockade can dampen the corticosterone-increasing effects of MAGL inhibition seen in *Experiment 1*. Treatment groups were 0 (Vehicle), 8 and 16 mg/kg JZL184, respectively, and half of the animals received 30 mg/kg metyrapone in each group, while other half received the vehicle of metyrapone. 40 min after treatment, subjects were decapitated and trunk blood was collected for corticosterone measurements. The selection of the lag time between treatment and blood sampling was based on the results of *Experiment 1a*, where JZL184 significantly increased basal corticosterone levels at 40 min. In *Experiment 2b* we studied if corticosterone-synthesis blockade is able to dampen the MAGL blockade-induced anxiolysis and increase in locomotor activity we reported earlier (Aliczki et al., 2012). Treatment groups were similar as described in *Experiment 2a*, 40 min after treatment subjects were studied for 5 min in the open-field and for 5 min in the elevated plus-maze. After behavioral testing, mice were decapitated and trunk blood was collected for corticosterone measurements. Group sample sizes were 8–10 in each group.

Statistical analyses

Data were presented as mean ± standard error of the mean. Plasma corticosterone levels in *Experiments 1a–c* were analyzed by repeated measures ANOVA, while corticosterone levels in *Experiments 2a–b* and behavioral data in *Experiment 2b* were analyzed by two-factor ANOVA (Factor 1: JZL184-treatment; Factor 2: metyrapone-treatment). ANOVA assumptions were evaluated by the Levene's test; where assumptions were not fulfilled, data were square root transformed. The Duncan test was performed for post-hoc analysis when main effect was significant. The Bonferoni correction was applied for multiple comparisons. P values lower than 0.05 were considered statistically significant.

Results

In *Experiment 1a*, there was a significant interactions between factors ($F_{Treatment * Stress}$ (2,45) = 4.71; p = 0.013). The highest dose of JZL184 increased basal corticosterone levels 40 min after treatment, but—albeit some increase was noticed—did not alter stress-induced increases in corticosterone (Fig. 1). basal corticosterone levels in *Experiments 1b* and *1c* were not affected by JZL184 (*120 min*: $F_{Treatment}$ (2,16) = 0.76; p = 0.48; $F_{Treatment*Stress}$ (2,16) = 1.30; p = 0.29; *240 min*: $F_{Treatment}$ (2,20) = 0.43; p = 0.65; $F_{Treatment * Stress}$ (2,20) = 0.58; p = 0.56). Stress exposure increased plasma corticosterone throughout (*40 min*: F_{Stress} (1,45) = 227.21; p<0.01; *120 min*: F_{Time} (1,16) = 118.35; p<0.01; *240 min*: F_{Time} (1,20) =

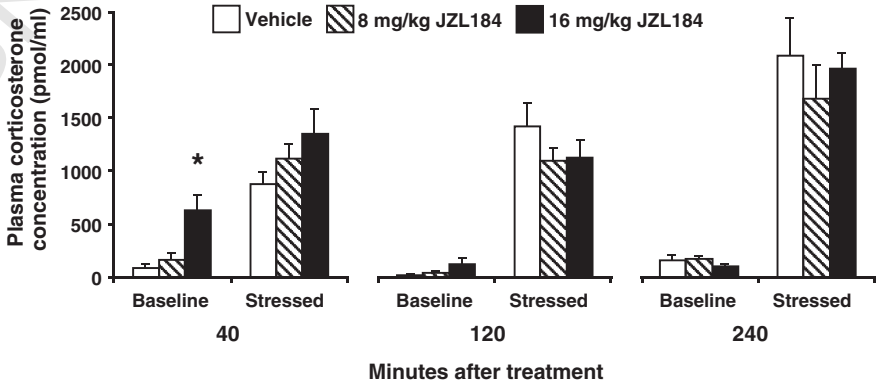

Fig. 1. Effects of JZL184 treatment on basal and stress-induced plasma corticosterone levels at 40, 120 and 240 min after treatment, respectively. *, significant difference from vehicle control (p < 0.05).

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Table 2
Behavioral variables by experimental groups in *Experiment 1*. Behavior was not affected by JZL184 treatment. The forced swimming procedure was used exclusively to stress the subjects.

Lag time	Variable	Vehicle	8 mg/kg JZL184	16 mg/kg JZL184	ANOVA
40 min	Floating time %	60.21 ± 6.52	68.54 ± 4.34	54.11 ± 10.14	$F(2,21) = 0.85$; $p = 0.44$
	Struggling time %	12.56 ± 2.86	10.2 ± 2.41	12.58 ± 4.11	$F(2,21) = 0.16$; $p = 0.84$
	Swimming time %	22.57 ± 4.66	17.22 ± 2.21	28.36 ± 7.01	$F(2,21) = 1.07$; $p = 0.35$
120 min	Floating time %	72.36 ± 5.12	64.91 ± 5.07	57.55 ± 10.55	$F(2,16) = 0.90$; $p = 0.42$
	Struggling time %	7.48 ± 1.7	9.08 ± 1.61	10.25 ± 3.58	$F(2,16) = 0.28$; $p = 0.75$
	Swimming time %	16.8 ± 3.62	21.51 ± 3.96	27.72 ± 6.48	$F(2,16) = 1.19$; $p = 0.32$
240 min	Floating time %	83.88 ± 1.72	73.91 ± 3.84	80.4 ± 3.41	$F(2,18) = 1.04$; $p = 0.37$
	Struggling time %	4.82 ± 0.68	10.13 ± 2.57	5.77 ± 1.59	$F(2,18) = 0.11$; $p = 0.89$
	Swimming time %	8.57 ± 1.02	12.38 ± 1.87	10.87 ± 1.67	$F(2,18) = 1.28$; $p = 0.30$

117.53; $p < 0.01$) (Fig. 1). Behavior in the forced swimming test was not affected by JZL184 treatment (Table 2).

In *Experiment 2a*, JZL184 increased baseline corticosterone levels 40 min after injection (F_{JZL184} (2,52) = 13.38; $p < 0.01$), while metyrapone remarkably decreased corticosterone levels ($F_{Metyrapone}$ (1,52) = 32.67; $p < 0.01$). A statistically significant interaction between the JZL184 and metyrapone treatment was also found ($F_{Interaction}$ (2,52) = 4.44; $p = 0.01$), as JZL184 was unable to increase corticosterone levels in metyrapone-treated groups (Fig. 2a). In *Experiment 2b*, JZL184 significantly increased locomotion in the open-field (F_{JZL184} (2,48) = 4.09; $p = 0.02$); the effect was independent of corticosterone-synthesis ($F_{Metyrapone}$ (1,48) = 0.14; $p = 0.70$; $F_{Interaction}$ (2,48) = 0.21; $p = 0.80$) (Fig. 2b). Metyrapone treatment did not alter, while JZL184 treatment caused a marginal increase in central area exploration ($F_{Metyrapone}$ (2,48) = 2.17; $p = 0.15$; F_{JZL184} (2,48) = 2.75; $p = 0.07$). A marginally significant interaction between the JZL184 and metyrapone treatment was also found ($F_{Interaction}$ (2,48) = 2.44; $p = 0.09$). This interaction prompted pairwise comparisons, which revealed that JZL184 increased central area exploration in vehicle-treated groups but metyrapone abolished these changes (Fig. 2c). In the elevated plus-maze, neither JZL184 nor metyrapone was able to alter closed arm entries (F_{JZL184} (2,48) = 1.04; $p = 0.35$; $F_{Metyrapone}$ (1,48) = 0.08; $p = 0.75$; $F_{Interaction}$ (2,48) = 0.09; $p = 0.91$) (Fig. 2d). The highest dose of JZL184 increased both the ratio of open arm entries (F_{JZL184} (2,48) = 3.29; $p = 0.04$) (Fig. 2e) and time spent in open arms (F_{JZL184} (2,48) = 3.81; $p = 0.02$) (Fig. 2f). These effects of JZL184 seem to be corticosterone-synthesis dependent as they were absent in groups treated with metyrapone (open arm entries ratio: $F_{Interaction}$ (2,48) = 3.27; $p = 0.04$; time spent in open arms: $F_{Interaction}$ (2,48) = 4.04; $p = 0.02$). (Fig. 2e. and f). JZL184 treatment also decreased risk assessment in protected areas of the elevated plus-maze (duration SAP: F_{JZL184} (2,48) = 3.55; $p = 0.03$; duration HD: F_{JZL184} (2,48) = 2.62; $p = 0.08$), however, these changes were independent of corticosterone synthesis (SAP: $F_{Interaction}$ (2,30) = 1.30; $p = 0.28$; HD: $F_{Interaction}$ (2,30) = 0.14; $p = 0.86$) (Table 3). Stress-induced levels of corticosterone were unaltered by JZL184 and were only decreased by metyrapone (F_{JZL184} (2,50) = 0.73; $p = 0.48$; $F_{Metyrapone}$ (1,50) = 75.73; $p < 0.01$; $F_{Interaction}$ (2,50) = 0.19; $p = 0.82$) (Table 3).

Discussion

MAGL blockade dose-dependently and dramatically increased basal corticosterone levels 40 min after treatment. Values returned to control levels within 2 h. The stress-induced corticosterone levels were unaltered by inhibition of MAGL. MAGL blockade also increased locomotor activity in a corticosterone-synthesis independent manner and exerted anxiolytic-related effects which were at least partly dependent on corticosterone. To our best knowledge, our study is the first to show that the inhibition of MAGL increases corticosterone levels under basal conditions. It was reported earlier that CB_1R agonists (e.g. Δ^9-tetrahydrocannabinol) and to a lesser extent the inhibition of AEA degradation by the FAAH inhibitor URB597 increased basal corticosterone levels (Johnson et al., 1978; Weidenfeld et al., 1994; Wenger et al., 1997, 2003; Zuardi et al., 1984). In addition to basal levels, CB_1R agonists increase stress responses as well (Sano et al., 2009), while FAAH inhibition decreases the same response (Hill et al., 2009, 2010). Intriguingly, the effects of MAGL inhibition mimicked the effects of CB_1R agonists on basal levels, but left stress responses unchanged which is different from the effects of both CB_1R agonists and FAAH inhibition.

The anxiolytic effects of MAGL blockade were in line with those reported earlier by our laboratory (Aliczki et al., 2012) and other studies (Busquets-Garcia et al., 2011; Sciolino et al., 2011). A slight difference occurred as it regards the timing of this effect, as in our earlier study, behavioral effects were evident 80 min after JZL184 administration only, while a similar effect occurred here after 40 min. This difference in timing is difficult to reconcile at present. We note however, that the behavioral effects of MAGL blockade showed similar slight differences in studies performed by the same group within a short time interval (Long et al., 2009a, 2009b, 2009c).

The present findings suggest that some, but not all the anxiety-related effects of MAGL blockade were secondary to the treatment-induced increase in basal corticosterone levels. The concurrent application of JZL184 and the corticosterone synthesis inhibitor metyrapone abolished the effects of MAGL blockade on central area exploration in the open-field and open arm exploration in the elevated plus-maze while effects on risk assessment were unchanged. This finding is especially intriguing, as it was reported earlier that risk assessment, but not open arm exploration is dependent of acute changes in corticosterone levels (Mikics et al., 2005). In contrast to the anxiety-like effects, the locomotion-enhancing effects of MAGL blockade did not depend on corticosterone, a finding which is in line with earlier observations on the lack of direct acute effects of corticosterone on open-field locomotion (Mikics et al., 2005). The mediation of anxiolytic effects by increased basal corticosterone might be surprising at the first sight, as stress is believed to enhance anxiety. This phenomenon, however, is valid for long-term increases in corticosterone. Acute stress responses promote the coping with challenging situations on the short run, which results in anxiolytic effects when the increase in corticosterone is acute (for a review see (Haller et al., 1998)).

MAGL blockade increased HPA-axis activity under basal but not under stressful conditions in our study, an endocrine effect that partly interfered with the behavioral consequences of JZL184 treatment. The mechanisms of these complex effects may be multiple, as cannabinoids affect the function of several brain areas involved in the modulation of emotions (McLaughlin et al., 2007; Rubino et al., 2008a, 2008b; Zarrindast et al., 2008) and also the activity of the HPA-axis (Cota et al., 2007; Di et al., 2003, 2005). The details of the mechanisms activated by MAGL blockade remains to be established in subsequent studies, especially the CB_1R-dependence of these mechanisms. It

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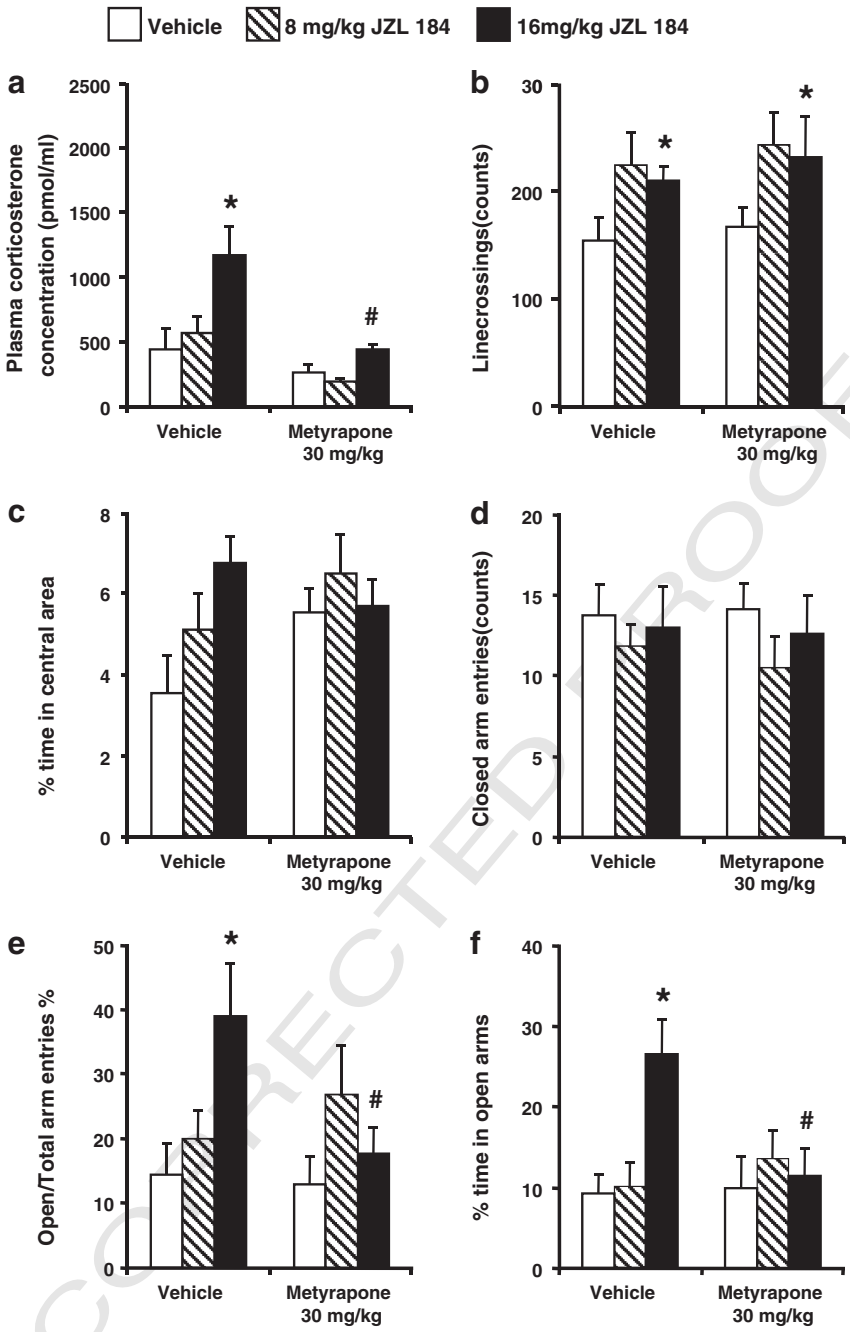

Fig. 2. Effects of combined JZL184 and metyrapone treatment on (a) basal corticosterone levels, (b) locomotor activity in the open-field test, (c) locomotor activity and (d and c) anxiety-related behavior in the elevated plus-maze test. In each panel, the left three columns represent experimental groups treated with the vehicle of metyrapone, while the three columns on the right represent groups treated with metyrapone. *, significant difference from vehicle control; #, significant difference from 16 mg/kg JZL184 group treated with the vehicle of metyrapone ($p < 0.05$ in both cases).

occurs, however, that those effects of JZL184 that were resistant to corticosterone synthesis inhibition were mediated by direct actions on neural processes.

Conclusion

The inhibition of MAGL activity—the indirect upregulation of 2-AG signaling—causes a rapid increase in basal corticosterone levels which disappears in less than 2 h. The comparison of this finding with earlier reports suggests that the overall stimulation of CB_1Rs, as well as the selective increase of AEA and 2-AG signaling produce partially overlapping but still different effects on corticosterone secretion. CB_1R agonists dramatically increase basal and stress-induced corticosterone secretion; enhanced anandamide signaling slightly increases basal levels but diminish stress responses, while increased 2-AG secretion dramatically increases basal levels but does not affect stress responses. Behavioral findings suggest that some of the putative anxiolytic effects of MAGL inhibition are in fact secondary to increased corticosterone secretion. At the same time, the locomotor enhancing effect of MAGL blockade appears to be an intrinsic effect of enhanced 2-AG signaling.

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Table 3
Variables by experimental groups in *Experiment 2b*. Bold text indicates statistically significant effect of JZL184 treatment or interaction of the two treatments.

Variable	Vehicle-Vehicle	Vehicle-JZL184 8 mg/kg	Vehicle-JZL184 16 mg/kg	Metyrapone-Vehicle	Metyrapone-JZL184 8 mg/kg	Metyrapone-JZL184 16 mg/kg	P-value of ANOVA
Basal corticosterone levels	**475.37 ± 19.29**	**617.44 ± 23.27**	**1445.05 ± 37.39**	**267.47 ± 14.81**	**153.03 ± 11.66**	**465.2 ± 21.25**	**$F_{JZL184}(2,54) = 17.43$; $p < 0.01$** **$F_{Metyrapone}(1,54) = 31.08$; $p < 0.01$** **$F_{Interaction}(2,52) = 4.44$; $p = 0.01$**
Stress-induced corticosterone levels	1374.95 ± 175.4	1206.76 ± 87.62	1348.87 ± 164.14	464.91 ± 52.79	439.76 ± 48.15	542.31 ± 64.02	$F_{JZL184}(2,50) = 0.73$; $p = 0.48$ **$F_{Metyrapone}(1,50) = 75.73$; $p < 0.01$** $F_{Interaction}(2,50) = 0.19$; $p = 0.82$
Linecrossings	**154 ± 22.03**	**223.8 ± 30.17**	**209.75 ± 13.1**	**167.77 ± 16.01**	**242.6 ± 30.89**	**231.55 ± 36.66**	**$F_{JZL184}(2,48) = 4.09$; $p = 0.02$** $F_{Metyrapone}(1,48) = 0.14$; $p = 0.70$ $F_{Interaction}(2,48) = 0.21$; $p = 0.80$
Closed arm entries	13.75 ± 7.91	11.8 ± 1.38	13 ± 2.54	14.11 ± 1.61	10.5 ± 1.84	12.55 ± 2.41	$F_{JZL184}(2,48) = 1.04$; $p = 0.35$ $F_{Metyrapone}(1,48) = 0.08$; $p = 0.75$ $F_{Interaction}(2,48) = 0.09$; $p = 0.91$
% time Closed arms	**73.53 ± 5.99**	**72.29 ± 6.29**	**56.43 ± 7.33**	**52.81 ± 8.83**	**54.64 ± 7.82**	**69.9 ± 5.61**	**$F_{JZL184}(2,50) = 3.08$; $p = 0.05$** $F_{Metyrapone}(1,50) = 0.15$; $p = 0.69$ $F_{Interaction}(2,50) = 1.26$; $p = 0.29$
Central platform entries	16.25 ± 2	16.88 ± 2.2	15.4 ± 1.73	14.7 ± 2.49	21.1 ± 2.04	15.88 ± 3.1	$F_{JZL184}(2,48) = 1.15$; $p = 0.32$ $F_{Metyrapone}(1,48) = 1.35$; $p = 0.25$ $F_{Interaction}(2,48) = 0.79$; $p = 0.45$
% time Central platform	**16.68 ± 4.49**	**17.16 ± 7.06**	**33.01 ± 6.74**	**33.15 ± 9.17**	**21.17 ± 4.26**	**18.05 ± 4.56**	**$F_{JZL184}(2,48) = 3.21$; $p = 0.04$** $F_{Metyrapone}(1,48) = 0.14$; $p = 0.71$ $F_{Interaction}(2,48) = 0.03$; $p = 0.96$
Open arm entries	**2.25 ± 0.79**	**3.2 ± 0.91**	**7.5 ± 1.7**	**2.66 ± 0.94**	**4 ± 1.22**	**3 ± 0.95**	$F_{JZL184}(2,48) = 2.26$; $p = 0.11$ $F_{Metyrapone}(1,48) = 0.81$; $p = 0.37$ $F_{Interaction}(2,48) = 1.68$; $p = 0.19$
% time Open arms	**9.18 ± 2.51**	**10.01 ± 3.2**	**26.5 ± 4.27**	**9.33 ± 3.78**	**13.48 ± 3.44**	**11.46 ± 3.41**	**$F_{JZL184}(2,48) = 3.81$; $p = 0.02$** $F_{Metyrapone}(1,48) = 0.87$; $p = 0.35$ **$F_{Interaction}(2,48) = 4.04$; $p = 0.02$**
% Open/total entries	**0.14 ± 0.04**	**0.2 ± 0.04**	**0.39 ± 0.08**	**0.13 ± 0.04**	**0.27 ± 0.07**	**0.18 ± 0.04**	**$F_{JZL184}(2,48) = 3.29$; $p = 0.04$** $F_{Metyrapone}(1,50) = 0.64$; $p = 0.42$ **$F_{Interaction}(2,48) = 3.27$; $p = 0.04$**
Protected SAP	13.25 ± 3.2	15.77 ± 2.61	16.1 ± 3.39	11.3 ± 2.06	9.1 ± 1.2	10.78 ± 2.12	$F_{JZL184}(2,48) = 1.73$; $p = 0.18$ $F_{Metyrapone}(1,48) < 0.01$; $p = 0.99$ $F_{Interaction}(2,48) = 1.23$; $p = 0.29$
% time Protected SAP	**5.86 ± 1.29**	**5.58 ± 1.47**	**2.83 ± 0.69**	**8.05 ± 1.54**	**4.03 ± 1.14**	**4.28 ± 1.01**	**$F_{JZL184}(2,48) = 3.55$; $p = 0.03$** $F_{Metyrapone}(1,48) = 0.31$; $p = 0.57$ $F_{Interaction}(2,30) = 1.30$; $p = 0.28$
Protected HD	15.37 ± 2.45	14.55 ± 3.86	11.2 ± 2.64	10.4 ± 1.77	8.9 ± 1.66	12.33 ± 2.55	$F_{JZL184}(2,48) = 1.78$; $p = 0.18$ $F_{Metyrapone}(1,48) = 0.05$; $p = 0.82$ $F_{Interaction}(2,48) = 0.54$; $p = 0.58$
% time Protected HD	4.66 ± 0.7	3.07 ± 0.66	2.67 ± 0.54	4.25 ± 0.87	3.33 ± 0.78	2.98 ± 0.71	$F_{JZL184}(2,48) = 2.62$; $p = 0.08$ $F_{Metyrapone}(1,48) < 0.01$; $p = 0.97$ $F_{Interaction}(2,30) = 0.14$; $p = 0.86$
Unprotected SAP	0.5 ± 0.38	0.33 ± 0.33	0.2 ± 0.13	1.6 ± 1.08	0.7 ± 0.3	0.89 ± 0.56	$F_{JZL184}(2,48) = 0.64$; $p = 0.53$ $F_{Metyrapone}(1,48) = 0.25$; $p = 0.61$ $F_{Interaction}(2,48) = 1.08$; $p = 0.34$
% time Unprotected SAP	0.12 ± 0.08	0.11 ± 0.11	0 ± 0	0.48 ± 0.29	0.16 ± 0.09	0.13 ± 0.07	$F_{JZL184}(2,48) = 0.19$; $p = 0.82$ $F_{Metyrapone}(1,48) = 0.93$; $p = 0.33$ $F_{Interaction}(2,48) = 2.16$; $p = 0.12$
Unprotected HD	4.37 ± 1.59	4 ± 1.55	6.8 ± 2.22	7.7 ± 2.52	9.9 ± 2.03	5.33 ± 1.85	$F_{JZL184}(2,48) = 1.63$; $p = 0.2$ $F_{Metyrapone}(1,48) = 0.69$; $p = 0.41$ $F_{Interaction}(2,48) = 0.74$; $p = 0.48$
% time Unprotected HD	0.95 ± 0.34	0.85 ± 0.42	1.37 ± 0.42	1.22 ± 0.36	2.06 ± 0.51	1.32 ± 0.53	$F_{JZL184}(2,48) = 1.57$; $p = 0.21$ $F_{Metyrapone}(1,48) = 0.98$; $p = 0.32$ $F_{Interaction}(2,48) = 0.31$; $p = 0.73$

Conflict of interest

The authors declare no conflict of interest.

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