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

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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.