Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Pharmacology 528 (2005) 99–102
www.elsevier.com/locate/ejphar

Short communication

Chronic corticosterone treatment increases the endocannabinoid 2-arachidonylglycerol in the rat amygdala

Matthew N. Hill [a], W.-S.Vanessa Ho [b], Sarah E. Meier [b], Boris B. Gorzalka [a], Cecilia J. Hillard [b,*]

[a] *Department of Psychology, University of British Columbia, Vancouver, B.C. Canada V6T1Z4*
[b] *Department of Pharmacology and Toxicology, Medical College of Wisconsin, Milwaukee, WI 53226, USA*

Received 27 June 2005; received in revised form 25 October 2005; accepted 28 October 2005

Abstract

This research was designed to examine the effect of three weeks of administration of corticosterone (20 mg/kg) on endocannabinoid content and cannabinoid CB_1 receptor binding in the amygdala. It was found that the endocannabinoid 2-arachidonylglycerol was significantly increased in the amygdala following chronic corticosterone treatment. However, there was no change in either the maximal binding (B_{max}) or binding affinity (K_D) of [^{3}H]-CP 55,940 to the CB_1 receptor in the amygdala. Given the role of amygdalar endocannabinoids in the regulation of emotionality, this suggests that the ability of glucocorticoids to influence affective behavior may involve interactions with regulation of endocannabinoid content.

Keywords: Corticosterone; Amygdala; Endocannabinoid; 2-arachidonylglycerol

1. Introduction

Accumulating evidence suggests that functional interactions exist between the endocannabinoid and corticosteroid systems. Administration of cannabinoid CB_1 receptor ligands has been shown to potently activate the hypothalamic–pituitary–adrenal axis and increase secretion of glucocorticoids such as corticosterone (Weidenfeld et al., 1994; Wenger et al., 1997). Alternatively, removal of adrenal steroids, inhibition of corticosteroidogenesis or pharmacological blockade of the glucocorticoid receptor result in a sensitization of the behavioral and physiological responses to cannabinoid administration (Gordon et al., 1978; Jackson and Murphy, 1997; Pryce et al., 2003). These data suggest that the cannabinoid CB_1 receptor may be under negative regulation by glucocorticoids. In support of this, it has been demonstrated that removal of adrenal steroids through adrenalectomy results in a significant upregulation of cannabinoid CB_1 receptor mRNA in the striatum, an effect that is reversed by subsequent glucocorticoid treatment (Mailleux and Vanderhaeghen, 1993). Recently, we demonstrated that exposure to 21 days of chronic unpredictable stress, a regimen which induces glucocorticoid hypersecretion (Hill et al., 2005), resulted in a pronounced downregulation of both the cannabinoid CB_1 receptor and the endocannabinoid 2-arachidonylglycerol (2-AG) in the hippocampus, but not the limbic forebrain (Hill et al., 2005). This indicates a region-specific effect of stress, and possibly glucocorticoids, in the regulation of the endocannabinoid system. It has also been demonstrated that chronic, but not acute, restraint stress results in an increase in 2-AG in the amygdala, while amygdalar content of the other major endocannabinoid, anandamide is reduced (Patel et al., 2005b). These findings from differing stress paradigms are interesting in light of in vitro work suggesting that endocannabinoid production and release may be facilitated by glucocorticoids (Di et al., 2003; Malcher-Lopes et al., 2004). Thus, the cannabinoid CB_1 receptor may be under negative regulation by glucocorticoids in the hippocampus and striatum, but not in the limbic forebrain. However, the direction of the regulation of endocannabinoids by glucocorticoids and stress is somewhat conflicting and unclear.

* Corresponding author. Tel.: +414 456 8493; fax: +414 456 6545.
E-mail address: chillard@mcw.edu (C.J. Hillard).

0014-2999/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2005.10.058

The amygdala is a critical locus for the expression of emotional behavior and several lines of evidence implicate the endocannabinoid system in emotionality (Haller et al., 2004; Marsicano et al., 2002; Uriguen et al., 2004). Cannabinoids are known to have a potent biphasic effect on emotionality such that relatively low doses reduce anxiety and higher doses increase anxiety (Hill and Gorzalka, 2004; Marco et al., 2004), an interaction likely subserved through their actions in the amygdala (Patel et al., 2005a). Glucocorticoids and stress are also known to influence amygdalar morphology and emotional behavior (Korte, 2001; Yang et al., 2005; Vyas et al., 2002). Knowledge of how these systems interact in the amygdala may be crucial in determining the importance of this interaction in the genesis of anxiety-based neuropsychiatric diseases. The current study investigated the effect of 21 days of treatment with the glucocorticoid corticosterone-21-acetate (20 mg/kg) on endocannabinoid content and cannabinoid CB_1 receptor binding parameters in the rat amygdala.

2. Methods

Twenty four, 70-day old male Long–Evans rats (300 g) housed in groups of three in triple mesh wire cages were used in this study. Colony rooms were maintained at 21 °C, and on a reverse 12 h light/dark cycle, with lights off at 0900 h. All rats were given ad libitum access to Purina Rat Chow and tap water. Subjects were randomly assigned to two groups and were either subjected to 21 days of subcutaneous injections of 20 mg/kg corticosterone-21-acetate (Sigma, Canada) or vehicle (1, 2-propanediol). Twenty four hours following the last injection, rats were rapidly decapitated, brains were removed and the amygdala were dissected over dry ice, flash frozen in liquid nitrogen and stored at − 80 °C until analysis. All treatments of animals were approved by the Canadian Council for Animal Care and the Animal Care Committee of the University of British Columbia.

Amygdala were homogenized and membranes were harvested and assayed for CB_1 receptor binding using a previously described protocol (Hill et al., 2005). A separate cohort of animals was prepared using the same injection regimen, and had their amygdala removed for endocannabinoid analysis. These amygdala samples were subjected to a lipid extraction process and endocannabinoid content of the lipid extracts was determined using isotope-dilution liquid chromatography/mass spectrometry as described previously (Patel et al., 2003).

Statistical comparisons of the effects of chronic corticosterone treatment on cannabinoid CB_1 receptor binding parameters and endocannabinoid content were done using an independent *t*-test. Significance was established against an alpha level of .05.

3. Results

Chronic corticosterone treatment did not affect the binding parameters of [^{3}H]-CP 55,940 for the cannabinoid CB_1 receptor in the amygdala. Specifically, there were no significant differences in the binding site density (B_{max}) [t (5)=0.278, P>.05] or [^{3}H]-CP 55,940 dissociation constant (K_D) [t (5)=0.443, P>.05] between animals treated with corticosterone and those with vehicle. However, three-week administration of corticosterone significantly increased amygdalar content of the endocannabinoid 2-AG [t (12)=3.10, P<.01]. Due to a contamination problem, anandamide levels could not be quantified. Data regarding the effects of chronic corticosterone treatment on the endocannabinoid system in the amygdala can be seen in Fig. 1.

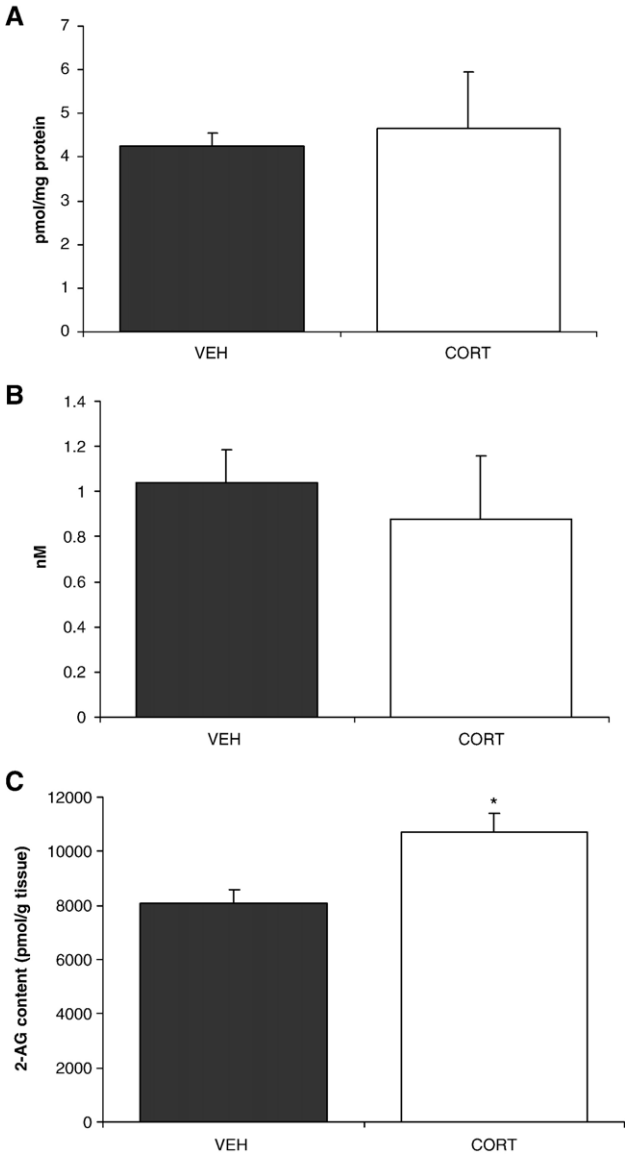

Fig. 1. The effect of 21 days of chronic corticosterone (CORT) or vehicle (VEH) on: A) the binding site density (B_{max}) of the CB_1 receptor in the amygdala; B) the dissociation constant (K_D) of [^{3}H]-CP 55,940 to the CB_1 receptor in the amygdala; C) 2-arachidonylglycerol (2-AG) content in the amygdala. *Significantly different from control (P<.05) (n=3–4/group for CB_1 receptor binding parameters; n=7/group for endocannabinoid quantification).

4. Discussion

In this study, it was found that chronic administration of the glucocorticoid corticosterone-21-acetate resulted in a significant

upregulation of the endocannabinoid 2-AG without affecting the density or binding affinity of the cannabinoid CB_1 receptor in the amygdala. These data do not support the hypothesis that glucocorticoids exert ubiquitous negative regulation over the cannabinoid CB_1 receptor, but perhaps that this effect is region specific. However, the increase in ligand content without concurrent downregulation of receptor, suggests that chronic glucocorticoid treatment results in an enhancement of endocannabinoid transmission in the amygdala. In vitro work has suggested that glucocorticoids increase synthesis and release of endocannabinoids in the hypothalamus through activation of a membrane bound, G-protein coupled receptor (Di et al., 2003; Malcher-Lopes et al., 2004). Our current data support the idea that glucocorticoids increase endocannabinoid synthesis; however it is important to note that these findings cannot be generalized to regions outside of the amygdala given the region-specific regulation of the endocannabinoid system (Hill et al., 2005; Patel et al., 2005b).

The present findings, taken together with evidence that repeated restraint stress increases 2-AG levels in the amygdala (Patel et al., 2005b), suggest the possibility that glucocorticoids could mediate this stress effect. However it must be noted that in that study, endocannabinoid measurements were made when the corticosterone response to stress had habituated (Patel et al., 2005b), suggesting that other factors may be involved. Chronic corticosterone treatment has been shown to enhance fear conditioning through actions in the amygdala (Conrad et al., 2004), however this is likely not mediated by changes in endocannabinoid content, as the endocannabinoids are not believed to be involved in the acquisition of fear conditioning, but instead play a crucial role in fear extinction (Marsicano et al., 2002). While the effects of chronic glucocorticoid treatment on fear extinction are not known, there is a recent study demonstrating that acute systemic or intra-amygdala infusion of glucocorticoid receptor agonists enhances fear extinction, whereas glucocorticoid synthesis inhibition or receptor antagonism impairs extinction (Yang et al., 2005). Given the possible role of glucocorticoids in regulating endocannabinoid levels in the brain (Di et al., 2003), and the critical role of endocannabinoids in the extinction of fear behavior (Marsicano et al., 2002) it would be interesting to determine how chronic corticosterone treatment affects fear extinction, and furthermore, if this effect is due to interactions with the endocannabinoid system. Given the importance of both endocannabinoids and corticosteroids in the expression of emotional behavior, understanding their interactions in brain regions regulating these behaviors is of great importance. In conclusion, this research provides the first evidence that prolonged glucocorticoid treatment enhances endocannabinoid transmission in the amygdala of the rat.

Acknowledgment

This research was supported by NIH grant DA16967 and by an Independent Investigator Award from NARSAD to CJH; by a Natural Sciences and Engineering Research Council of Canada (NSERC) operating grant to BBG and by a NSERC postgraduate scholarship and a Michael Smith Foundation for Health Research Biomedical Trainee Award to MNH. The authors would like to thank Amanda Savoie, Maric Tse and Jane Sun for their technical assistance.

References

Conrad, C.D., MacMillan 2nd, D.D., Tsekhanov, S., Wright, R.L., Baran, S.E., Fuchs, R.A., 2004. Influence of chronic corticosterone and glucocorticoid receptor antagonism in the amygdala on fear conditioning. Neurobiol. Learn. Mem. 81, 185–199.

Di, S., Malcher-Lopes, R., Halmos, K.C., Tasker, J.G., 2003. Nongenomic glucocorticoid inhibition via endocannabinoid release in the hypothalamus: a fast feedback mechanism. J. Neurosci. 23, 4850–4857.

Gordon, J.H., Bromley, B.L., Gorski, R.A., Zimmermann, E., 1978. Delta9-Tetrahydrocannabinol enhancement of lordosis behavior in estrogen treated female rats. Pharmacol. Biochem. Behav. 8, 603–608.

Haller, J., Varga, B., Ledent, C., Freund, T.F., 2004. CB1 cannabinoid receptors mediate anxiolytic effects: convergent genetic and pharmacological evidence with CB1-specific agents. Behav. Pharmacol. 15, 299–304.

Hill, M.N., Gorzalka, B.B., 2004. Enhancement of anxiety-like responsiveness to the cannabinoid receptor agonist HU-210 following chronic stress. Eur. J. Pharmacol. 499, 291–295.

Hill, M.N., Patel, S., Carrier, E.J., Rademacher, D.J., Ormerod, B.K., Hillard, C. J., Gorzalka, B.B., 2005. Downregulation of endocannabinoid signaling in the hippocampus following chronic unpredictable stress. Neuropsychopharmacology 30, 508–515.

Jackson, A.L., Murphy, L.L., 1997. Role of the hypothalamic–pituitary–adrenal axis in the suppression of luteinizing hormone release by delta-9-tetrahydrocannabinol. Neuroendocrinology 65, 446–452.

Korte, S.M., 2001. Corticosteroids in relation to fear, anxiety and psychopathology. Neurosci. Biobehav. Rev. 25, 117–142.

Mailleux, P., Vanderhaeghen, J.J., 1993. Glucocorticoid regulation of cannabinoid receptor messenger RNA levels in the rat caudate-putamen. An in situ hybridization study. Neurosci. Lett. 156, 51–53.

Malcher-Lopes, R., Di, S., Marcheselli, V.L., Bazan, N.G., Tasker, J.G., 2004. Regulation of endocannabinoid release in the hypothalamus by convergent glucocorticoid-and activity-dependent mechanisms. Abstr.-Soc. Neurosci. 892.15.

Marco, E.M., Perez-Alvarez, L., Borcel, E., Rubio, M., Guaza, C., Ambrosio, E., File, S.E., Viveros, M.P., 2004. Involvement of 5-HT1A receptors in behavioural effects of the cannabinoid receptor agonist CP 55,940 in male rats. Behav. Pharmacol. 15, 21–27.

Marsicano, G., Wotjak, C.T., Azad, S.C., Bisogno, T., Rammes, G., Cascio, M. G., Hermann, H., Tang, J., Hofmann, C., Zieglgansberger, W., Di Marzo, V., Lutz, B., 2002. The endogenous cannabinoid system controls extinction of aversive memories. Nature 418, 530–534.

Patel, S., Rademacher, D.J., Hillard, C.J., 2003. Differential regulation of the endocannabinoids anandamide and 2-arachidonylglycerol within the limbic forebrain by dopamine receptor activity. J. Pharmacol. Exp. Ther. 306, 880–888.

Patel, S., Cravatt, B.F., Hillard, C.J., 2005a. Synergistic interactions between cannabinoids and environmental stress in the activation of the central amygdala. Neuropsychopharmacology 30, 497–507.

Patel, S., Roelke, C.T., Rademacher, D.J., Hillard, C.J., 2005b. Inhibition of restraint stress-induced neural and behavioral activation by endogenous cannabinoid signaling. Eur. J. Neurosci. 21, 1057–1069.

Pryce, G., Giovannoni, G., Baker, D., 2003. Mifepristone or inhibition of 11beta-hydroxylase activity potentiates the sedating effects of the cannabinoid receptor-1 agonist Delta(9)-tetrahydrocannabinol in mice. Neurosci. Lett. 341, 164–166.

Uriguen, L., Perez-Rial, S., Ledent, C., Palomo, T., Manzanares, J., 2004. Impaired action of anxiolytic drugs in mice deficient in cannabinoid CB1 receptors. Neuropharmacology 46, 966–973.

Vyas, A., Mitra, R., Shankaranarayana Rao, B.S., Chattarji, S., 2002. Chronic stress induces contrasting patterns of dendritic remodeling in hippocampal and amygdaloid neurons. J. Neurosci. 22, 6810–6818.

Weidenfeld, J., Feldman, S., Mechoulam, R., 1994. Effect of the brain constituent anandamide, a cannabinoid receptor agonist, on the hypothalamo–pituitary–adrenal axis in the rat. Neuroendocrinology 59, 110–112.

Wenger, T., Jamali, K.A., Juaneda, C., Leonardelli, J., Tramu, G., 1997. Arachidonyl ethanolamide (anandamide) activates the parvocellular part of hypothalamic paraventricular nucleus. Biochem. Biophys. Res. Commun. 237, 724–728.

Yang, Y.-L., Chao, P.-K., Lu, K.-T., 2005. Systemic and intra-amygdala administration of glucocorticoid agonist and antagonist modulate the extinction of conditioned fear. Neuropsychopharmacology, doi:10.1038/sj.npp.1300899.