European Journal of Pharmacology 602 (2009) 54–57

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Neuropharmacology and Analgesia

5-HT_{2A} receptor mediated neuronal activation within the paraventricular nucleus of the hypothalamus is desensitized following prolonged glucocorticoid treatment

Tiffany T.-Y. Lee, Van A. Redila, Matthew N. Hill, Boris B. Gorzalka *

Department of Psychology, University Of British Columbia, Vancouver, B.C. Canada V6T1Z4

ARTICLE INFO

Article history:
Received 13 September 2008
Received in revised form 28 October 2008
Accepted 17 November 2008
Available online 25 November 2008

Keywords:
HPA axis
Corticosterone
5-HT
Raphe
Fos
Depression

ABSTRACT

An organism's ability to adapt successfully to stress reflects an equilibrium that requires not only an appropriate response, but also an ability to control that response. The hypothalamic–pituitary–adrenal (HPA) axis contributes to these homeostatic actions. Previous research implicates involvement of the serotonergic 5-HT_{2A} receptors of the hypothalamic paraventricular nucleus (PVN) in HPA axis activation. However, the sensitivity of these receptors to activate the PVN under conditions of chronically elevated glucocorticoids is not known. To this extent, we investigated the effects of chronic corticosterone administration on c-fos expression induced by the serotonergic 5-$HT_{2A/2C}$ receptor agonist DOI within the PVN. Under resting conditions, DOI evokes a robust activation of the PVN; however, following chronic treatment with corticosterone, this response is abolished. These results indicate that chronically elevated glucocorticoid levels desensitize serotonergic 5-HT_{2A} receptors within the PVN, a phenomenon which may contribute to HPA axis suppression following protracted glucocorticoid hypersecretion.

1. Introduction

Dysregulation of the hypothalamic–pituitary–adrenal (HPA) axis is consistently associated with affective disorders such as depression (Holsboer, 2000). The HPA axis plays a critical role in the body's response to stress via direct influence and feedback mechanisms. Briefly, when an organism is faced with an aversive stimulus, this stimulates neurons of the hypothalamic paraventricular nucleus (PVN) to synthesize and secrete two hormonal peptides, corticotropin-releasing hormone (CRH) and vasopressin into the portal blood. These hormones then induce release of adrenocorticotropin-releasing hormone (ACTH) into the general circulation from the anterior lobe of the pituitary gland. The adrenal cortices produce and release glucocorticoid hormones, cortisol or corticosterone (in humans and rats, respectively) in response to circulating ACTH. In the short term, regulation of this mechanism occurs as negative feedback in which glucocorticoids suppress CRH and ACTH release in the PVN and pituitary gland (Pecoraro et al., 2006). This finely tuned system also exhibits dynamic responses to acute versus repeated stress. For example, long-term elevations in circulating glucocorticoid hormones results in a dampening of HPA axis output (Calogero et al., 1991); however, the mechanisms that drive this HPA axis suppression are largely unknown.

There are several lines of converging evidence suggesting a functional relationship between the serotonergic (5-hydroxytryptamine; 5-HT) system and HPA axis (van de Kar and Blair, 1999; Chaouloff, 1995). Serotonergic fibers arising from the raphe nuclei innervate the CRH neurosecretory cells within the PVN directly (Liposits et al., 1987), and a wealth of evidence suggests that 5-HT signaling is recruited by stressors to promote HPA axis activity (Feldman et al., 1995). With respect to 5-HT receptor subtypes, the serotonergic 5-HT_{2A} receptor has received particular attention. Expression analysis studies have revealed that the serotonergic 5-HT_{2A} receptor is localized within the PVN proper (Zhang et al., 2002), situating it in an ideal location to regulate HPA axis activity. Consistently, the serotonergic 5-$HT_{2A/2C}$ receptor agonist, DOI ((±)1-(2,5-dimethoxy-4-iodophenyl)-2 aminopropane) potently activates the HPA axis as revealed by both an increase in adrenocortical secretion and the induction of immediate early genes (such as c-fos) within the PVN (van de Kar et al., 2001; Zhang et al., 2002; Bagdy, 1996). Despite the lack of selectivity of DOI for the 5-HT_{2A} receptor, subsequent research employing specific antagonists to the 5-HT_{2A} and 5-HT_{2C} receptors revealed that it is the activation of 5-HT_{2A} receptors, exclusively, which mediates the ability of DOI to induce neuronal activation within the PVN (Zhang et al., 2002). Similarly, under conditions of acute stress, antagonism of the serotonergic 5-HT_{2A} receptor is capable of attenuating activation of the HPA axis (Saphier et al., 1995; Feldman et al., 1998), indicating that serotonergic 5-HT_{2A} receptor signalling may be an endogenous stimulator of the HPA axis. To date, there is no research regarding the ability of the serotonergic 5-HT_{2A} receptor to activate the HPA axis under conditions of prolonged glucocorticoid hypersecretion. Given that the serotonergic 5-HT_{2A} receptor provides excitatory tone over the HPA axis, and that HPA axis activation is diminished under conditions of chronically elevated glucocorticoids, we sought to determine if chronic corticosterone administration modulated the ability of the serotonergic 5-HT_{2A} receptor to activate the HPA axis.

* Corresponding author. Tel.: +1 604 822 3095; fax: +1 604 822 6923.
E-mail address: bgorzalka@psych.ubc.ca (B.B. Gorzalka).

0014-2999/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2008.11.035

2. Materials and methods

2.1. Animals

Sixteen adult male Long–Evans rats were obtained between 300–350 grams (Charles River, Montreal, Canada). Rats were housed in groups of three in triple wire mesh cages. Colony rooms were maintained at 21° on a 12:12 light cycle (lights on 0900 h). Purina Rat Chow and tap water were provided *ad libitum*. Rats were randomly assigned into two conditions: 1) corticosterone-21-acetate (20 mg/kg; Sigma-Aldrich, Canada); or 2) vehicle (100% propylene glycol). Dosage of corticosterone was determined based on previous research demonstrating that 20 mg/kg corticosterone is capable of regulating serotonergic 5-HT_{2A} receptor binding in the cortex and behavioural stereotypies elicited by serotonergic 5-HT_{2A} receptor activation (Kuroda et al., 1992; Gorzalka et al., 2001). Rats were subcutaneously injected with vehicle or corticosterone daily for 14 days using 26 gauge 1/2 in. needles (between 1000 h and 1100 h when the corticosterone diurnal rhythm is at its trough; Girotti et al., 2007). Twenty four h following the final injection, animals in both treatment conditions were subdivided into two groups and administered a single challenge dose of either 1) vehicle (0.9% Saline); or 2) DOI (2.5 mg/kg; Sigma), similar to that employed by van de Kar and colleagues (2001). All injections were counterbalanced across conditions and occurred between 1000 h and 1200 h. Following this injection schedule, rats were transcardially perfused 2 h later with 4% paraformaldehyde. Brains were removed and placed in fixative overnight at 4 °C. Following this, brains were cryoprotected in a 20% sucrose solution for a week. Brains were then sliced using a vibratome (Leica), coronally sliced into 40 µm sections and processed immunohistochemically free-floating. All treatments of animals were approved by the Animal Ethics Committee of the University of British Columbia and were consistent with the standards of the Canadian Council on Animal Care.

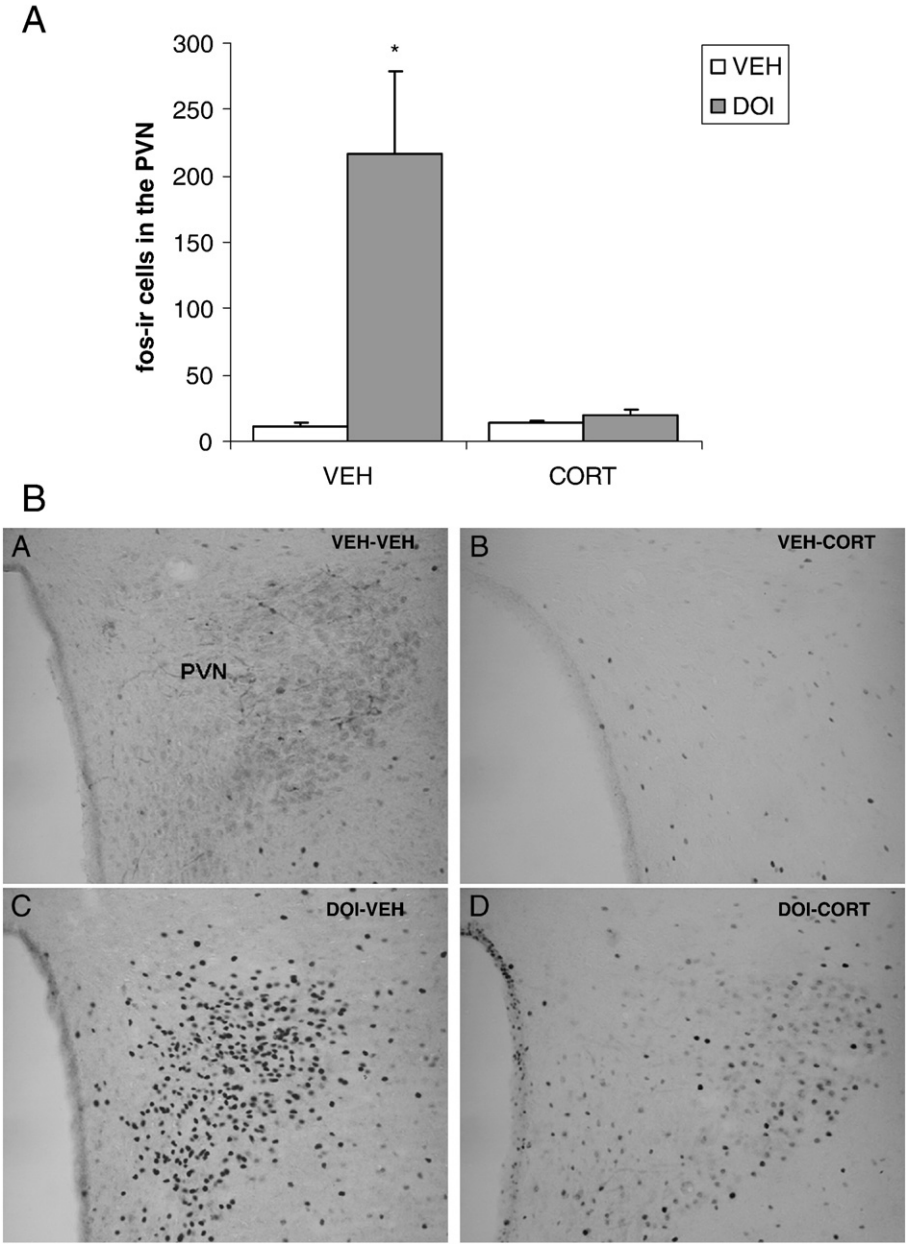

Fig. 1. A) The effect of an acute challenge with the 5-$HT_{2A/2C}$ receptor agonist DOI (2.5 mg/kg), or vehicle (VEH), on fos immunoreactive (fos-ir) cells within the PVN following 14 days administration of corticosterone (CORT; 20 mg/kg/day), or VEH. Values are denoted as mean fos-ir cell counts ± S.E.M. * denotes significant differences relative to all other treatment conditions (n=4 for each condition). B) Representative photomicrographs of fos-ir cells in the PVN in each treatment condition.

2.2. Immunohistochemical analysis

Sections were washed in 0.05 M Tris buffered saline (TBS; pH 7.4) and incubated in 0.3% peroxide, then washed again in TBS. The sections were then blocked with 4% goat serum and 0.2% Triton X-100 in TBS solution for 2 h. Following this, sections were incubated for 48 h at 4 °C in primary antibody of polyclonal rabbit antisera against residues 4–17 of human Fos protein (Oncogene Labs, Cambridge, MA, USA) at 1:10,000. After this period, sections were washed in TBS and incubated in biotinylated, goat anti-rabbit IgG (1:400) for 2 h, and again washed in TBS. Tissue was then developed with Vector elite ABC kit (Vector Laboratories, Burlinghame, CA, USA) and visualized using a nickel ammonium sulfate intensified 3,3′-diaminobenzidine kit as per manufacturer's instructions. Tissue was subsequently mounted and coverslipped. Counts of cells which were positive for fos immunoreactivity were taken by a blind observer in 200 μm intervals using C-imaging high performance Imaging software (Compix Inc. Imaging Systems, Cranberry Township, PA, USA).

2.3. Statistical analysis

Data regarding fos immunoreactive cell counts was analyzed by univariate analysis of variance with corticosterone and DOI as fixed factors, followed by Tukey's post hoc comparisons. All data are represented as group means±S.E.M.

3. Results

Analyses revealed a significant interaction between DOI administration and corticosterone treatment on fos immunoreactive cells in the PVN [$F_{(1,12)}$=8.08, P=0.015]. Post-hoc comparisons demonstrated that administration of DOI to animals previously receiving vehicle evoked a robust induction of fos immunoreactivity within the PVN (P>0.001 relative to all other treatment conditions); however, following chronic corticosterone administration, DOI had no significant effect on fos immunoreactivity in the PVN (P>0.05 relative to vehicle-DOI and corticosterone-vehicle). Data regarding the effects of corticosterone treatment and DOI administration on fos immunoreactivity in the PVN can be seen in Fig. 1A, while representative pictures of fos immunoreactive cells in the PVN for all treatment conditions can be seen in Fig. 1B.

4. Discussion

This study demonstrated that the ability of the serotonergic 5-$HT_{2A/2C}$ receptor agonist DOI to induce fos immunoreactivity within the PVN is abolished by chronic pre-treatment with the glucocorticoid hormone corticosterone. Previous research has indicated that serotonergic 5-HT_{2A} receptors, and not 5-HT_{2C} receptors, localized directly within the PVN mediate the ability of DOI to both induce fos immunoreactivity within the PVN and adrenocortical secretion (van de Kar et al., 2001; Zhang et al., 2002). Thus, the current data imply that prolonged elevations in circulating glucocorticoid hormones result in a desensitization of the ability of serotonergic 5-HT_{2A} receptors within the PVN to activate the HPA axis.

Basal activity of the HPA axis (both glucocorticoid secretion and fos immunoreactivity in the PVN) exhibits a diurnal rhythm with a peak occurring in the dark phase and the trough occurring at the onset of the light phase (Girotti et al., 2007). The measurements in the current study were taken during the trough of the HPA axis daily rhythm (which corresponds to the onset of the inactive phase of the day) to allow maximal detection of HPA axis activation. Future work should examine if these effects are maintained when experimental manipulations occur during the dark phase when the HPA axis exhibits a higher level of basal activity.

Persistently elevated glucocorticoid levels result in an attenuation of HPA axis output, presumably in an attempt to limit subsequent adrenocortical secretion (Calogero et al., 1991). At the systems level, this is often seen as a habituation of the neuroendocrine response to stress (Armario, 2006); however, the neural mechanisms subserving the ability of glucocorticoids to dampen HPA axis function are not well characterized. Glucocorticoid mediated suppression of CRH transcription within the PVN is believed to contribute to this phenomenon (Makino et al., 1995), but other mechanisms remain to be identified. Under conditions of acute stress, serotonergic 5-HT_{2A} receptor signalling contributes to HPA axis activation (Saphier et al., 1995; Feldman et al., 1998). Thus, based on the current data, it seems reasonable to presume that one mechanism by which prolonged glucocorticoid hypersecretion dampens activity of the HPA axis is potentially via a desensitization of serotonergic 5-HT_{2A} receptors within the PVN. Future research is required to determine if chronic stress elicits comparable alterations in serotonergic 5-HT_{2A} receptor activity within the PVN, and in turn if this phenomenon is contributing to the habituation of the neuroendocrine response to stress.

It should be noted that this apparent ability of glucocorticoids to desensitize serotonergic 5-HT_{2A} receptors within the PVN is in contrast to the results of several other studies demonstrating that in the cerebral cortex, chronically elevated glucocorticoids increase serotonergic 5-HT_{2A} receptor binding (Kuroda et al., 1992; Fernandes et al., 1997). Similarly, chronic administration of corticosterone potentiates serotonergic 5-HT_{2A} receptor mediated behavioural stereotypes (Gorzalka et al., 2001), which in part are believed to be driven by cortical serotonergic 5-HT_{2A} receptor activity (Willins and Meltzer, 1997). Thus, the ability of glucocorticoids to modulate serotonergic 5-HT_{2A} receptor function appears to be regionally specific. Given that several studies have suggested a pertinent role of both glucocorticoids and the serotonergic 5-HT_{2A} receptor in affective disorders (Holsboer, 2000; Stein et al., 2007), investigating the reciprocal relationships between these two systems may provide a better understanding of how their interaction relates to the development of pathological affective states.

Acknowledgements

This research was supported by an operating grant to BBG from the Natural Sciences and Engineering Research Council of Canada (NSERC), and graduate scholarships to MNH by NSERC and the Michael Smith Foundation for Health Research. The authors would like to thank Katia Sinopoli for her technical assistance.

References

Armario, A., 2006. The hypothalamic–pituitary–adrenal axis: what can it tell us about stressors? CNS Neurol. Disord. Drug Targets 5, 485–501.
Bagdy, G., 1996. Role of the hypothalamic paraventricular nucleus in 5-HT1A, 5-HT2A and 5-HT2C receptor-mediated oxytocin, prolactin and ACTH/corticosterone responses. Behav. Brain Res. 73, 277–280.
Calogero, A.E., Liapi, C., Chrousos, G.P., 1991. Hypothalamic and suprahypothalamic effects of prolonged treatment with dexamethasone in the rat. J. Endocrinol. Invest. 14, 277–286.
Chaouloff, F., 1995. Regulation of 5-HT receptors by corticosteroids: where do we stand? Fundam. Clin. Pharmacol. 9, 219–233.
Feldman, S., Conforti, N., Weidendfeld, J., 1995. Limbic pathways and hypothalamic neurotransmitters mediating adrenocortical responses to neural stimuli. Neurosci. Biobehav. Rev. 19, 235–240.
Feldman, S., Newman, M.E., Gur, E., Weidenfeld, J., 1998. Role of serotonin in the amygdala in hypothalamo–pituitary–adrenocortical responses. Neuroreport 9, 2007–2009.
Fernandes, C., McKittrick, C.R., File, S.E., McEwen, B.S., 1997. Decreased 5-HT1A and increased 5-HT2A receptor binding after chronic corticosterone associated with a behavioural indication of depression but not anxiety. Psychoneuroendocrinology 22, 477–491.
Girotti, M., Weinberg, M.S., Spencer, R.L., 2007. Differential responses of hypothalamus–pituitary–adrenal axis immediate early genes to corticosterone and circadian drive. Endocrinology 148, 2542–2552.
Gorzalka, B.B., Hanson, L.A., Hong, J.J., 2001. Ketanserin attenuates the behavioral effects of corticosterone: implications for 5-HT(2A) receptor regulation. Eur. J. Pharmacol. 428, 235–240.
Holsboer, F., 2000. The corticosteroid receptor hypothesis of depression. Neuropsychopharmacology 23, 477–501.
Kuroda, Y., Mikuni, N., Ogawa, T., Takahashi, K., 1992. Effect of ACTH, adrenalectomy and the combination treatment on the density of 5-HT2 receptor binding sites in neocortex of rat forebrain and 5-HT2 receptor-mediated wet-dog shake behaviours. Psychopharmacology 108, 27–32.

Liposits, Z., Phelix, C., Paull, W.K., 1987. Synaptic interaction of serotonergic axons and corticotropin releasing factor (CRF) synthesizing neurons in the hypothalamic paraventricular nucleus of the rat. A light and electron microscopic immunocytochemical study. Histochemistry 86, 541–549.

Makino, S., Schulkin, J., Smith, M.A., Pacak, K., Palkovits, M., Gold, P.W., 1995. Regulation of corticotropin-releasing hormone receptor messenger ribonucleic acid in the rat brain and pituitary by glucocorticoids and stress. Endocrinology 136, 4517–4525.

Pecoraro, N., Dallman, M.F., Warne, J.P., Ginsberg, A.B., Laugero, K.D., la Fleur, S.E., Houshyar, H., Gomez, F., Bhargava, A., Akana, S.F., 2006. From Malthus to motive: how the HPA axis engineers the phenotype, yoking needs to wants. Prog. Neurobiol. 79, 247–340.

Saphier, D., Farrar, G.E., Welch, J.E., 1995. Differential inhibition of stress-induced adrenocortical responses by 5-HT1A agonists and by 5-HT2 and 5-HT3 antagonists. Psychoneuroendocrinology 20, 239–257.

Stein, D.J., Hemmings, S., Moolman-Smook, H., Audenaert, K., 2007. 5-HT2A: its role in frontally mediated executive function and related psychopathology. CNS Spectr. 12, 512–516.

van de Kar, L.D., Blair, M.L., 1999. Forebrain pathways mediating stress-induced hormone secretion. Front. Neuroendocrinol. 20, 1–48.

van de Kar, L.D., Javed, A., Zhang, Y., Serres, F., Raap, D.K., Gray, T.S., 2001. 5-HT2A receptors stimulate ACTH, corticosterone, oxytocin, renin and prolactin release and activate hypothalamic CRF and oxytocin expressing cells. J. Neurosci. 21, 3572–3579.

Willins, D.L., Meltzer, H.Y., 1997. Direct injection of 5-HT2A receptor agonists into the medial prefrontal cortex produces a head-twitch response in rats. J. Pharmacol. Exp. Ther. 282, 699–706.

Zhang, Y., Damjanoska, K.J., Carrasco, G.A., Dudas, B., D'Souza, D.N., Tetzlaff, J., Garcia, F., Sullivan Hanley, N.R., Scripathirathan, K., Petersen, B.R., Gray, T.S., Battaglia, G., Muma, N.A., van de Kar, L.D., 2002. Evidence that 5-HT$_{2A}$ receptors in the hypothalamic paraventricular nucleus mediate neuroendocrine responses to (−)DOI. J. Neurosci. 22, 9635–9642.