Behavioural Brain Research 235 (2012) 30–35

Contents lists available at SciVerse ScienceDirect

Behavioural Brain Research

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

Short communication

Corticosterone attenuates conditioned fear responses and potentiates the expression of GABA-A receptor alpha-2 subunits in the brain structures of rats selected for high anxiety

A. Wisłowska-Stanek[a,*], M. Lehner[b], A. Skórzewska[b], P. Maciejak[a,b], J. Szyndler[a], D. Turzyńska[b], A. Sobolewska[b], A. Płaźnik[a,b]

[a] Department of Experimental and Clinical Pharmacology, Medical University of Warsaw, 26/28 Krakowskie Przedmieście Street, 00-927 Warsaw, Poland
[b] Department of Neurochemistry, Institute of Psychiatry and Neurology, 9 Sobieskiego Street, 02-957 Warsaw, Poland

HIGHLIGHTS

- Corticosterone decreased expression of conditioned fear in high anxiety rats (HR).
- Fear increased expression of alpha-2 subunits of GABA-A receptor in limbic structures.
- Corticosterone potentiated effects of conditioned fear on alpha-2 subunit expression.

ARTICLE INFO

Article history:
Received 30 May 2012
Received in revised form 6 July 2012
Accepted 10 July 2012
Available online 20 July 2012

Keywords:
GABA-A receptor alpha-2 subunits
Immunocytochemistry
Brain structures
Corticosterone
Conditioned fear
Individual differences

ABSTRACT

The aim of the experiment was to assess the effects of an acutely administered corticosterone on the expression of GABA-A receptor alpha-2 subunits in the brain structures of high (HR) and low (LR) anxiety rats (divided according to their conditioned fear-induced freezing response) subjected to a second conditioned fear session (1 week after fear conditioning). We found that corticosterone (20 mg/kg, sc) given to rats prior to the second conditioned fear session significantly enhanced a decrease in fear expression in the HR group. The behavioural effect of fear was accompanied by the increased expression of alpha-2 subunits in the basolateral amygdala (BLA) and the dentate gyrus of the hippocampus (DG) of the HR group. Corticosterone potentiated the effect of fear on alpha-2 subunit expression in the BLA, DG, the cingulate cortex area 1 and the secondary motor cortex (areas Cg1 and M2). The current study provides insight into the mechanisms that may be responsible for the beneficial effects of glucocorticoids in the therapy of some anxiety disorders.

Acute glucocorticosteroid administration facilitates active behavioural coping in threatening situations [1]. We previously found that a single dose of corticosterone given to rats before training in a conditioned fear test significantly attenuated the freezing response when examined 24 h later [2,3]. Similarly, the acute administration of corticosterone before a session in an elevated plus maze produced an anxiolytic effect in rats [4]. It is also noteworthy that cortisol (20 mg) administered orally 1 h before each extinction-based session of psychotherapy significantly reduced anxiety symptoms during exposure to a phobic situation [5].

Recently, using a model of individual differences in fear responses of rats selected according to low and high freezing responses in the contextual fear test (defined as 'low- and high anxiety' rats; LR and HR groups, respectively), we found that HR rats had deficits in the activity of the brain structures that control the cognition necessary to cope with stress (i.e., the prefrontal cortex, as measured by c-Fos expression) and increased activity of the amygdalar nuclei that enhance the stress response (c-Fos/glucocorticoid receptors-ir) [6]. We also observed that some behavioural or pharmacological interventions attenuated the increased fear responses of HR rats. For example, we found that the administration of D-cycloserine and midazolam before the testing session attenuated the freezing of HR rats and increased the expression of alpha-2 subunits of the GABA-A receptor in limbic areas and the prefrontal cortex [7]. Moreover, other preclinical data suggest that a decrease in conditioned fear is correlated with the upregulation of GABAergic markers (i.e., alpha-2 subunits of the GABA-A receptor) in the amygdala [8]. The role of alpha-2 subunits in fear processing is evidenced by the finding that mice with point-mutated alpha-2 GABA-A receptor subunits are resistant to the anxiolytic-like effects

* Corresponding author. Tel.: +48 22 8262116; fax: +48 22 8262116.
E-mail address: awislow@yahoo.com (A. Wisłowska-Stanek).

0166-4328/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bbr.2012.07.018

of benzodiazepines and display a greater behavioural response to fear-conditioned stimuli [9]. In light of these data, we decided to test a hypothesis that the corticosterone-induced decrease in rat freezing responses is related to the drug and stress-induced changes in the expression of alpha-2 subunits in limbic and cortical areas.

The experiments were performed on a cohort of 71 male Wistar rats (180–200 g body weight) purchased from a licenced breeder and housed in standard laboratory conditions under a 12 h light/dark cycle (lights on at 7 a.m.). The experiments were performed in accordance with the European Communities Council Directive of November 24th, 1986 (86/609 EEC). The Local Committee for Animal Care and Use at Warsaw Medical University, Poland approved all experimental procedures using animal subjects.

After 4 days of acclimatisation, 40 animals (S group) were subjected to a conditioned fear test as previously described by Wisłowska-Stanek et al. [7]. In short, the experiment was performed over 3 consecutive days. On the first day, the animals were individually placed in a training box for 2 min to adapt to the experimental conditions. The following day, after 5 min of habituation to the training box, animals underwent a fear conditioning procedure that consisted of 3 footshocks (0.7 mA, 1 s, repeated every 59 s), and the animals were removed from the testing box 3 min after the last footshock was delivered. Conditioned fear was tested on the third day (test day, T1) by re-exposing rats to the testing box and recording the freezing response over 10 min. Freezing behaviour was measured by photo beams (10 Hz detection rate) controlled by a fear conditioning PC-program. The animals were divided according to their context-induced freezing responses into LR, low-anxiety rats with total durations of freezing responses one SEM or more below the mean (i.e., <224.1 s, mean = 246 s and SEM = 21.9 s), and an HR group consisting of high-anxiety rats with total durations of freezing responses one SEM or more above the mean (i.e., >267.9 s mean = 246 s, SEM = 21.9 s). These animals were defined as the LR-T1 ($n = 18$) and HR-T1 ($n = 16$) group, respectively. The control group, C ($n = 6$), was not conditioned but only placed in the conditioning boxes (mean freezing duration = 92.7 s, SEM = 24.45). After testing, animals remained undisturbed in their home cages for 7 days and were then subjected to the aversive context in a second fear testing session (T2). Before the T2 session, the pre-selected groups, LR-T1 and HR-T1, were further divided into the following subgroups: LR-T2-v, low-anxiety rats given vehicle ($n = 7$); LR-T2-cort, low-anxiety rats given corticosterone ($n = 9$); HR-T2-v, high-anxiety rats given vehicle ($n = 8$); and HR-T2-cort, high-anxiety rats given corticosterone ($n = 8$). The corticosterone (20 mg/kg; Sigma–Aldrich, Poland) was suspended in sesame oil and administered subcutaneously (sc) to the nape of the neck 30 min before the T2 session. Control animals were administrated sesame oil (Sigma–Aldrich, Poland). All rats were decapitated 2 h after drug or vehicle administration.

A control study was performed on a second group of animals ($n = 25$). The rats [LR < 230.7 s (256.4 – 25.7 s); HR > 282.1 s (256.4 + 25.7 s)] were divided into four groups: LR-v, low-anxiety rats given vehicle ($n = 6$); LR-cort, low-anxiety rats given corticosterone ($n = 5$); HR-v, high-anxiety rats given vehicle ($n = 6$); and HR-cort, high-anxiety rats given corticosterone ($n = 8$). The design of the second part of the experiment was the same as the first part of the experiment with the exception that the second fear test session was not performed (T2). Thus, these animals were injected with vehicle or corticosterone 7 days after T1 and decapitated 2 h later.

Immunocytochemical staining for the alpha-2 subunit of the GABA-A receptor was performed on slide-mounted frozen brain sections. Staining and counting techniques have been described previously by Wisłowska-Stanek et al. [7]. Cells were counted in the following subregions: the cingulate cortex (areas 1 and 2), the secondary motor cortex (AP: 1.20; Cg1, Cg2, M2), the basolateral amygdala and the dentate gyrus of the hippocampus (AP – 3.14; DG, BLA) [10] (Fig. 1). Three slices from each section of the brain were taken. Western blot analyses performed previously with the same antibody confirmed the specific binding of this antibody to the alpha-2 subunit. Examples of alpha-2 subunit GABA-A receptor immunopositive cells have been published by Lehner et al. [11].

The behavioural and biochemical data are presented as the means ± the SEM. The differences between the conditioned group (S) and the control group (C) were analysed by Student's *t*-test. The differences in freezing response durations during the first and second conditioned fear test (T1 and T2) in the LR and HR groups were analysed by one-way repeated measures ANOVA followed by Tukey's post hoc test. The behavioural data from the second test session were analysed by two-way ANOVA followed by Tukey's post hoc test. The immunocytochemical data were analysed by three-way ANOVA followed by Tukey's post hoc test. For correlation analyses, a Pearson coefficient was calculated. Statistical analyses

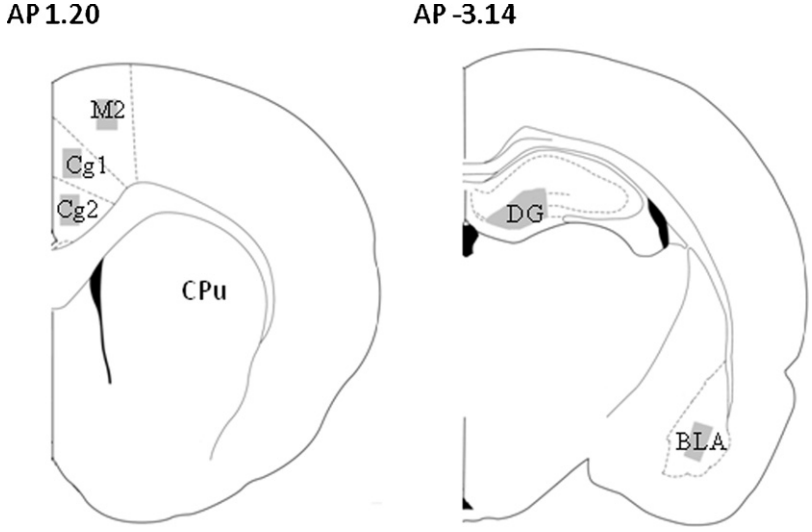

Fig. 1. Diagrams adapted from Paxinos and Watson [10] showing regions of the brain analysed for expression of alpha-2 subunits. BLA: basolateral amygdala; Cg1, Cg2: cingulate cortex area 1 and 2; CPu: caudate putamen; DG: dentate gyrus of the hippocampus; M2 area: prefrontal cortex, secondary motor cortex. Shaded areas indicate the analysed brain regions.