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Research report

Behavioral effects and CRF expression in brain structures of high- and low-anxiety rats after chronic restraint stress

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HIGHLIGHTS

- Chronic restraint increased immobility in Porsolt test in high anxiety rats (HR).
- Chronic restraint increased anxiety of HR rats in the open field test.
- Chronic restraint decreased sucrose solution consumption in HR rats.
- HR restraint had decreased CRF density in paraventricular nucleus of hypothalamus.
- HR restraint had decreased CRF expression in dentate gyrus of hippocampus.

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ABSTRACT

The aim of our study was to investigate the influence of chronic restraint stress (5 weeks, 3 h/day) on behavior and central corticotropin-releasing factor (CRF) expression in rats selected for high (HR) and low anxiety (LR). The conditioned freezing response was used as a discriminating variable. Moreover, we assessed the influence of acute restraint on CRF expression in the brain in HR and LR rats. We found that chronic restraint induced symptoms of anhedonia (decreased consumption of 1% sucrose solution) in HR rats. In addition, HR restraint rats showed an increased learned helplessness behavior (immobility time in the Porsolt test) as well as neophobia in the open field test vs. LR restraint and HR control rats. These behavioral changes were accompanied by a decreased expression of CRF in the paraventricular nucleus of the hypothalamus (pPVN) and the dentate gyrus of the hippocampus (DG) compared to the HR control and LR restraint rat groups, respectively. The acute restraint condition increased the expression of CRF in the pPVN of HR rats compared to the HR control group, and enhanced the expression of CRF in the CA1 area and DG of LR restraint animals compared to the HR restraint and LR control rats, respectively. The present results indicate that chronic restraint stress in high anxiety rats attenuated CRF expression in the pPVN and DG, which was probably due to detrimental actions on the hippocampus-hypothalamus-pituitary-adrenal gland feedback mechanism, thus modulating the stress response and inducing anhedonia and depressive-like symptoms.

1. Introduction

An individual's genetic predisposition has been implicated in the sensitivity or resiliency to the development of stress-related depressive symptoms [1–3]. In preclinical studies, chronic stress induces depressive-like symptoms, e.g., learned helplessness behavior and anhedonia, only in some animals but not the entire animal population. Nevertheless, the molecular basis underlying individual differences in the behavioral responses to stressors still remains unresolved [2–5]. The incidence of depressive symptoms in almost every situation involves the dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis, which is initiated by the activity corticotrophin-releasing factor (CRF) neurons in the hypothalamic paraventricular nucleus (PVN) [6–9]. The CRF system, which is highly responsive to the environment, has been suggested to serve as a key interface between environmental stressors and

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the development of depression or depressive-like behavior [10,11]. Differences in the functioning of the CRF system due to genetic variability may lead to differences in an individual's response to stressful events [6,9]. CRF stimulates the secretion of adrenocorticotropin hormone (ACTH), which induces the production of glucocorticoids in the adrenal cortex [12]. The increase in plasma glucocorticoids reflects the activation of the HPA axis and initiates a negative feedback effect on the hippocampus, hypothalamus and anterior pituitary, as well as a positive feedback effect on the amygdala [13]. CRF may also act as an inducer of aversive processes in the limbic system, regardless of its hormonal effect on ACTH [14–16].

In recent years, we have studied the central mechanisms responsible for individual vulnerability to stressors by employing a model that assigns rats to high- (HR) or low- (LR) anxiety groups based on the duration of their conditioned freezing response in a contextual fear test. In our previous studies, we found that high- and low-anxiety rats differ in their susceptibility for developing anxiety- and depressive-like behavior upon 21 daily sessions of chronic restraint. HR restraint rats revealed an increased neophobia in open field test (OFT) and immobility time in the Porsolt test compared to LR restraint rats, which was accompanied by central effects, e.g., a lower concentration of corticosterone in the prefrontal cortex and a lower density of alpha-2 subunit of GABA-A receptor in the prefrontal cortex and dentate gyrus of the hippocampus, and a higher subunit expression in the basolateral amygdala, compared to that in LR restraint rats [17]. HR rats were also more susceptible to the anxiogenic (in the open field test of neophobia) and depressive (in the Porsolt test) effects of 21 chronic injections of corticosterone (20 mg/kg). These effects were associated with decreased expression of alfa-2 subunit of GABA-A receptors in the medial prefrontal cortex and increased density in the basolateral amygdala, suggesting an impaired control of the prefrontal cortex over the basolateral amygdala [18].

Considering these previous results and those reported in the literature, we decided to test in the present study the hypothesis that HR rats are more likely to develop anxiety- and depression-like behavior after 5 weeks of chronic restraint stress, and these effects are accompanied by selective changes in CRF expression in the brain regions involved in the expression of affective responses and regulation of hormonal axis stimulated by stressors (hypothalamus, medial prefrontal cortex, hippocampus). We would like to take advantage of the fact that the model of chronic immobilization induces the depressive-like behavior only in predisposed animals, but not in the whole animal population, so it is a good tool to assess individual differences in the susceptibility to the stress [1,2]. Thus, the important new point of the study is the analysis of the central CRF expression in individuals differing in susceptibility to the fear evoking stimuli.

An analysis of the effects of the stress induced with immobilization, which is considered a useful model of affective pathology, could allow for a more general conclusion about the individual differences in the central mechanisms of emotional reactions.

2. Materials and methods

2.1. Animals

The experiment was performed on 67 adult male Wistar rats (220–240 g at the beginning of the experiment) that were bought from a licensed breeder. Animals were housed in standard laboratory conditions under a 12-h light/dark cycle (lights on at 7 a.m.) and at a constant temperature (21 ± 2 °C). The experiment was performed in accordance with the European Communities Council Directive of 24 November 1986 (86/609 EEC). The Local Committee for Animal Care and Use at Warsaw Medical University, Poland, approved all experimental procedures using animal subjects.

2.2. Experimental protocol (Fig. 1)

The experiment was conducted in two parts. In the first part of the experiment, we evaluated the influence of chronic restraint on the behavior and brain CRF expression of HR and LR rats. The aim of the second part of the study, which was the control, was to assess the influence of an acute restraint on CRF expression in the brain structures of rats with a diverse anxiety phenotype.

In the chronic restraint procedure, after seven days of acclimatization, 35 animals were divided into HR and LR rats according to their behavior in the conditioned fear test (CFT). Next, HR and LR groups were randomized into restraint (HR restraint, n = 8; LR restraint n = 8) and control groups (HR control n = 8; LR control, n = 9). Two rats did not meet criterion for either LR or HR rats. Animals were restrained for 35 consecutive days (3 h/day), while controls were only handled 10 min per day. The body weights of all rats were measured weekly. Once a week, beginning with the 25th day of the experiment (14th day of the chronic restraint), animals were exposed for 20 h to a 1% sucrose solution (1 bottle test) [19]. On the 47th day, 24 h after the last session of chronic restraint, training for the Porsolt test was performed, and 24 h later, on the 48th day, the open field test (OFT) was performed, which was followed 1 h later by the Porsolt test. Next, 45 min after performing the Porsolt test and resting in their home cages, the animals were decapitated (to avoid an influence of acute stress on CRF protein production) and their brains were removed and frozen at −70° C.

In the acute restraint experiment, which was performed seven days after acclimatization, 32 animals were assigned to low- or high-anxiety rat groups (LR, HR) according to their behavior in the conditioned fear test. One week later, the HR and LR animals were randomized into 3 h acute restraint (HR restraint, n = 8; LR restraint n = 6) or control groups (HR control, n = 8; LR control, n = 6). Four rats did not meet the criterion for either LR or HR rats. The animals were then sacrificed 90 min after the end of the acute restraint procedure. The brains were retrieved as described above for the immunochemistry analysis. The schemes of the experimental protocols are shown in Fig. 1.

2.3. Contextual fear test and division of the animals into HR and LR groups

The fear-conditioning experiment was performed using a computerized fear-conditioning system (TSE, Bad Homburg Germany) in two experimental cages (36 × 21 × 20 cm, *w/l/h*) and in the presence of constant white noise (65 dB). The experiment was conducted over three consecutive days. On the first day, the rats were individually placed in a training box for 2 min for habituation to the experimental conditions. The following day, after 2 min, the animals underwent the fear-conditioning procedure, and each animal received three footshocks (stimulus: 0.7 mA, 1 s, repeated every 60 s) during a 10-min session. The conditioned fear was tested on the third day by examining the freezing response of rats during a 10-min context fear test that involved re-exposure to the testing box. Footshocks were not delivered at this stage of the experiment. The conditioned response (i.e., the freezing response) was recorded and analyzed by the fear-conditioning system. Freezing behavior was measured by photo beams (10-Hz detection rate) controlled by the fear-conditioning PC program. Photo beams were spaced 1.3 and 2.5 cm apart in the direction of the x- and y-axes, respectively. The absolute duration of freezing was defined as no interruption of the photo beams for 5 s, and the total freezing time was calcu-