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The effect of chronic administration of corticosterone on anxiety- and depression-like behavior and the expression of GABA-A receptor alpha-2 subunits in brain structures of low- and high-anxiety rats

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ABSTRACT

The aim of this study was to examine changes in rat emotional behavior and determine differences in the expression of GABA-A receptor alpha-2 subunits in brain structures of low- (LR) and high-anxiety (HR) rats after the repeated corticosterone administration. The animals were divided into LR and HR groups based on the duration of their conditioned freezing in a contextual fear test. Repeated daily administration of corticosterone (20 mg/kg) for 21 days decreased activity in a forced swim test, reduced body weight and decreased prefrontal cortex corticosterone concentration in both the LR and HR groups. These effects of corticosterone administration were stronger in the HR group in comparison with the appropriate control group, and compared to LR treated and LR control animals. Moreover, in the HR group, chronic corticosterone administration increased anxiety-like behavior in the open field and elevated plus maze tests. The behavioral effects in HR rats were accompanied by a decrease in alpha-2 subunit density in the medial prefrontal cortex (prelimbic cortex and frontal association cortex) and by an increase in the expression of alpha-2 subunits in the basolateral amygdala. These studies have shown that HR rats are more susceptible to anxiogenic and depressive effects of chronic corticosterone administration, which are associated with modification of GABA-A receptor function in the medial prefrontal cortex and basolateral amygdala. The current data may help to better understand the neurobiological mechanisms responsible for individual differences in changes in mood and emotions induced by repeated administration of high doses of glucocorticoids or by elevated levels of these hormones associated with chronic stress or affective pathology.

Introduction

Glucocorticoids are released in response to physical, emotional and/or metabolic stressors, and many of the effects of glucocorticoids are thought to serve as adaptive responses to stressful events (Levy and Tasker, 2012). The chronic stress or repeated corticosterone administration may lead to profound maladaptive changes in emotional behavior, thereby mimicking human mental disorders such as anxiety and depression (Erickson et al., 2003; Korte, 2001; McEwen, 2005; Mitchell and O'Keane, 1998; Swaab et al., 2005). We previously found that chronic corticosterone administration decreased plasma corticosterone concentration, inhibited exploratory behavior, enhanced freezing behavior on a retest of fear conditioning (with the final injection being given 90 min before contextual fear conditioning training), and produced complex changes in c-Fos expression: a decrease in the magnocellular neurons of the paraventricular hypothalamic nucleus, and an increase in the secondary motor cortex and in the central nucleus of amygdala (CeA) (Skórzewska et al., 2006).

Preclinical findings indicate reciprocal regulation of emotional behavior by GABA-A receptors and glucocorticoids (Lussier et al., 2013; Mody and Maguire, 2012; Orchinik et al., 1995; Verkuyl et al., 2005). The structure of GABA-A receptors causes differences in their pharmacological and functional characteristics. It seems that the effect of GABA on emotional behavior depends on activation of specific GABA-A receptor subunits. For example, it has been shown that activation of alpha-2 subunits of GABA-A receptors is involved in the regulation of anxiety and depression-like behavior (Lussier et al., 2013; cf. Smith and Rudolph, 2012). In this context it is noteworthy that glucocorticoids can alter the uptake and release of GABA and can also decrease benzodiazepine receptor binding in the hippocampus and amygdala (Drugan et al., 1989; Miller et al., 1988; Orchinik et al., 1995; Wilson and Biscardi, 1994).

We have recently been studying the central mechanisms responsible for individual vulnerability to stress by employing a model that divides

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rats into high- (HR) and low-anxiety (LR) groups based on the duration of their conditioned freezing response in a contextual fear test. We found that HR rats had deficits in 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) (Lehner et al., 2009a). The HR rats additionally showed enhanced concentrations of CRF-positive cells in the basolateral amygdala (BLA) and parvocellular neurons of the paraventricular hypothalamic nucleus (pPVN) and had higher basal concentrations of GABA-A receptor alpha-2 subunits in the amygdala (compared with an unconditioned control group) (Lehner et al., 2008, 2010a). We also observed that some behavioral interventions (extinction and relearning of a conditioned fear response) and pharmacological interventions (D-cycloserine, midazolam, corticosterone) attenuated the increased fear responses of HR rats (Lehner et al., 2009b, 2010a, 2010b).

The aim of this study was to test the hypothesis that animals more vulnerable to stress are more likely to develop anxiety- and depression-like behavior following chronic corticosterone exposure. Thus, in the present study, we sought to determine if there are individual differences in rat emotional behavior and expression of GABA-A receptor alpha-2 subunits in the brain structures of low- (LR) and high-anxiety (HR) rats after repeated administration of corticosterone. We concentrated particularly on the medial prefrontal cortex and amygdalar nuclei to verify and extend previous findings that emphasized the role of these areas in mediating the central effects of stress in HR animals.

Materials and methods

Animals

The experiments were performed on 30 male Wistar rats (280–300 g body weight), purchased from a licensed breeder (The Center for Experimental Medicine of the Medical University, 24A Skłodowskiej-Curie Str., Białystok, Poland) and housed under standard laboratory conditions with a 12 h light/dark cycle (lights on at 7 a.m.) at a constant temperature (21 ± 2 °C). The experiments were 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 the Warsaw Medical University, Poland approved all experimental procedures using animals.

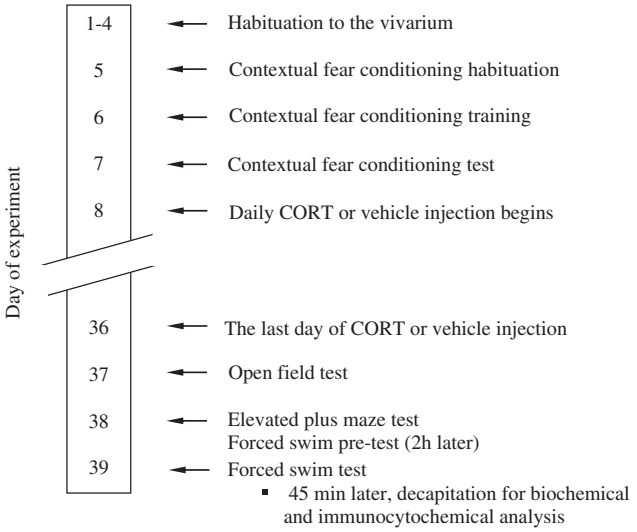

Fig. 1. Treatment scheme.

Experimental protocol (Fig. 1)

After four days of acclimatization to the vivarium, the animals were subjected to a contextual fear-conditioning test to assess individual responses to conditioned aversive stimuli (Lehner et al., 2010a, 2010b, 2011).

Contextual fear-conditioning test

The fear-conditioning experiment was performed using a computerized fear-conditioning system (TSE, Bad Homburg, Germany; FCS 04.11) in a Plexiglas cage (36 × 21 × 20 cm, w/l/h) with a steel foot-shock grid (the 38 floor bars are 0.4 cm in diameter and are spaced 0.5 cm apart), under constant white noise (65 dB) and constant illumination (12 V, 10 W halogen lamp ~150 lx). Freezing behavior was recorded by an infrared photobeam system (10 Hz detection rate) controlled by the fear-conditioning system. Photobeams were spaced 1.3 cm in the direction of the x-axis and 2.5 cm in the direction of the y-axis. This method and equipment have been used in our and other laboratories for years and have been validated pharmacologically using many clinically effective and experimental anxiolytic and anxiogenic agents (Maciejak et al., 2008; Stiedl et al., 2000).

The absolute duration of inactivity was calculated by the fear-conditioning system. Absolute duration was defined as no interruption of any photobeam over 5-s periods, which was then summarized for the whole experimental session to yield total time of freezing. The box was cleaned with 95% ethanol after each trial. The experiment was performed on three consecutive days in the same testing box and experimental chamber. On the first day, the animals were placed separately for 2 min in a training box without aversive stimulation to adapt to the experimental conditions. On the second day, a training day, animals were placed for 10 min in the training box. After a 5-min pause, the animals received four 1-s footshocks repeated every 59 s (each consisted of a train of stimuli: 0.8 mA, 150/300 ms) delivered through the stainless steel floor grid. The animals were removed from the testing boxes 1 min after the last shock was delivered. On the third experimental day, the freezing behavior of rats was observed for 10 min in the same box. The conditioned response (freezing reaction) was analyzed and recorded by the fear-conditioning system. The absolute duration of inactivity was calculated from the activity plots and expressed as total time during which the animals were inactive. The computerized method, based on the latency between photobeam interruption measures obtained during contextual fear-conditioning tests, was highly correlated with hand-scored freezing (r values ranged from 0.87 to 0.94) (Takahashi, 2004; Valentinuzzi et al., 1998). The rats were divided into two experimental groups according to the duration of context-induced freezing responses. The LR (low anxiety) group had a total duration of freezing responses one S.E.M. or more below the mean (265.04–24.95, i.e., <240.09). The HR (high anxiety) group had a total duration of freezing responses one S.E.M. or more above the mean (265.04 + 24.95, i.e., >289.99). Thus, the LR and HR animals did not overlap with respect to the duration of their conditioned fear responses. Two rats did not meet either criterion.

The rats were then divided into four experimental groups: HRveh (high-anxiety rats given a vehicle, n = 7); LRveh (low-anxiety rats given a vehicle, n = 7); HRcort (high-anxiety rats given corticosterone at a dose of 20 mg/kg, n = 7) and LRcort (low-anxiety rats given corticosterone at a dose of 20 mg/kg, n = 7). The rats received one injection of corticosterone or vehicle per day, for a total of 21 injections over 29 days. All rats were tested in the open field test 24 h after the last injection. They were then tested on the following day in the elevated plus maze test and 2 h later in the forced swim pretest. Finally, they were tested 24 h later in the forced swim test. All animals (n = 28) were decapitated 45 min after the forced swim test, and their brains were removed and frozen at −70 °C for immunocytochemistry and corticosterone analysis. The immunocytochemical and corticosterone analyses were performed on the same group of animals. The body