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Impaired glucocorticoid-mediated HPA axis negative feedback induced by juvenile social isolation in male rats

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ABSTRACT

We previously demonstrated that socially isolated rats at weaning showed a significant decrease in corticosterone and adrenocorticotropic hormone (ACTH) levels, associated with an enhanced response to acute stressful stimuli. Here we shown that social isolation decreased levels of total corticosterone and of its carrier corticosteroid-binding globulin, but did not influence the availability of the free active fraction of corticosterone, both under basal conditions and after acute stress exposure. Under basal conditions, social isolation increased the abundance of glucocorticoid receptors, while it decreased that of mineralocorticoid receptors. After acute stress exposure, socially isolated rats showed long-lasting corticosterone, ACTH and corticotrophin releasing hormone responses. Moreover, while in the hippocampus and hypothalamus of group-housed rats glucocorticoid receptors expression increased with time and reached a peak when corticosterone levels returned to basal values, in socially isolated rats expression of glucocorticoid receptors did not change. Finally, social isolation also affected the hypothalamic endocannabinoid system: compared to group-housed rats, basal levels of anandamide and cannabinoid receptor type 1 were increased, while basal levels of 2-arachidonoylglycerol were decreased in socially isolated rats and did not change after acute stress exposure. The present results show that social isolation in male rats alters basal HPA axis activity and impairs glucocorticoid-mediated negative feedback after acute stress. Given that social isolation is considered an animal model of several neuropsychiatric disorders, such as generalized anxiety disorder, depression, post-traumatic stress disorder and schizophrenia, these data could contribute to better understand the alterations in HPA axis activity observed in these disorders.

1. Introduction

Alterations in steroidogenesis and dysregulation of hypothalamic-pituitary-adrenal (HPA) axis function have been observed in several neuropsychiatric disorders (Heim and Nemeroff, 2001; Jacobson, 2014). Indeed, HPA axis hyperactivity may be implicated in melancholic depression (Gold and Chrousos, 2002), obsessive-compulsive disorder (Gustafsson et al., 2008), schizophrenia (Walker et al., 2008), and autism spectrum disorders (Jansen et al., 2006). In contrast, HPA axis activity seems to be blunted in post-traumatic stress disorder (PTSD) (Yehuda and Seckl, 2011) and atypical depression (Gold and Chrousos, 2002). Social isolation rearing, which involves isolation of the animals with no handling, starting at weaning and for an extended period, mimics several neuropsychiatric disorders. Thus, rats deprived of social contact with other rats at a young age experience a form of prolonged stress that leads to a plethora of behavioural, physiological, functional and molecular changes (Fone and Porkess, 2008; Pibiri et al., 2008; Marsden et al., 2011).

We have previously demonstrated that juvenile social isolation

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decreased plasma concentrations of adrenocorticotropic hormone (ACTH) and corticosterone (CORT) (Serra et al., 2005; Pisu et al., 2016), and induced an enhanced response to acute stressful stimuli (Serra et al., 2000, 2003) in rats. It also increased the sensitivity of the pituitary to exogenous corticotrophin-releasing hormone (CRH), and it impaired HPA negative feedback tested with the dexamethasone suppression test (Serra et al., 2005). The general aim of this paper is to further characterize the hyperresponsiveness to acute stress, and to investigate the mechanisms involved in negative feedback regulation of the HPA axis in socially isolated (SI) rats.

The main effector of the stress response is represented by CRH release from hypothalamus, the most important secretagogue of ACTH, which stimulates the synthesis and secretion of corticosteroids from the adrenal glands (Tasker and Herman, 2011). Elevated CRH concentrations are found in patients affected by depression and anxiety disorders (Hauger et al., 2009); similarly, elevated CRH induces depression- and anxiety-like behaviour in animals (Bale and Vale, 2004). Likewise, ACTH and CORT are also dysregulated in several mood disorders. We thus examined the time course of foot-shock stress-induced changes in hypothalamic CRH, as well as plasma ACTH and CORT in SI rats. We specifically focused on variations in total and free CORT. In the systemic circulation CORT is coupled to different carriers, mainly corticosteroid binding globulin (CBG) (Hammond, 1995), whose major role has been proposed to be the maintenance of the circulating CORT pool to be delivered to target tissues (Moisan et al., 2014). In addition, CBG acts as a "buffer protein" to protect tissues from excess CORT, by sequestering it in an inactive complex (Perogamvros et al., 2012). Under stress conditions, only the free fraction of CORT binds to mineralocorticoid (MR) and glucocorticoid receptors (GR) in several brain targets; thus, changes in plasma CBG levels reflect the available CORT. Previous studies indicated that both acute and chronic stress exposure decreases CBG levels, leading to a substantial increase in free CORT (Tinnikov, 1999). We thus measured the total and free fractions of CORT, as well as CBG levels, in SI rats under basal conditions and 30 min after acute foot-shock stress exposure.

Suppression of stress-induced activation of HPA axis is exerted by CORT through two types of negative feedback regulation: a "slow" action, mediated by classical delayed transcriptional effects of intracellular receptors, and a "rapid" non-genomic action, which involves membrane-associated MR (mMR) and GR (mGR) (Di et al., 2003). We sought to investigate the effect of social isolation on mMR and mGR expression in the hippocampus and hypothalamus under basal conditions; we also examined the temporal availability of mGR after foot-shock stress in these same areas from SI and group-housed (GH) rats. Moreover, we tested GR function using the non-selective antagonist mifepristone.

We further evaluated the endocannabinoid (eCB) system by measuring the expression of cannabinoid receptors type 1 (CB1R), as well as anandamide (AEA) and 2-arachidonoylglycerol (2-AG) concentrations in the hypothalamus of SI rats. In fact, glucocorticoid-induced eCB regulation of excitatory synaptic inputs to paraventricular nucleus (PVN) neuroendocrine cells seems to be a predominant mechanism in this area: activation of GR on CRH neurons stimulates the synthesis and release of AEA and 2-AG, which act as retrograde messengers on CB1R localized in glutamatergic and GABAergic interneurons (Di et al., 2003; Tasker and Herman, 2011). Given that 2-AG plays a key role in the activation of HPA axis negative feedback to terminate the stress response (Tasker and Herman, 2011; Morena et al., 2016), we also measured 2-AG content 30 min after foot-shock stress exposure. Finally, we explored CB1R activity with the antagonist AM251.

2. Material and methods

2.1. Animals

Male Sprague-Dawley CD rats (Charles River, Calco, IT) at 25–30 days of age (postnatal day PND 25–30), immediately after weaning, were housed for 30 days either in groups of five per cage (59 cm × 38 cm × 20 cm) (group-housed or GH rats), or individually in smaller cages (42 cm × 26 cm × 15 cm) (isolated or SI rats). They were maintained under an artificial 12-h light, 12-h dark cycle (light on 08:00–20:00 h) at a constant temperature of 23 ± 2 °C and 65% humidity. Food and water were freely available ad libitum. SI animals were left undisturbed in their cages and received the minimal handling associated with fortnightly husbandry. All the experiments were performed after the isolation period (PND 55–60), in the morning between 08:30 h and noon (see Fig. 1 for the timeline of the experimental procedures). Adequate measures were taken to minimize pain or discomfort of animals whose care and handling throughout the experimental procedures were in accordance with the European Parliament and the Council Directive of 22 September 2010 (2010/63/UE), and were approved by the Italian Ministry of Health according to the Italian Legislative Decree no. 26 of 4 March 2014.

2.2. Acute foot-shock stress

Animals were exposed to acute foot-shock stress at the end of the isolation period (SI rats) or at 55–60 days of age (GH rats, controls). Foot-shock stress consisted of a series of electrical impulses delivered in individual boxes with floors made of brass rods, 2 cm apart. Shocks (0.2 mA for 500 ms) were delivered every second over a period of 5 min. Separate groups of rats were sacrificed at different time points thereafter.

2.3. Pharmacological treatments

Mifepristone (#M8046, Sigma-Aldrich, Milan, Italy) was dissolved in absolute ethanol (5% final volume) and propylene glycol (Glavas et al., 2006). Animals received a single subcutaneous injection of mifepristone (120 mg/2 ml/kg bw) or vehicle (propylene glycol/5% ethanol) 60 min prior to foot-shock stress exposure, and were sacrificed 30 min later. Non-shocked controls were sacrificed 90 min after treatment.

AM251 (#S2819, Selleck Chemicals, Munich, Germany) was dissolved in DMSO, Tween-80 and physiological saline (0.9%) in a 1:1:8 ratio, respectively (Newsom et al., 2012). Rats received a single intraperitoneal injection of AM251 (2 mg/1 ml/kg bw) or vehicle (DMSO/Tween-80/0.9% saline) 30 min prior to foot-shock stress exposure, and were sacrificed 30 min later. Non-shocked controls were sacrificed 60 min after drug or vehicle injection.

2.4. ELISA

All animals were sacrificed by decapitation. Blood was collected from the trunk into K3-EDTA tubes, centrifuged at 900×g for 10 min at 4 °C and frozen at −80 °C until use. The enzyme-linked immunosorbent assay (ELISA) was used to quantify plasma CORT (#RE52211; IBL International, Hamburg, Germany), CBG (#SEB226Hu; Cloud-Clone Corp., Huston, TX, USA), and ACTH (#EK-001-21; Phoenix Pharmaceuticals Inc., Burlingame, CA, USA) levels. ELISA assays were performed according to the manufacturer's instructions using a 96-well plate pre-coated with polyclonal antibodies against an antigenic site on the CORT, CBG or ACTH molecules. The kits also provided a seven-point standard curve using two-fold serial dilutions. Each sample was run in duplicate.