Behavioural Brain Research 326 (2017) 121–131

Contents lists available at ScienceDirect

Behavioural Brain Research

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

Research report

Repeated corticosterone injections in adult mice alter stress hormonal receptor expression in the cerebellum and motor coordination without affecting spatial learning

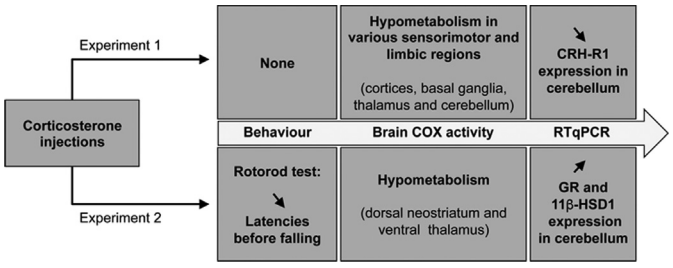
Guillaume Harlé[a], Robert Lalonde[b], Coralie Fonte[a], Armelle Ropars[a], Jean-Pol Frippiat[a], Catherine Strazielle[a,c,d,*]

[a] EA7300, Lab. «Stress, Immunity, Pathogens», Lorraine University, 54500 Vandoeuvre-les-Nancy, France
[b] EA4699, Lab. ICONES, University of Rouen, 76821 Mont-Saint-Aignan, France
[c] Service of Microscopy, Lorraine University, 54500 Vandoeuvre-les-Nancy, France
[d] CHRU de Nancy, 54500 Vandoeuvre-les-Nancy, France

HIGHLIGHTS

- Acute corticosterone induced specific motor coordination deficit in rotorod test.
- Glucocorticoid exposure decreased CRH-R1 transcription in cerebellum.
- Corticosterone effects on cerebellar CRH/CRH-R1 possibly caused motor alteration.
- Corticosterone decreases energy metabolism in efferent cerebellar circuitry.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 30 November 2016
Received in revised form 18 February 2017
Accepted 21 February 2017
Available online 2 March 2017

Keywords:
Glucocorticoid
Corticotropin releasing hormone
Motor coordination
Cognitive function
Brain metabolism

ABSTRACT

Receptors for glucocorticoid (GR) and corticotropin-releasing hormone (CRH) are largely found in brain sensorimotor structures, particularly in cerebellum, underlining a potential role of stress hormones in the regulation of motor function. Since CRH is involved in neuroplasticity, known for its trophic effect on synapses, we investigated how manipulations in corticosterone serum levels can modulate the CRH system in the cerebellum and affect motor coordination. Corticosterone at doses of either 15 or 30 mg/kg was injected in mice and the status of hormonal expression evaluated in cerebellum, hippocampus, and hypothalamus in undisturbed housing conditions or after different behavioral tests. Under both conditions, metabolic activity in numerous brain regions involved in motor functions and emotion was measured by means of cytochrome oxidase (COX) activity labeling. After six consecutive days of corticosterone administration, CRH-R1 transcription was downregulated in hypothalamic and cerebellar regions and hypometabolic changes were observed in mice treated with the higher dose for several limbic and sensorimotor circuitries, notably basal ganglia, deep cerebellar nuclei, and red nucleus. Corticosterone did not modify motor activity, anxiety, and spatial orientation, but decreased latencies before falling from the rotorod and prevented mice from reaching targets in the coat-hanger test. In addition, COX activities were similar to control mice except in ventromedial thalamus and dorsal neostriatum, possibly

* Corresponding author at: Laboratoire «Stress, Immunité, Pathogènes» (EA7300), Faculté de Médecine, Bâtiment A/B, 9 avenue de la Forêt de Haye, 54500 Vandoeuvre-les-Nancy, France.
E-mail address: catherine.strazielle@univ-lorraine.fr (C. Strazielle).

http://dx.doi.org/10.1016/j.bbr.2017.02.035

indicating that physical activity protected brain energy metabolism against the stress hormone. The present findings showed that the CRH/CRH-R1 system might play a role in mediating the effects of stress on cerebellar function, affecting especially motor learning tasks.

1. Introduction

Glucocorticoids, such as corticosterone (CORT) in rodents or cortisol in humans, are the resulting products of the adrenal gland cortex in response to activation by stress of the hypothalamic-pituitary-adrenal (HPA) axis from paraventricular nucleus corticotropin releasing hormone (CRH) secretory cells [1]. To avoid glucocorticoid excess, the HPA axis is under the influence of several regulation circuitries. Negative feedback control by glucocorticoids themselves and by CRH acts on secretory cells of the hypothalamus and pituitary gland [1]. Another regulation involves expression and activity of 11β-hydroxysteroid dehydrogenases (11β-HSD) 1 and 2, two antagonist enzymes producing or inhibiting active or inactive forms of glucocorticoids [2]. Catecholaminergic inputs and limbic neurocircuits, including the medial prefrontal cortex, hippocampus, and amygdala, also modulate the HPA axis [1,3].

Limbic structures are vulnerable to stress because of elevated densities in glucocorticoid receptors (GR) [4]. Anxious behavior appeared after a single dose of CORT [5,6] while an anxio-depressive phenotype occurred after chronic administration [7–9]. Neuroplastic alterations [10,11], oxidative damage [12], and tissue atrophy [13] in the hippocampus were observed under stressful conditions, associated with cognitive impairments following an inverted U-shape dose-response relationship [14].

Motor functions are not spared by stress [15]. High GR densities are present in sensorimotor cortex, basal ganglia, and cerebellum [4] and some studies reported stress-induced alterations in locomotor activity, postural adjustments, and motor coordination [15–18]. In cerebellar cortex, GRs are localized on granule and Purkinje cells [4], the latter being particularly vulnerable to oxidative damage. Chronic mild stress in rats inhibited mitochondrial oxidative phosphorylation in cerebral cortex and cerebellum [19]. Cerebellar atrophy associated with deficits on a rotating bar was observed in adult mice after CORT administration during infancy [20].

CRH is another important regulator of stress responses. Besides the hormonal axis, the neuropeptide acts as a neuromodulator via CRH-R1 and CRH-R2 receptors, with an approximately 13-fold higher affinity for the former. They are widely diffused in brain, especially the hippocampus and cerebellum where expression levels are high [21,22]. Mice deficient for *Crhr1* encoding CRH-R1 developed locomotor alterations not compensated by CRH injections, whereas acute CRH injections induced locomotor activation in the wild-type [23]. Moreover, *Crh* overexpressing mice were impaired on the rotorod test for motor coordination [24] and displayed regional atrophy, especially in the cerebellum [25].

The intent of the present study was to examine the impact of CORT on the CRH/CRH-R1 system, especially in the cerebellum. A first experimentation was performed with daily CORT injections for six days. One hour after the last injection, we evaluated the status of hormonal expression (especially the CRH-R1 and GR) in hypothalamus, cerebellum, and hippocampus. Following the obtained results and to determine whether these alterations affect cerebellar function, a second experiment was carried out with the same protocol of CORT injections followed by an evaluation of behavioral tests. In both experiments, metabolic activity in numerous brain regions involved in motor functions and emotion was measured by quantitative histochemistry of cytochrome oxidase (COX) activity labeling. COX is a mitochondrial enzyme involved in the respiratory chain leading to ATP production and a reliable marker of neuronal activity [26], strongly governed by excitatory glutamatergic synapses [27]. This type of cartography has all the more interest in that glucocorticoids can inhibit metabolic activity as shown by COX labeling, especially in CNS and muscle [28,29].

2. Materials and methods

2.1. Animals and drug administration

C57Bl/6J male mice (Charles River, L'Arbresles, France), controlled for age (14–18 weeks), were housed 3 per cage and maintained under a 12/12-h light-dark cycle with free access to food and water. Corticosterone (Tocris Bioscience, R&D system, France), dissolved in 0.9% NaCl with 100 mM DMSO as the control vehicle, was administered i.p. with an injection volume of 5 mL/kg at doses of 15 (low-dose) and 30 (high-dose) mg/kg between 13:00 and 14:00 h. Two experiments were conducted according to the protocol described in Fig. 1. *In experiment 1*, the drugs were injected to three groups of mice (control vehicle, low-dose CORT-treated, and high-dose CORT-treated, $n = 7$/group) during 6 consecutive days. On the last day, the mice were euthanized 1 h after drug administration. *In experiment 2*, the three groups of mice ($n = 10$/group) were injected in the same manner and assessed on behavioral tests 1 h after the injection following the chronological pattern presented in Fig. 1. On the last day, the mice were euthanized at the end of the test. Besides these experiments, a single CORT-injection ($n = 5$/group, three groups of mice) was applied on mice, euthanized 1 h after drug administration (1) to obtain a mean serum CORT level at the time when the animals have performed the behavioral tests and (2) to compare this level with that obtained in experiment 1 with a cumulative 6-day CORT administration.

The research protocol adhered to guidelines of the European Council Directive (2010/63/UE) and animal care regulations in force at the Ethical Committee of the University of Lorraine (CELMEA-2012-0008).

2.2. Behavioral tests

The tests were carried out according to protocols described previously by Lalonde and collaborators [30]. The weekly order of the tests was settled on the basis of starting with the least anxiogenic (exploratory activity) one to the most anxiogenic (water maze). Moreover, for the two first weeks, each group of mice was divided into three sub-groups, those performing the tasks in 3 different orders, to avoid possible test interference on performances. In the exploratory and motor tests, the apparatus was wiped clean with a damp cloth and dried between entry of each animal.

2.2.1. Exploration activity tests (1st week – days 1–3)

The photocell activity chamber (Letica model LE8811, Bioseb, France), an automated version of the open-field, contained a black Perspex floor, 45 cm × 45 cm in size and with 36 cm high transparent Perspex walls. The device distinguished between fast (>10 cm/s)