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Impaired adrenal medullary function in a mouse model of depression induced by unpredictable chronic stress

Magda M. Santana[a,d], Joana Rosmaninho-Salgado[a], Vera Cortez[a], Frederico C. Pereira[b], Manuella P. Kaster[c], Célia A. Aveleira[a], Marisa Ferreira[a], Ana Rita Álvaro[a], Cláudia Cavadas[a,d,*]

[a]*CNC – Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal*
[b]*IBILI – Faculty of Medicine, University of Coimbra, Coimbra 3000-548, Portugal*
[c]*Department of Biochemistry, Universidade Federal de Santa Catarina (UFSC), Florianópolis, Brazil*
[d]*Faculty of Pharmacy, University of Coimbra, Coimbra, Portugal*

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Abstract

Stress has been considered determinant in the etiology of depression. The adrenal medulla plays a key role in response to stress by releasing catecholamines, which are important to maintain homeostasis. We aimed to study the adrenal medulla in a mouse model of depression induced by 21 days of unpredictable chronic stress (UCS). We observed that UCS induced a differential and time-dependent change in adrenal medulla. After 7 days of UCS, mice did not show depressive-like behavior, but the adrenal medullae show increased protein and/or mRNA levels of catecholamine biosynthetic enzymes (TH, DβH and PNMT), Neuropeptide Y, the SNARE protein SNAP-25, the catecholamine transporter VMAT2 and the chromaffin progenitor cell markers, Mash1 and Phox2b. Moreover, 7 days of UCS induced a decrease in the chromaffin progenitor cell markers, Sox9 and Notch1. This suggests an increased capacity of chromaffin cells to synthesize, store and release catecholamines. In agreement, after 7 days, UCS mice had higher NE and EP levels in adrenal medulla. Opposite, when mice were submitted to 21 days of UCS, and showed a depressive like behavior, adrenal medullae had lower protein and/or mRNA levels of catecholamine biosynthetic enzymes (TH, DβH, PNMT), catecholamine transporters (NET, VMAT1), SNARE proteins (synthaxin1A, SNAP25, VAMP2), catecholamine content (EP, NE), and lower EP serum levels, indicating a reduction in catecholamine synthesis, re-uptake, storage and release. In conclusion, this study suggests that mice exposed to UCS for a period of 21 days develop a depressive-like behavior accompanied by an impairment of adrenal medullary function.

*Correspondence to: CNC – Center for Neuroscience and Cell Biology, University of Coimbra, Rua Larga, 3000-517 Coimbra, Portugal. Tel.: +351 963928766.

E-mail address: ccavadas@uc.pt (C. Cavadas).

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1. Introduction

Depression is a common and disabling psychiatric disorder with a lifetime prevalence of about 20% (WHO, 2008). According to the World Health Organization, depression is among the five leading causes of global disease and estimations predict that by the year of 2030 depression will be the second leading cause of disease burden worldwide (WHO, 2008). Moreover, depressive disorders significantly influence the outcome of other co-morbid medical illnesses, such as cardiovascular disease, diabetes, obesity, cancer, and neurodegenerative diseases (Benton et al., 2007). Despite the considerable prevalence and high impact of disease, the underlying causes of depression are far from being completely understood. Nevertheless, stress is considered determinant in the etiology of depression (Hammen, 2005).

The physiological responses to stress are mediated by two main systems: the hypothalamic–pituitary–adrenocortical (HPA) axis and the sympathoadrenal system. The adrenal gland, as part of both systems, plays a pivotal role in homeostasis maintenance, by secreting glucocorticoids and catecholamines, from adrenal cortex and adrenal medulla, respectively (Goldstein, 2010). Although these hormones are initial beneficial and required for survival during acute stress, when stress is frequent or prolonged they may induce changes that alters the normal functioning of physiological systems. Indeed, dysfunction of stress systems has been consistently demonstrated in depressed patients and animal models of depression (Krishnan and Nestler, 2010; Saveanu and Nemeroff, 2012). However, these studies are largely focused on central and peripheral characterization of HPA axis structure (Hill et al., 2012; Saveanu and Nemeroff, 2012), including adrenal cortex morphology and function (Ulrich-Lai et al., 2006). On the other hand, there is scarce research regarding the study of adrenal medulla function in depression. Catecholamines, released from chromaffin cells of the adrenal medulla, are implicated in the development of several stress-related disorders (Chrousos, 2009), such cardiovascular disease and metabolic disorders, which are associated with depressive states (Bradley and Rumsfeld, 2015). In this context, we aimed to investigate the adrenal medulla in an animal model of depression induced by unpredictable chronic stress (UCS). The UCS paradigm is a widely used rodent model of depression, which emphasizes the role of stress in the etiology of depression. In this model, animals gradually develop a chronic depressive-like state due to sequential and unpredictable exposures to different stressors, over a sustained period of time. In opposition to other models of chronic stress (such social stress and restraint stress), which are not yet so extensively characterized as depression models, UCS paradigms showed to reproduce several behavioral and neurobiological changes that parallel dysfunctions observed in depressed patients (Hill et al., 2012). Moreover, UCS paradigms are depression models with construct, face and predictive validity and are a suitable animal model to investigate the biology of depression (Hill et al., 2012; Krishnan and Nestler, 2011). The detailed study of adrenal medulla in this animal model represents an improvement to the field, being relevant to identify mechanisms underlying the pathophysiology of depression.

2. Experimental procedures

2.1. Animals

Male, 9-weeks old C57/BL6 mice (Charles River, Barcelona) were individually housed under a 12 h light/dark cycle in a humidity/temperature controlled room, with *ad libitum* access to a standard chow diet and water, except when food and water deprivation was specified by the stress protocol. Animals were allowed 5 days to acclimatize to the surroundings before each UCS protocol. All experimental procedures were performed in accordance with the European Union Directive 86/609/EEC for the care and use of laboratory animals.

2.2. Unpredictable chronic stress (UCS) protocol

For 7 or 21 days, UCS mice were maintained in cages without enrichment and exposed to different stressors, accordingly to Table 1. In turn, the control mice were maintained in enriched cages and daily handled to minimize stress induced by manipulation.

2.3. Forced swimming test (FST)

FST was performed 24 h after the last stressor. Mice were dropped individually into glass cylinders (height: 25 cm, internal diameter: 10 cm) containing 15 cm water, maintained at 23–25 °C, and the immobility time (ceased struggling and remained floating motionless in the water) for a 6-min period was measured.

2.4. Serum and adrenal medulla collection

Mice were anesthetized and killed by decapitation 24 h after the last stressor. Blood was collected from trunk and serum was separated by centrifuging (2000*g* for 15 min at 4 °C) and then kept at −80 °C.

For adrenal gland volume and weight determination, 12 animals were used (6 controls and 6 UCS) and adrenal glands were collected intact. Left adrenals were used for weight measurements and right adrenals for adrenal gland volume estimation.

For western blot and adrenal medulla catecholamine content quantification, 12 animals were used (6 controls and 6 UCS). Adrenal glands were removed and adrenal medullae were dissected from adrenal cortex. Samples were immediately frozen on dry ice and kept at −80 °C until use.

For RNA extraction, 24 animals were used (12 controls and 12 UCS). Adrenal glands were removed; adrenal medullae were dissected from adrenal cortex, protected from degradation with RNAlater (Qiagen, Germany), according to the manufacturer's instruction, and kept at −80 °C until use. Four adrenal medullae (2 animals) were used per individual sample (*n*).

2.5. Estimation of adrenal gland volume

The volume of adrenal gland and its subregions (cortex and medulla) was estimated using the Cavalieri method. For that purpose, adrenal glands were collected, fixed with 4% paraformaldehyde and embedded in optimal cutting temperature (OCT) medium before being frozen at −80 °C. Adrenal gland sections of 15 μm thickness were cut using a cryostat (Leica, Germany) and collected in super frost plus slides (Thermo scientific, USA). For analysis with the Cavalieri estimator probe of the Stereo Investigator Software, the sections chosen were sampled in order obtain 10–15 sections covering the adrenal medulla subregion. The first

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