Physiology & Behavior 103 (2011) 540–546

Contents lists available at ScienceDirect

Physiology & Behavior

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

Altered aortic vascular reactivity in the unpredictable chronic mild stress model of depression in mice
UCMS causes relaxation impairment to ACh

Elsa Isingrini [a,*], Alexandre Surget [a], Catherine Belzung [a], Jean-Louis Freslon [b], Jefferson Frisbee [c,d], James O'Donnell [e], Vincent Camus [a,f], Alexandre d'Audiffret [d,g]

[a] INSERM U930 and, Université François Rabelais de Tours, 37200 Tours, France
[b] EA4433, Université François Rabelais, 37200 Tours, France
[c] Department of Physiology and Pharmacology, West Virginia University, School of Medicine, Morgantown, WV 26506, USA
[d] Center for Interdisciplinary Research in Cardiovascular Sciences, West Virginia University, School of Medicine, Morgantown, WV 26506, USA
[e] Department of Behavioral Medicine & Psychiatry, West Virginia University, School of Medicine, Morgantown, WV 26506, USA
[f] Clinique psychiatrique universitaire, CHRU de Tours, Tours, France
[g] Department of Surgery, Division of Vascular and Endovascular Surgery, West Virginia University, School of Medicine, Morgantown, WV 26506, USA

ARTICLE INFO

Article history:
Received 2 September 2010
Received in revised form 20 March 2011
Accepted 4 April 2011

Keywords:
Depression
Cardiovascular disease
Endothelial dysfunction
Vasorelaxation
Rodent model
Chronic stress

ABSTRACT

Major depression is an independent risk factor for the development of cardiovascular disease. This impact of depression on vascular function seems to be mediated by the endothelial dysfunction, defined as an impairment of endothelium-dependent vasorelaxation, which represents a reliable predictor of atherosclerosis and has been regularly found to be associated with depression. This study aimed at investigating aortic vascular reactivity in mice submitted to the unpredictable chronic mild stress (UCMS) procedure, a reliable model of depression. The results confirm the effectiveness of the UCMS procedure to induce neuroendocrine, physical and behavioral depression-like alterations as well as a significant decrease of acetylcholine-induced vasorelaxation without any effect on phenylephrine-induced vasoconstriction. In this study, we reveal an altered vascular reactivity in an animal model of depression, demonstrating an endothelial dysfunction reminiscent to the one found in depressed patients.

1. Introduction

In developed countries, major depression is the second leading cause of death and disability after coronary heart disease [1]. Interestingly, these two pathologies have a high co-morbidity [2,3]. Several hypotheses have been proposed to explain depression-induced cardiovascular disease including neurohormonal activation, autonomic dysfunction, immuno-inflammatory processes, platelet activation and endothelial dysfunction [4,5].

The vascular endothelium plays a key role in maintaining vascular homeostasis and consequently regulates several physiological functions, including vascular tone, smooth muscle cell proliferation, inflammation, platelet aggregation, thrombosis, fibrinolysis and oxidation. Nevertheless, the term endothelial dysfunction is limited to describe the impairment of endothelium-dependant vasorelaxation caused by a loss of nitric oxide (NO) bioavailability. While NO has vasorelaxant properties and exerts protective effects on the vasculature, endothelial dysfunction leads to a modification in the vascular homeostasis which contributes to atherosclerosis formation [6], specifically in large conductive arteries such as aorta where NO is the most important vasorelaxant mediator.

The functional and structural assessment of endothelial dysfunction is based on studies demonstrating that normal endothelium mediates the vasodilatation effect of acetylcholine (ACh). When the endothelium is removed or altered, either ACh exerts a vasoconstrictive effect by a direct action on smooth muscles [7] or the endothelium releases contractile factors instead of relaxing factors. Consequently, the vasomotor response to various pharmacological or physical stimuli can be considered as surrogate markers of NO bioavailability. Using high resolution ultrasound equipment for assessing the vascular remodeling of the blood vessel, several studies demonstrated endothelial function altered in depressed patients [8–10]. Moreover, the level of biological surrogate markers of endothelial dysfunction in patients with depression frequently shows increased concentrations of the soluble chemokine monocyte chemotactic protein-1, E-selectin and ICAM-Is [9,11], as well as decreased plasma levels of NO metabolites and endothelial nitric oxide synthase (eNOS)

* Corresponding author at: INSERM U930 – Team 4, Affective disorders, Université François Rabelais de Tours, UFR Sciences et Techniques, Parc grandmont (Bat O), 37200 Tours, Cedex 01, France. Tel.: +33 247 47 36 69 96.
E-mail address: Isingrini.elsa@voila.fr (E. Isingrini).

0031-9384/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.physbeh.2011.04.002

activity [12]. This endothelial dysfunction is particularly interesting in the context of the main mechanistic hypothesis of the relationship between depression and the increased risk of cardiovascular disease.

The unpredictable chronic mild stress (UCMS) animal model is valid, reliable and sensitive [13] for studying depressive disorders in rodents. UCMS involves subjecting mice to a period of mild socio-environmental stressors. This procedure replicates several depression-related behavioral and physiological impairments which are reversed by chronic, but not acute, antidepressant treatment [14].

The aim of the present study was to investigate the effect of UCMS, intended to induce depression-like behaviors, on the vaso-reactive properties of the thoracic aorta, including the vasoconstriction to phenylephrine (L-Phe) and the vasodilatation to acetylcholine (ACh). We hypothesize that UCMS, as observed in depression, can alter endothelium vascular reactivity by impairing acetylcholine-induced vasodilatation.

2. Methods

2.1. Animals

Seven week-old male BALB/cJ mice were housed in groups of 4 at their arrival and allowed to become used to their new environment for 2 weeks. The animals had free access to water and food in standard laboratory conditions (12 h day/night cycle, light on at 08:00; $T = 22 \pm 2$ °C). All protocols were approved by the West Virginia University IACUC.

2.2. General procedure

The mice were randomized into the control group ($n = 18$), kept in standard laboratory conditions or the UCMS group ($n = 18$) subjected to 8 weeks of random daily socio-environmental stressors. A 7–9 week of UCMS protocol is commonly used in order to demonstrate that UCMS-induced behavioral disturbance can be reverse by antidepressant treatment [14–16]. In the present study, 8-week UCMS procedure without any antidepressant treatment was used. The control mice were housed in groups of 4 in standard laboratory cages ($42 \text{ cm} \times 28 \text{ cm} \times 18 \text{ cm}$) while mice in the UCMS group were singularly housed in individual cages ($8 \text{ cm} \times 13.5 \text{ cm} \times 8.1 \text{ cm}$). The coat state and body weight of each animal were evaluated every Monday in a blind manner. The total score of the coat state was computed by attributing a score of 0 (clean coat) or 1 (dirty coat) to eight different body parts (head, neck, dorsal coat, ventral coat, tail, forelimb, hind-limb, and genital region) [14,17]. At the seventh week, after performance in the reward test (anhedonic test), 8 animals per group were sacrificed for a plasma corticosterone radioimmunoassay. During the last week, after the splash test, the novelty suppression of feeding (NSF) test and locomotor activity test, the other half of the animals (UCMS, $n = 10$ and control $n = 10$) were euthanized for the vascular reactivity study.

2.3. Unpredictable chronic mild stress

The UCMS regimen used is a variation of the procedure previously described [18–21] and adapted to mice. Several times a day during the 8 weeks of the UCMS procedure, the mice were exposed to various socio-environmental mild stressors (between two and four stressors per day). The environmental stressors were: altered bedding (change or removal of sawdust, damp sawdust, substitution of sawdust with 2 cm of 21 °C water), cage tilting (45°) and altered length and time of light/dark cycle (inversion of the light/dark cycle and lights on for a short time during the dark phase). The social stressors were: isolation (control mice were housed in groups of 4 while UCMS mice were singularly housed) and social stress (mice were placed in the empty cage of another male). The presentation of the different stressors was randomized each week to maximize their unpredictability (Table 1). For ethical reasons, the stress procedure did not involve food and water deprivation.

2.4. Behavioral testing

All behavioral testing was performed during the light phase of the light/dark cycle and were recorded using video equipment.

2.4.1. Reward test

The *reward test* is a model of anhedonia, used to assess UCMS-induced effects on the motivation to obtain a reward. It was demonstrated that exposure to repeated unpredictable stress prevents the acquisition of an appetitive behavior induced and maintained by a highly palatable food [22]. The reward test requires a device containing three aligned compartments with the same dimension ($20 \times 20 \times 20$ cm). Only the colors of the walls and the floor are different: white for the first one, gray for the second one and black for the third one. The three compartments are linked by two openings controlled by the experimenters. The device is illuminated with 200-lux white light. Four and half weeks before the first session, a small portion ($2 \text{ g} \pm 1$) of a chocolate cookie (Pepito, Lu, France) was placed in the home cage every two days during 2.5 weeks in order to familiarize the mice with the palatable stimulus. The last two weeks before the first session were cookie-free. One hour before testing, food was removed in order to avoid inter-individual differences in the drive for feeding (hunger). At the time of testing, a small amount ($2 \text{ g} \pm 1$) of chocolate cookie (or of regular food in a supplemental control experiment) was positioned at the center of the black room. The white room was the compartment of the departure where the mouse is placed with its head facing the opposite side to the opening. Four sessions of testing are performed within 9 days, each session being separated from the previous one by 3 days. Each session lasted 5 min; the door of the first opening was closed after the transition of the mouse within a maximum time of 2 min (at 2 min, the mouse was gently guided to the second room when required). The cookie consumption (number of bites) was recorded within the 5 min test period. The validation of this test was demonstrated by the stronger drive to chew a chocolate cookie than to chew a regular food pellet as a robust increase of the chewing frequency with the chocolate cookie when compared with the regular food [18,23]. This test allowed measuring the locomotion and exploratory behavior (number of passages through the second door over the session) and anhedonia (number of bites over the session).

2.4.2. Splash test

The *splash test*, performed under a red light (15 W), consists of squirting two sprays of an atomizer containing a 10% sucrose solution on the dorsal coat of a mouse in its home cage. Because of its viscosity, the sucrose solution dirties the mouse's fur and animals initiate grooming behavior. After applying sucrose solution, the grooming latency and frequency were recorded for a period of 5 min as an index of self-care and motivational behavior. The splash test, pharmacologically validated, demonstrates that UCMS decreases grooming behavior [17,19,23,24], which can be interpreted as a loss of motivational behavior considered to parallel some symptoms of depression such as apathic behavior [25].

2.4.3. NSF test

The *NSF test* is a modified version of the test used by Santarelli et al. [19]. This test induces a motivational conflict between eating behavior and the fear of novelty. Latency to initiation of sniffing and eating a food pellet is used as an index of depressive-like behavior since chronic antidepressant treatment decreases this measure [14,17]. This test was conducted in an open-field ($30 \times 30 \times 30$ cm) with a sawdust covered floor and under a red light (230 V, 15 W). Twelve hours before testing, the mice were food restricted. At the start of testing, a