Physiology & Behavior 106 (2012) 476–484

Contents lists available at SciVerse ScienceDirect

Physiology & Behavior

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

Chronic social isolation in the prairie vole induces endothelial dysfunction: implications for depression and cardiovascular disease

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ARTICLE INFO

Article history:
Received 7 March 2012
Accepted 17 March 2012

Keywords:
Acetylcholine
Endothelial dysfunction
Cardiovascular disease
Depression
Prairie vole
Social isolation

ABSTRACT

Humans with depression show impaired endothelium-dependent vasodilation; one recent demonstration of which was in the form of a reduced acetylcholine (ACh)-induced relaxation of adrenergically-precontracted small arteries biopsied from older depressed patients. Results from such uses of ACh in general have been validated as the most predictive marker of endothelium-related cardiovascular diseases. Accordingly, we examined vascular reactivity to ACh in the socially isolated prairie vole, a new animal model relevant to human depression and cardiovascular disease. Thoracic aortas were carefully dissected from female prairie voles after one month of social isolation (versus pairing with a sibling). Only aortas that contracted to the adrenergic agent phenylephrine (PE) and then relaxed to ACh were evaluated. Among those, ACh-induced relaxations were significantly reduced by social isolation ($p<0.05$), with maximum relaxation reaching only 30% (of PE-induced precontraction) compared to 47% in aortas from paired (control) animals. Experimental removal of the endothelium from an additional set of aortic tissues abolished all ACh relaxations including that difference. In these same tissues, maximally-effective concentrations of the nitric oxide-donor nitroprusside still completely relaxed all PE-induced precontraction of the endothelial-free smooth muscle, and to the same degree in tissues from isolated versus paired animals. Finally, in the absence of PE-induced precontraction ACh did not relax but rather contracted aortic tissues, and to a significantly greater extent in tissues from socially isolated animals if the endothelium was intact ($p<0.05$). Thus, social isolation in the prairie vole may 1) impair normal release of protective anti-atherosclerotic factors like nitric oxide from the vascular endothelium (without altering the inherent responsiveness of the vascular smooth muscle to such factors) and 2) cause the endothelium to release contracting factors. To our knowledge this is the first demonstration of this phenomenon in an animal model of depression induced solely by social isolation. These findings have implications for understanding mechanisms involved in depression and cardiovascular disease.

1. Introduction

Evidence obtained from both epidemiological and clinical investigations in humans and of experimental investigations in non-human animals suggests that there is a bidirectional association between mood and cardiovascular dysfunction. Cardiovascular pathophysiology significantly increases the likelihood of developing depressive disorders; and conversely depressive disorders are risk factors for cardiac morbidity and mortality [1–5]. The association between altered mood and cardiovascular disease has been observed in individuals both with and without a history of cardiac pathophysiology, and is independent of traditional cardiovascular risk factors such as high cholesterol, increased body mass index, family history, and disease severity [2,4,6,7].

Despite evidence that a significant relationship exists between mood and cardiovascular regulation, the precise behavioral and neurobiological mechanisms underlying this association remain unclear. One proposed mechanism of interaction includes arterial endothelial dysfunction, an early precursor of atherosclerosis [8,9]. Further, dysfunction of the vascular endothelium may interact with behavioral mechanisms, including inappropriate responses to environmental and social stressors, to mediate the relationship between depression and cardiovascular disease. Social isolation and perceived loneliness are associated with maladaptive grief, mood disorders, and autonomic dysfunction, as well as altered interactions among these variables [10–14]. For example, individuals with smaller social networks and fewer meaningful social connections show increased depressive symptomatology [15], cardiovascular risk factors (including coronary artery calcification, increased blood glucose levels, hypertension, and diabetes) [16,17], and increased cardiovascular mortality

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doi:10.1016/j.physbeh.2012.03.019

[15,16,18–20]. Experimental investigations with rodents and non-human primates also demonstrate that negative social experiences (such as acute social stressors, long-term isolation, and social subordination stress) produce several behavioral and physiological consequences including depressive behaviors, autonomic dysregulation, atherosclerosis, immune system activation, oxidative stress, and exaggerated behavioral and cardiac reactivity to stressors [21–27].

Experimental protocols with valid and reliable animal model systems will promote an increased understanding of the interactions among negative social experiences, vascular endothelial dysfunction, and the risk of cardiovascular disease. Recent studies have shown that the prairie vole *(Microtus ochrogaster)* may be a useful animal system for the study of these inter-relationships. The prairie vole is a highly social rodent species that is dependent on social interactions for the regulation of behavior, endocrine function, and cardiovascular regulation. This socially monogamous species shares with humans several physiological and behavioral characteristics including the capacity to form social bonds and to develop extended families, coupled with a high level of parasympathetic regulation of the heart, therefore offering a powerful translational model for understanding the mechanisms through which social experiences influence behavior and cardiovascular function [28,29]. For instance, in this species social isolation from family members or opposite-sex partners induces depression-relevant behaviors that mimic those observed in humans, including anhedonia (i.e., reduced responsiveness to pleasurable stimuli) and learned helplessness (i.e., behavioral "despair") [21,30,31]. Social isolation also sensitizes prairie voles to several autonomic and cardiovascular disturbances, including increased heart rate, reduced heart rate variability, cardiac arrhythmias, and sympathovagal imbalance [22,32,33].

Given its relevance to the study of social experiences, mood, and cardiovascular function, the prairie vole is a useful model system in which to investigate interactions of social stressors and vascular endothelial dysfunction. One well established marker of vascular endothelial dysfunction is impaired endothelium-dependent vasodilation [8,34–41]. The first evidence of this phenomenon in depressed patients was observed in the form of impaired flow-mediated dilation of the brachial artery in the forearm [36–39]. More recently, this same phenomenon was demonstrated in the form of impaired acetylcholine (ACh)-induced relaxation of adrenergically-precontracted small arteries biopsied from depressed patients [40,41] and thoracic aortas removed from a mouse model of depression induced by exposure to chronic unpredictable stressors [42]. In humans, results from such uses of ACh have been validated as the most prognostic marker of endothelium-related cardiovascular diseases [34].

In the present study, we sought to evaluate vascular reactivity to ACh in the abovementioned socially isolated prairie vole, because of its current status as a newer animal model relevant to clinical depression and cardiovascular disease [43]. We tested arterial tissues from these animals not only for impaired ACh-induced relaxation of adrenergically-induced precontraction as a measure of impaired endothelial release of relaxing substances, but also for enhanced ACh-induced contractions (detectable only in the absence of adrenergic or any other form of precontraction) as a measure of abnormal endothelial release of contracting substances. As a control measure, these same tests were performed in additional arterial tissues in which the endothelium was deliberately removed. We also examined all tissues for the ability of the nitric oxide-donor nitroprusside (NP) to relax adrenergically-induced precontraction, another widely-used control measure in ACh tests (for approximating endothelium-independent relaxation). Blood samples were collected and analyzed for elevated cholesterol, a long-recognized risk factor for endothelial dysfunction [35], and heart-to-body weight ratios were quantified. All these tests were performed after first exposing the animals to social isolation for four weeks, which has been shown to induce the depressive signs and cardiac dysfunction described above [22,31].

2. Methods

2.1. Animals

Fifty-five adult (73 ± 2 days of age) female prairie voles were used for the experiments described here. All animals were descendants of a wild stock caught near Champaign, Illinois, and maintained on a 14/10 h light/dark cycle (lights on at 0630 h) with a temperature of 25 ± 2 °C and relative humidity of 30 ± 5%. All animals were allowed food (Purina rabbit chow) and water ad libitum. Offspring were removed from breeding pairs at 21 days of age and housed in same-sex sibling pairs until the commencement of the experimental procedures. For all procedures described herein, only one animal from each sibling pair was studied. All procedures were conducted according to the National Institutes of Health's *Guide for the Care and Use of Laboratory Animals* and approved by the local university Institutional Animal Care and Use Committees.

2.2. Experimental treatment

Prairie voles were randomly divided into two independent groups of either paired (control; n = 28) or isolated (n = 27) conditions. Isolated animals were separated from their respective siblings and housed individually for four weeks, while paired animals were continually housed with the same siblings that they had lived with since weaning. This time period was chosen to be consistent with previous studies demonstrating that four weeks of social isolation in female prairie voles results in a disruption of affective behaviors (e.g., depression-relevant behaviors) [31,44] and resting cardiac function [22]. All handling, cage changing, collection and preparation of samples, and testing of tissues and plasma (described below) were matched between the two groups.

2.3. Experimental tissues

All prairie voles were subjected to the following procedures for removal, preparation, and testing of aortic and other tissues, following 4 weeks of either social isolation or social pairing (control condition). Anesthesia was achieved with a mixture of ketamine and xylazine as described previously [31]. Blood was sampled as described previously [31], centrifuged at 4 °C, at 3500 rpm, for 15 min to obtain plasma. Plasma was stored for later analysis of cholesterol as described below. Then the chest of each animal was opened, lungs and large veins discarded, residual blood in its cavity flushed out with cold physiological buffer, prepared as described previously [45], and the thoracic aorta removed with as much care as possible to minimize stretching. Unfortunately, some stretching was unavoidable as we found that the aorta in this animal was surrounded by considerably more connective and fat tissue than we have encountered in larger species [46]. The heart was also removed and weighed for later calculation of heart weight-to-body weight ratio. The aortas were transported on ice in cold physiological buffer to another laboratory for the vascular reactivity tests described below. The connective and fat tissue adhering to the outer wall of each aorta were removed, with as much care as possible to avoid excess stretching of the vessel (although again some was unavoidable). Each cleaned aorta was then sectioned into a pair of 3-millimeter cylindrical rings, using a bound set of evenly-spaced scalpel blades to optimize length uniformity. Each of these rings was mounted between two tungsten wire stirrups, which are strong enough not to bend during ring contraction yet thin enough not to damage the inner monolayer of endothelial cells [45,47]. For a select number of aortas, this inner endothelial cell layer was deliberately removed from one ring of each pair (before mounting on stirrups) by rubbing it off with a roughened hypodermic needle inserted through the lumen of the vessel as described previously [47]. The mounting of each tissue ring on stirrups allowed for