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Chronic unpredictable mild stress generates oxidative stress and systemic inflammation in rats

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HIGHLIGHTS

- Stress is risk factor for pathologies that are considered priority health problems.
- CUMS alter redox state in the liver and pancreas.
- CUMS producing low-grade systemic inflammation.
- Resistance period between 20 and 40 days of stress is surpassed at 60 days.

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ABSTRACT

Stress is considered to be a causal agent of chronic degenerative diseases, such as cardiovascular disease, diabetes mellitus, arthritis and Alzheimer's. Chronic glucocorticoid and catecholamine release into the circulation during the stress response has been suggested to activate damage mechanisms, which in the long term produce metabolic alterations associated with oxidative stress and inflammation. However, the consequences of stress in animal models for periods longer than 40 days have not been explored. The goal of this work was to determine whether chronic unpredictable mild stress (CUMS) produced alterations in the redox state and the inflammatory profile of rats after 20, 40, and 60 days. CUMS consisted of random exposure of the animals to different stressors. The following activities were measured in the liver and pancreas: reduced glutathione (GSH), lipid peroxidation (LPO), superoxide dismutase (SOD), catalase (CAT), total antioxidant capacity (TAC), and protein oxidation. Similarly, serum cytokine levels (IL-6, TNF-α, IL-1β, and IL-10) were determined. CUMS activated the stress response from day 20 until day 60. In the liver and pancreas, GHS levels were decreased from day 40, whereas protein lipid peroxidation and protein oxidation were increased. This is the first work to report that the pancreas redox state is subject to chronic stress conditions. The TAC was constant in the liver and reduced in the pancreas. An increase in the TNF-α, IL-1β, and IL-6 inflammatory markers and a decrease in the IL-10 level due to CUMS was shown, thereby resulting in the generation of a systemic inflammation state after 60 days of treatment. Together, the CUMS consequences on day 60 suggest that both processes can contribute to the development of chronic degenerative diseases, such as cardiovascular disease and diabetes mellitus. CUMS is an animal model that in addition to avoiding habituation activates damage mechanisms such as oxidative stress and low-grade chronic inflammation, which allows the study of physio-pathological stress aspects over prolonged time periods of at least 60 days.

1. Introduction

Stress has been associated with the development of chronic degenerative diseases, such as cardiovascular disease, rheumatoid arthritis, Alzheimer's, diabetes mellitus, and metabolic syndrome [1–4]. Although oxidative stress and inflammation have been implicated in the development of diverse pathologies due to chronic stress, the activation process of this system is not clear.

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Animal models (mainly rats) have been utilized to study the physiopathological aspects related to stress. The most commonly used stressors are immobilization, cold, and cold-water immersion, which can be applied for different exposition times. The chronic unpredictable mild stress (CUMS) model is a stress model that is also used as a depression animal model [5]. In this model, the animals are randomly exposed to different stressors with the goal of diminishing the animals' habituation. To date, its behavioral effects have only been studied for up to day 40 [6].

The stress response induces the activation of the hypothalamic–pituitary–adrenal axis and increases glucocorticoids levels; corticosterone (the main glucocorticoid in rodents) is released from the adrenal cortex [7]. Homeostasis reestablishment or allostasis maintenance under stress conditions involves neuro-chemical and endocrine regulation that results in metabolic alterations [7,8]. In this sense, the liver and pancreas are regulators of metabolism and therefore their functions are also altered in the stress response.

Understanding the molecular and cellular pathways activated in the response to stress is very important for the development of pharmacological interventions for the prevention and treatment of diseases induced by physiological stress. One of the most important mechanisms of stress is the production of reactive oxygen species (ROS) [6,9]. ROS in cells are neutralized by both enzymatic and non-enzymatic antioxidant defense systems. Glutathione reduction (GSH) represents a principal marker for the non-enzymatic antioxidant mechanisms because it is widely distributed among organisms. Among the enzymatic antioxidant mechanisms, superoxide dismutase (SOD) and catalase (CAT) are the most studied [9]. On many occasions, the general redox state of an organism is used to represent the total antioxidant capacity (TAC), which denotes a balance between the generation of ROS and the hen ROS production exceeds the antioxidant capacity, resulting in the generation of lipid peroxidation (LPO), protein oxidation and DNA damage that can lead to cell death. This mechanism of damage has been associated with the development of several chronic degenerative pathologies, including diabetes mellitus, hypertension, and cardiovascular disease [9]. Several studies have shown that the alteration of antioxidant capacity in stress induced by immobilization, cold, and cold water immersion for 21 days in Wistar rats is associated with oxidative stress in different organs (liver, kidney, heart, stomach, lung, and brain) and effects on erythrocytes [11–13].

The chronic stress increment favors neuronal apoptosis and reduces neurogenesis in the hippocampus [14], which leads to a reduction in the hippocampal volume [14,15]. Specifically, exposure to CUMS for 40 days increased protein oxidation in the prefrontal cortex, hippocampus and striatum and lipid peroxidation (LPO) in the cerebellum and striatum [6].

Although oxidative stress is involved in the appearance and complication of various diseases, at present there is no experimental evidence of a pancreas condition or oxidative stress induced by physiological stress markers in the pancreas. However, the pancreas is heavily involved in the pathogenesis of metabolic chronic degenerative diseases.

The interaction of the stress response and the immune system differs with the exposure time. The activation of the β-adrenergic receptor produces an acute inflammatory process, whereas the function of glucocorticoids is anti-inflammatory [16]. Chronic stress can also increase pro-inflammatory cytokine levels, such as tumor necrosis factor alpha (TNFα), interleukin 1β (Il-1β) and interleukin 6 (IL-6) [4], which are potential sources of ROS [17]. Excessive ROS production due to the chronic stress effect is also related to complications observed in patients with diabetes mellitus [8,18].

Although alterations such as chronic hyperglycemia have been demonstrated to favor oxidative stress [8] and inflammation [18], the damage mechanisms implicated in the metabolic alterations generated by physiological stress have not been sufficiently studied. The purpose of this work was to determine the redox state of the liver and pancreas and the inflammatory profile in rats subjected to 20, 40, and 60 days of CUMS. Reduced glutathione (GSH), lipid peroxidation (LPO), superoxide dismutase (SOD), catalase (CAT), total antioxidant capacity (TAC), and protein oxidation activity were detected. Additionally, the inflammatory profile was measured through the quantification of serum cytokine levels (IL-6, TNFα, IL-1β, and IL-10).

2. Materials and methods

Three-month-old male Wistar rats with weights ranging from 250 g to 300 g were obtained from the experimental animal center at the UAM-Iztapalapa and housed with a light-darkness cycle of 12 h (9 am–9 pm) with water and food (Harlan) provided ad libitum. The animals were separated into a control group (each time point had its own control group, $n = 15$) and groups subjected to 20, 40, and 60 stress days ($n = 5$). The animals were maintained in 50 × 30 × 20 cm boxes (5 rats/box). Because no differences were found in the control groups between time points, the control group data are presented for only one group. The handling of the laboratory animals and experimental procedures were performed according to the national and international rules (NIH guidelines for the handling and care of animals), including the Official Mexican Rule (NOM-062-ZOO-999, revised 2001). This protocol was approved by the Ethic Committee at the Metropolitan Autonomous University (Session 1514, verified 3–15–14-2010-2018).

2.1. Chronic unpredictable mild stress (CUMS) model

The animals were separated into two groups and placed into separate rooms. The control group was manipulated only for box changing, whereas the experimental group was subjected to the CUMS model for 20, 40, and 60 days according to the protocol described by Willner [5,19] and used by Lucca et al. [6] with some modifications. The stressors used were: food privation (12 h), water privation (12 h), immobilization (1 h − 3 h), cold (4 °C, 3 h), immobilization plus cold (4 °C, 1.5 h–2.5 h), cold water immersion (15 °C, 15 m), continuous light (24 h), isolation (2–3 days), strange object (plastic balls 10 cm in diameter, 3 h–5 h), box inclination (40°, 5 h), overcrowding (15 animals per box, 8 h–24 h), wet bed (5 h–24 h) and noise (40 dB, 3 h–5 h). Stressors were applied randomly, with the stressor varying in frequency and time to avoid the animals' habituation (Table 1). The stressors were applied preferably at the beginning of the light phase between 9 am and 11 am.

To discard the effect of acute stress, the animals were sacrificed by decapitation one day after finalizing the stress procedure. Blood was collected and the organs extracted to process and analyze the samples. Serum was obtained by centrifuging the blood at 1500 × *g* for 10 min. The serum was stored at − 70 °C prior to corticosterone and cytokine analyses.

2.2. Corticosterone

For corticosterone determination, the animals were sacrificed 24 h after the stress procedure between 10 am and 11 am (one hour into the light phase). The corticosterone determination was performed in serum with a commercial ELISA kit (ALPCO Diagnostics, immunoassays, Salem, NH, USA). The optic density was determined photometrically at 450 nm.

2.3. Tissue analysis

The liver and pancreas were dissected and washed in a saline solution at 4 °C and separated. A total of 0.1 g of tissue was homogenized in 600 μl of phosphate buffer (0.067 M, pH 7.8) and a protease inhibitor stock solution (Roche, Indianapolis, IN, USA) for the determination of the SOD, CAT, TAC and protein oxidation activities. A total of 0.1 g of tissue was homogenized in 600 μl of sulfosalicylic acid for the GSH determination, and 0.1 g of tissue was homogenized in HPLC water for the