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Immobilization stress causes extra-cellular oxidant—antioxidant imbalance in rats: Restoration by L-NAME and vitamin E

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Abstract Stress has been shown to be associated with altered homeostasis that may lead to oxidant–antioxidant imbalance. Non-enzymatic antioxidants are important regulators of reactive oxygen species produced in extra-cellular milieu and represent the first line of defense against them. Extra-cellular non-enzymatic antioxidants may be disturbed by the production of superoxide and nitric oxide and this has not been studied in stressful situation previously. In the present study, effects of immobilization stress (IS), both acute (IS × 1) and repeated (IS × 7) were assessed on extra-cellular total antioxidant capacity measured as plasma ferric reducing antioxidant power (FRAP) and protein sulfhydryls, and oxidative stress measured as leukocyte superoxide generation, plasma nitric oxide production (total nitrates and nitrites, NOx) and lipid peroxides in rats. Effects of pretreatment with nitric oxide synthase (NOS) inhibitors and vitamin E were also studied on these biochemical parameters. The results showed that both IS × 1 and IS × 7 resulted in extra-cellular oxidant–antioxidant imbalance as oxidant generation was increased and non-enzymatic antioxidants were depleted. Pretreatment either with NOS inhibitors or vitamin E restored stress-induced extracellular oxidant–antioxidant imbalance implying their potential role as antioxidants. Our data suggest that there is extra-cellular oxidant–antioxidant imbalance in the stressed rats, with greater magnitude of severity in repeated stress paradigm. Augmentation of antioxidant defenses might be beneficial in long-term stress.

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1. Introduction

Stress is usually recognized as a state of altered physiological homeostasis and the ability to cope with such stressful stimuli is a crucial determinant of health and disease. The limbic system, the hypothalamopituitary adrenal axis and several components of the visceral system react to a variety of stress inputs and complex interactions between them plays a vital role in determining the outcome of the stress response (Mc Ewen, 2000).

Free radicals and antioxidants have been the focus of research for the past decade. An imbalance between oxidants and antioxidants has been implicated in various disorders including gastric ulcerogenesis (Nishida et al., 1998), impaired cognitive function (Abidin et al., 2004) and neurodegenerative disorders (Liu et al., 1994) and emotional stress is believed to play a major role in these. Nitric oxide (NO) is a versatile molecule with diverse functions. It can act both as pro-oxidant and antioxidant depending upon the simultaneous production of superoxide radical (Beckman and Koppenol, 1996; Muijsers et al., 1997) and has also been found to be one of the factors for stress-induced oxidative stress (Matsumoto et al., 1999). While the role of NO has been explored in many studies, there is a lacuna in the literature on the studies of superoxide radical along with NO in stressful conditions. The free radical superoxide is released during respiratory burst of phagocytes by NADPH oxidase activity in response to several stimuli (Babior, 2000). Superoxide radical can be detrimental to biological molecules on its own and can also combine with NO to form peroxynitrite, which is a potent oxidant (Beckman et al., 1990; Radi et al., 1991a).

Organisms have endogenous mechanisms to neutralize any increase in the production of oxidants, as they possess wide array of both enzymatic and non-enzymatic antioxidants. Previous studies have also documented the existence of altered endogenous antioxidants both in the brain and blood (Gumuslu et al., 2002; Oishi et al., 1999; Zaidi et al., 2003; Zaidi and Banu, 2004), but they have mainly concentrated on enzymatic antioxidants. Biological antioxidants such as vitamin E, ascorbic acid, uric acid, bilirubin and protein thiols are the major non-enzymatic antioxidants present in extra-cellular fluids such as plasma (Frei et al., 1988). These antioxidants act in synergy to form an integrated network of antioxidant defense collectively forming total antioxidant capacity (TAC) and are considered to be first line of defense against any increase in the production of reactive oxygen species because of being consumable in nature (Halliwell and Gutteridge, 1990; Serafini et al., 2000).

In the present study, our aim was to assess the role of single (IS × 1) and repeated (IS × 7) immobilization stress on total antioxidant capacity (TAC) and oxidative stress to create a picture on extra-cellular oxidant—antioxidant balance. Single and repeated stress paradigms were used to evaluate the effects of different durations of stressors on the biochemical parameters as both intensity and duration of the stressor is known to determine the outcome of the stress response. To our knowledge, there is no previous data on investigation of extra-cellular TAC with oxidant generation in a stressful situation. Furthermore, we used NOS inhibitors and vitamin E to examine their possible antioxidant actions on stress-induced changes in extra-cellular oxidant—antioxidant balance in rats.

2. Materials and methods

2.1. Animals

Inbred male Wistar rats (150—220 g) were obtained from the Central Animal House Facility of Hamdard University, New Delhi, India. They were housed in standard laboratory conditions (12 h light—12 h dark cycle) at a temperature of 22 ± 2 °C. They had free access to food and water. Each experimental group comprised of 6—8 animals. Care of animals was taken as per guidelines in Care and Use of Animals in Scientific Research prepared by the Indian National Science Academy (INSA), New Delhi. The study had the approval of the Institutional Animal Ethical Committee (IAEC).

2.2. Experimental procedure

Rats were exposed to immobilization stress (IS) between 11 A.M and 1 P.M in a quiet room in the animal house. Acute immobilization stress, 1 h for a single day (IS × 1) and repeated immobilization stress, 1 h for seven consecutive days (IS × 7) was accomplished by placing the rats in specific Plexiglas restrainers. This method involves minimum pain with minimum movement including that of the tail. This immobilization technique is a standard procedure for stress and is widely accepted as a reliable method for inducing emotional and physical stress (Pare and Glavin, 1986). Control unstressed rats (No IS) were handled at the same time in the cages everyday. Immediately after completion of IS procedure, the rats were anesthetized with pentobarbitone sodium (50 mg/kg, i.p.) for collection of blood in heparinized tubes through exsanguination.

2.3. Drugs

N-nitro-L-arginine methyl ester (L-NAME), aminoguanidine hydrochloride and vitamin E (all from Sigma Chemical Co. Banglore, India) were dissolved in appropriate vehicle and injected intraperitoneally (i.p.) in a volume of 1 ml/kg, 30 min before the stress procedure.

2.4. Biochemical estimations

Unless otherwise stated all the chemicals were purchased from Sigma Chemical Co. (Banglore, India).

2.4.1. Leukocyte harvesting

Five milliliters of blood was withdrawn and mixed with 3% (*w* / *v*) dextran (509,000 mw) and allowed to stand for 45 min at room temperature. The resulting leukocyte-rich plasma was centrifuged at 250 × *g* (4 °C) for 12 min to obtain leukocytes. Contaminating red cells were lysed by 0.2% (*w* / *v*) NaCl for 30 s followed by restoration of molarity by addition of 1.6% (*w* / *v*) NaCl. After centrifugation, the leukocytes were washed twice in Krebs—Ringer Phosphate buffer, pH-7.35 containing 0.2% (*w* / *v*) dextrose and finally suspended in it at a concentration of 5 million cells/ml. The preparation at this point contains about 90% neutrophils (Markert et al., 1984). The remainder of the cells consists of monocytes, lymphocytes and eosinophils. The viability of leukocytes harvested with this technique was greater than 95% as determined by means of trypan blue exclusion.