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Original Article

THE EFFECT OF REPEATED SWIMMING STRESS ON ORGAN WEIGHTS AND LIPID PEROXIDATION IN RATS

Nayanatara A K[1], Nagaraja H S[2], and Anupama B K[1]

[1] Department of Physiology, Kasturba Medical College, Mangalore and [2] Department of Human Biology, International Medical University, Malaysia

Stressful situations induce physiological and behavioral changes in an organism to maintain the homeostasis. Chronic stress exposure has detrimental effect on several cell functions. The present study was to investigate the influence of repeated swimming stress on body weight, organ weights, and lipid peroxidation in brain regions, liver, kidneys, and adrenal glands. Adult male Wistar rats (n = 12) were exposed to forced swimming stress daily (45 minutes duration at 20^{o} C) for 7 days. The control was unstressed animals housed in the same condition (n = 12). Swimming stress significantly decreased total body weight (198.8 ± 5.75 g) when compared to unstressed control (218.20 ± 4.60 g) ($p < 0.01$). There was a significant increase in liver, kidney, adrenal gland, and cerebral cortex weights. A significant elevation in the lipid peroxidation in liver, kidneys, adrenal glands, and different brain regions like cerebral cortex, cerebellum, and hypothalamus was also observed. The present data suggest that repeated stress in the form of forced swimming activates the free radical processes leading to an increase in lipid peroxidation in many tissues.

Key words: stress, swimming, lipid peroxidation, organ weights

Exposure to stressful situations is among the most common human experiences. In response to stressors, a series of behavioral, neurochemical, and immunological changes occur that ought to serve in an adaptive capacity (Anisman, 1999). However, if those systems become overly taxed, the organism may become vulnerable to pathology. Likewise, the biological changes, if sufficiently sustained, may themselves adversely affect the organism's well being (Brown, 1993). The stress response, regarded as a positive adaptive process, comprises a set of functional and behavioral reactions to cope with challenging situations. A coordinated and adequate set of responses to stress is crucial for the survival of the organism in these situations. However, exaggerated responses to stress appear to be related to a wide range of physiological and psychological dysfunctions such as hypertension, stroke, depression, etc. (Vogel, 1993).

Swimming in small laboratory animals has been widely used for studying the physiological changes and the capacity of the organism in response to stress (Greenen et al., 1988; Tan et al., 1992). Swimming has got a number of advantages over other types of exercise such as treadmill running. The amount of work done during swimming exercise is far greater than that during the treadmill running of identical time duration. Swimming is not always a simple exercise stress, because emotional factors are difficult to be eliminated (Kramer et al.,

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Correspondence should be addressed to Dr. H. S. Nagaraja
Department of Human Biology
International Medical University, Plaza Komanwel, Bukit Jalil
Kuala Lumpur, Malaysia
E-mail: hsnagaraja@rediffmail.com

1993; Nagaraja and Jeganathan, 1999). The forced swimming stress developed by Porsolt et al. (1977) has now become widely accepted model for studying physical stress in animals. Water temperature is another important factor in forced swimming test. By varying the water temperature, Richter (1957) found that rats could survive as long as 80 hours in lukewarm water (36^{o}C). Increasing or decreasing the water temperature above or below this point influences the overall behavior of the animal and changes the involvement of glucocorticoids (Abel, 1991).

The conversion of oxygen during normal metabolism to the by products, hydrogen peroxide, super oxide, and hydroxyl radical occurs by successive electron additions to oxygen (Alho et al., 1998). Toxic free radicals have been implicated as important pathological factors in cardiovascular diseases, pulmonary disorders, autoimmune diseases, cancer, metabolic disorders, and aging (Latini et al., 2003; Prior and Cao, 1999).

It has been reported that exposure to stress situations can stimulate numerous pathways, leading to increased production of oxygen free radicals (Liu and Mori, 1999). Free radicals generate a cascade producing lipid peroxidation. Lipid peroxidation is one of the main events induced by oxidative stress. Lipid peroxidation can produce a range of enzymatically damaging consequences (Kovacheva and Ribarov, 1995). Extensive lipid peroxidation is shown to cause membrane disorganization, by peroxidizing mainly the polyunsaturated fatty acids and phospholipids leading to alterations in the ratio of polyunsaturated fatty acids to other fatty acids. Thus, lipid peroxidation is considered as a serious consequence of free radical toxicity leading to profound changes in the membrane structure and function that may even cause cellular death (Alessio, 1993; Ji, 1999).

Stress in the form of immobilization was accompanied by an increase in the lipid peroxidation intermediates in the myocardium (Davydov and Shvets, 2001; Shvets and Davydov, 2003). It is supposed that chronic stress in aged rats was accompanied by the decreased effectiveness of initial processes of free radical reactions (Shvets and Davydov, 1995). Markova and Misula (1998) have reported that, on exposure to stress, rats displayed high activity of lipid peroxidation and low activity of anti-oxidative system in the heart 18-60 hours post stress. Concentrations of TBA (thiobarbituric acid) reactive substances were increased in various brain regions like hypothalamus and limbic system when rats were exposed to acute stress of forced isolation in the restraining plastic cages for one hour (Sosnovskii and Kozlov, 1992). Voronych and Iemelianenko (1994) reported that lipid peroxidation in various brain regions of albino rats was very sensitive to acute immobilization stress.

The effect of short-term stress on oxidative stress and production of free radicals has been investigated in previous studies. However, the influence of repeated stress on lipid peroxidation is not clear. This study tested the hypothesis that repeated stress brings about a stress-induced injury in various organs and a significant change in the free radical production in an organism.

Materials and Methods

Adult male Wistar rats weighing between 225-250 g were divided into two groups as Control (n =12) and Stress (n =12). All the rats were given standard rat chow and tap water *ad libitum* and were housed at 25 ± 2 ^{o} C on a 12-hour dark/light cycle. All the experimental procedures were approved by the Institutional Animal Care and Usage Committee.

Stress procedures:

Rats were exposed to forced swimming stress daily for duration of 45 minutes between 09.00AM to11.00AM until 7 days. They were forced to swim in plastic tanks (length 100cm, width 40 cm, depth 60 cm) containing tap water maintained at a temperature of 20 ^{o} C. The depth of water in the tank was 30 cm. A maximum of two rats were allowed to swim together during stress session. The control rats were housed under the same conditions and they were handled as often as

the stressed group.

The body weight of each rat was measured before and after the stress period. At the end of experiment, all rats were anaesthetized with sodium pentobarbital (40 mg/kg body weight) and then liver, kidney, adrenal gland, and brain were quickly removed and weighed. From the whole brain, cortex, cerebellum, and hypothalamus were carefully separated. The specimens were stored at -80^{o} C until assay. The wet weight of each organ was expressed as 100 g of body weight.

Biochemical analysis:

The collected tissues (liver, kidney, cortex, cerebellum, and hypothalamus) were homogenized with a motor driven glass homogenizer in ice-cold phosphate buffer at 0^{o} C for lipid peroxidation analysis. Lipid peroxidation in liver, kidney, cerebral cortex, cerebellum, and hypothalamus was estimated spectrophotometrically by thiobarbituric acid reactive substance (TBARS) as previously described (Stocks and Dormandy, 1971). TBARS measurements were done immediately after tissue sampling. Results were expressed as nanomoles per gram of protein in tissue. The protein contents of tissues were analyzed with the method of Lowry et al. (1955).

Statistical analyses

Data were computed for mean values ± standard error of the mean (SEM). Comparisons between control and stress were performed by Mann Whitney U test with a significant criteria of $p<0.05$.

Results

Both whole body weight and food intake significantly decreased ($p<0.01$) at the end of repeated swimming stress. In contrast, such forced swimming stress significantly increased various organ weights that expressed as percent of whole body weight. These included liver ($p<0.01$), kidneys ($p<0.01$), adrenal gland ($p<0.05$), and cerebral cortex ($p<0.05$). However, the weights of the cerebellum and hypothalamus did not differ between the groups.

Table 1. Stress-induced alterations in body weight, food intake, and organ weights

Parameters	Control (n= 12)	Stress (n=12)
Body weight (g)	218.20 ± 4.60	198.8 ± 5.75 **
Food intake (g/d)	20.40 ± 0.42	12.66 ± 0.52 **
Liver (g/100g BW)	3.285 ± 0.15	3.721 ± 0.224 **
Kidneys (g/100g BW)	0.547 ± 0.161	0.657 ± 0.142 **
Adrenals (g/100g BW)	0.0265 ± 0.002	0.0364 ± 0.005 *
Cerebral cortex (g/100 BW)	0.239 ± 0.083	0.296 ± 0.014 *
Cerebellum (g/100g BW)	0.190 ± 0.011	0.194 ± 0.037
Hypothalamus (g/100g BW)	0.182 ± 0.027	0.175 ± 0.062

Values are mean ± SEM; BW = body weight; n= number of rats; * P<0.05, ** P<0.01, *** P<0.001 when compared to control

In all the tissues studied, TBARS levels were significantly increased after swimming stress for 7 days (Table 2). These included liver, kidneys, and brain areas, indicating increased lipid peroxidation following repeated swimming stress. Among them, the oxidative stress in cerebellum seems to be most sensitive to repeated exercise (about 200% increment).

Table 2. Stress-induced alterations in lipid peroxidation (nmol/g protein) in various organs

Parameters	Control (n= 12)	Stress (n=12)
Liver	28.107 ± 0.800	46.094 ± 0.730 **
Kidneys	21.242 ± 0.655	25.046 ± 0.216 **
Cerebral cortex	102.228 ± 1.785	122.369 ± 0.450 ***
Cerebellum	69.234 ± 1.139	134.676 ± 2.636 ***
Hypothalamus	80.645 ± 0.575	99.239 ± 0.607 **

Values are mean ± SEM; n= number of rats; * P<0.05, ** P<0.01, *** P<0.001 when compared to control.

Discussion

The effect of acute exercise on free radical production and the antioxidant system in rats has been intensively investigated (Ji et al., 1998; Polidori et al., 2000) but it is still controversial about the effect of repeated stress on lipid peroxidation in different organ systems. The present study indicate that repeated swimming stress for 7 days significantly increased the oxidative damage biomarker of lipid peroxidation in liver, kidneys, and different brain regions in rats. The increased level of lipid peroxidation is the evidence most frequently cited in support of the involvement of oxidative stress in tissues (Haliwell, 1992; Liu and Mori, 1994). The data suggest that there is the activation of the free radical precursors in all the investigated tissues with maximum changes in the brain regions and minimum changes in the kidneys.

Increases in lipid peroxidation in the liver after stress confirm the earlier observation by Alptekin et al. (1996) who observed a similar increase in lipid peroxidation in the liver after acute stress in rats. However, the present observation is in contrast to that report by Hu et al. (2000) who observed no change in the lipid peroxidation in the liver after chronic swimming stress. The increased lipid peroxidation in hypothalamus is in agreement with the studies by Sosnovskii and Kozlov (1992) who reported an increased lipid peroxidation in the early stages of stress exposure in rats.

Repeated stress on a daily basis may impair the antioxidant defenses in the body leading to oxidative damage by changing the balance between oxidant and antioxidant factors. The increased levels of reactive oxygen species under stress conditions could be due to the increased concentration of glucocorticoids. It has been reported that these hormones exacerbate reactive oxygen species (ROS) generation in the body (McIntosh and Sapolsky, 1996). Decreased activities of the antioxidant enzymes have been observed in the brain of rats treated with glucocorticoids (McIntosh et al., 1998). An increase in lipid peroxidation in the present data (liver, kidney, and brain regions) indicates the increase in the free radical generation due to repeated stress and a decrease in resistance against stress. Increasing stress was reported to enhance stress-induced tissue damage

and malfunction (Lakatta, 1980; Davydov and Shvets, 2001). Oxidative stress is a central feature of many diseases and stress-induced damage to these tissues may be the cause of severe stress disorders after repeated stress exposure. Numerous studies have shown that strenuous physical exercise can increase free radical production and cause oxidative damage (Bejma and Ji, 1999; Reid et al., 1992). Apart from repeated swimming exercise, decreased water temperature also produced severe stress in rats (Abel, 1991) and these two factors together might have caused increased free radical production in various tissues. Further experiments need to separate the effect of low water temperature alone from that of swimming stress.

Short-term swimming significantly decreased the whole body weight as compared to unstressed control. This effect is in accordance to other reports in chronic exercise (Jain and Stevenson, 1991; Marti et al., 1993). The decreased body weight could be due to the decreased food intake in the rats under the influence of stress. Corticotrophin-releasing hormone (CRH) is commonly released during stress and might be a factor that suppressed food appetite in the repeated swimming (Levine et al., 1983). Increased weight of the liver during stress could be due to the increased secretion of the stress hormones, which are known to increase the metabolic activities and mRNA levels in the hepatic cells. Changes in the homeostatic mechanism such as increased cardiac output and blood pressure during stress might have contributed to the increased kidney weight after stress (Chang et al., 1995; Nagaraja and Jeganathan, 1999). Stress-induced adrenal hypertrophy is a well-established phenomenon. Strong stimulation of the adrenal glands during prolonged stress situations is known to cause adrenal hyperplasia and hypertrophy (Marti et al., 1993; Tuli et al., 1995).

In summary, the present data indicate that repeated stress on a daily basis for a period of 7 days decreased the whole body weight and food intake and increased weights of liver, kidneys, and adrenal glands. Short-term stress significantly elevated lipid peroxidation in liver, kidneys, and different brain regions, indicating increased generation of free radicals following repeated stress. The present study confirms the shift of oxidants and antioxidants balance towards the oxidative stress in short-term exercise. The assessment of the different antioxidants during stress will be an interesting step to identify the mechanisms involved.

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