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FORCED SWIMMING STRESS-INDUCED CHANGES IN THE PHYSIOLOGICAL AND BIOCHEMICAL PARAMETERS IN ALBINO RATS

H. S. NAGARAJA AND P. S. JEGANATHAN*

Department of Physiology,
Kasturba Medical College,
Mangalore - 575 001

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Abstract : The effects of fresh water swimming and cold water swimming for one day, 7 days and 15 days were studied on the total body weight, weight of the heart, kidney, adrenals, blood sugar level, serum cholesterol level, total leucocyte count, absolute neutrophil count and absolute eosinophil count in albino rats. The stress session in each day lasted for 45 min. Though the body weight of the animals decreased significantly during the initial period, when the stress period was prolonged, there was recovery in the body weight. There was a significant increase in the weight of the heart, kidney and adrenal glands after both types of stress. A significant leucopenia, eosinopenia and neutropenia along with significant hypoglycemia and hypocholesterolemia was observed in all the subgroups. A maximum stress response was seen upto a period of 7 days and the stress response decreased when the stress period was prolonged to 15 days in the physiological parameters studied. This could be due to the adaptation or habituation for the continued stress. There was no sign of adaptation in the case of biochemical parameters studied. A statistically significant physiological and biochemical changes were observed even after one day of forced swimming stress.

Key words : stress-fresh water swimming cold water swimming

INTRODUCTION

In the 1930's, Hans Selye defined stress as the reaction of an organism to various stimuli. These diverse stimuli together can be called as stressors (1). Stress would manifest itself as physiological changes, biochemical changes and behavioural changes. Intense or chronic stress would then lead to the well known stress related diseases such as hypertension, diabetes, stroke, cancer, depression etc., (2) Stressors are mainly of two types : Acute stress, where animals are subjected to short or single exposure to stress and chronic stress i.e. repeated exposure to stress on a daily basis (3). A short single experience of stress can have long term consequences for the animal's stress responses. A single stress experience can produce profound changes in stress responsiveness and behaviour (4). One predominant feature of chronic stress is the finding that repeated stress leads to adaptation or habituation. Hence repeated

*Corresponding Author

exposure to the same stressor evokes lesser response to each session. Chronic or repeated stress can cause a wide range of physiological and neuroendocrine changes (5).

Swimming in small laboratory animals has been widely used for studying the physiological changes and the capacity of the organism in response to stress (6,7). 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 (8). The forced swimming test developed by Porsolt et al (9) has now become a widely accepted model for studying physical stress in animals. Water temperature is another important factor in the forced swimming test. By varying the water temperature, Richter (10) found that rats could survive as long as 80 hours in lukewarm water (36°C). Increase or decreasing the water temperature above/below this point influences the overall behaviour of the animal and changes the involvement of glucocorticoids (11).

Though there are number of studies on the effect of cold stress, the literature is scanty regarding the effect of cold water swimming and fresh water swimming stress. Hence, the present work was undertaken to study the effect of both fresh water swimming (FWS) and cold water swimming (CWS) stress on some of the physiological and selected biochemical parameters in albino rats.

METHODS

Adult male albino rats of Wistar strain with body weight ranging between (180-250 g) were used for all the experiments. Rats were housed under standard laboratory conditions with food and water provided ad libitum.

Control Group : This group of rats (n=12) were kept under ideal laboratory conditions served as normal control animals.

Fresh water swimming stress : Rats were forced to swim in the plastic tubs (height : 60 cm, diameter : 40 cm) containing tap water, maintained at room temperature (28°C). Depth of the water in the plastic tub was 30 cm. The swimming session lasted for 45 minutes daily. Experiments were done between 10 AM and 12 noon to minimise the circadian variability. FWS stress was studied in three subgroups : In the first subgroup of rats (n=10) the stress period lasted for 15 days. The second subgroup (n=10) was subjected to FWS for 7 days and in the third subgroup (n=9), the forced swimming stress in fresh water was done for 45 minutes only and this was taken as one day fresh water swimming subgroup.

Cold water swimming stress : Rats were forced to swim in the cold water maintained at 10°C. Like FWS stress, cold water swimming stress was also studied in three different subgroups for a period of 15 days, 7 days and one day respectively with each subgroup containing 10 animals.

The body weight of the rats were measured before and after the stress period in all the experimental groups and also in the control group. The animals were sacrificed by decapitation and blood samples were collected. The wet weight of the organs were expressed per 100g of body weight. From the blood samples, total leucocyte count, absolute eosinophil count and absolute neutrophil count were estimated by standard physiological methods. Blood sugar level and serum total cholesterol level were measured by calorimetric method.

Statistical analysis was done by one way analysis of variance (ANOVA). P value less than 0.05 was considered statistically significant.

RESULTS

There was a significant decrease in the body weight after 7 days of stress in FWS stress and also in CWS stress (Table I). No significant change in the body weight was observed after 15 days of FWS stress. But in the case of CWS stress, body weight increased significantly ($F = 17.96$, $P < 0.00001$) more than in one day and 7 days of CWS stress and 15 days of FWS stress.

A significant increase ($F = 46.80$, $P < 0.00001$) in the heart weight was observed in all the three durations of FWS stress and CWS stress (Table I). After 7 days, CWS stress resulted in increased heart weight than in FWS stress for the same period.

Kidney weight increased significantly ($F = 6.52$, $P < 0.005$) in all the three subgroups of FWS stress. In the case of CWS stress, a significant increase in the kidney weight was observed only in the 15 days stress subgroup (Table I).

There was a significant increase in the adrenal gland weight ($F = 24.80$, $P < 0.00001$) in all the three subgroups, both in FWS stress and CWS stress (Table I). FWS stress for 15 days was having significantly higher adrenal gland weight than CWS stress for the same period. In the FWS stress group, 7 days subgroup was having significantly higher adrenal gland weight than one day and 15 days subgroup.

Total leucocyte count decreased significantly ($F = 61.32$, $P < 0.00001$) in all the three subgroups in both FWS and CWS stress. When one day stress subgroups were compared, FWS stress was having significantly lower count than one day CWS

TABLE I : Body weight and organ weight (g/100g of body wieght) in fresh water swimming and cold water swimming stress.

Parameter	Control (n=12)	*Fresh water swimming* 1 day (n=9)	*Fresh water swimming* 7 days (n=10)	*Fresh water swimming* 15 days (n=10)	*Cold water swimming* 1 day (n=10)	*Cold water swimming* 7 days (n=10)	*Cold water swimming* 15 days (n=10)
Body weight	216.8±1.8	213.2±12.6	196.8±11.8*	220.2±19.6	223.7±6.2	199.7±4.8**	259.2±9.1**
Heart	0.319±0.003	0.390±0.014**	0.403±0.008**	0.396±0.009**	0.374±0.010**	0.453±0.010**	0.366±0.010**
Kidney	0.310±0.003	0.347±0.012*	0.344±0.007*	0.334±0.005*	0.316±0.009	0.317±0.007	0.351±0.010*
Adrenals	0.005±0.00001	0.009±0.001**	0.013±0.001**	0.011±0.001**	0.011±0.001**	0.011±0.001**	0.008±0.001**

Values are expressed as Mean ± SEM
Significance : *P<0.05 **P<0.00001

TABLE II : Leucocyte, Eosinophil and Neutrophil count in fresh water swimming and cold water swimming stress.

Parameter	Control (n=12)	Fresh water swimming: 1 day (n=9)	Fresh water swimming: 7 days (n=10)	Fresh water swimming: 15 days (n=10)	Cold water swimming: 1 day (n=10)	Cold water swimming: 7 days (n=10)	Cold water swimming: 15 days (n=10)
Total leucocyte count	3685.8±54.8	2422.2±112.8**	2075.0±128.9**	2712.0±86.7**	2958.0±39.8**	1387.5±27.7**	2080.0±74.6**
Absolute eosinophil count	140.4±4.9	37.9±3.7**	20.9±2.0**	32.0±8.8**	46.2±3.9**	73.7±3.7**	52.8±4.9**
Absoulte neutrophil count	1440.3±58.5	467.4±37.0**	413.5±38.5**	539.9±35.0**	605.5±17.6**	319.4±10.0**	410.8±20.8**

Values are expressed as Mean±SEM
Significance : *P<0.05 **P<0.00001

stress (Table II). After 7 days and 15 days period, CWS was having more significant leucopenia than in FWS stress for the same duration.

There was a significant fall in the absolute eosinophil count ($F = 99.53$, $P < 0.00001$) after exposing the animals for one day, 7 days and 15 days of CWS stress and FWS stress (Table II). FWS stress produced significantly lower eosinophil count than in CWS stress for the same period.

A significant reduction ($F = 124.59$, $P < 0.00001$) in the absolute neutrophil count was seen in all three subgroups of both the types of stress. After 7 days of stress exposure, CWS stress produced significantly more fall in the neutrophil count than that observed in FWS stress for the corresponding period (Table II). After one day stress exposure, FWS stress produced significant neutropenia than in CWS stress.

A significant fall in the blood sugar level ($F = 144.35$, $P < 0.00001$) was observed in all the subgroups of both types of stress (Fig I). The reduction in the blood sugar level was significantly more after 15 days

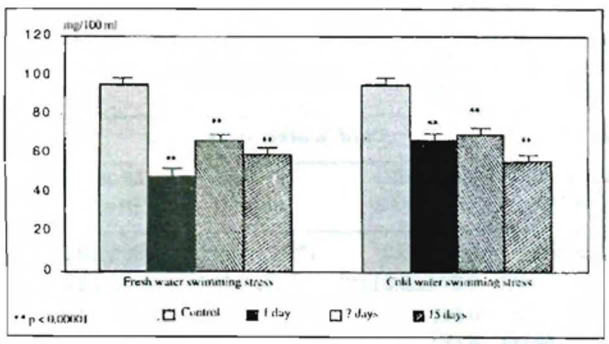

Fig. 1: Blood sugar level in fresh water swimming and cold water swimming stress.

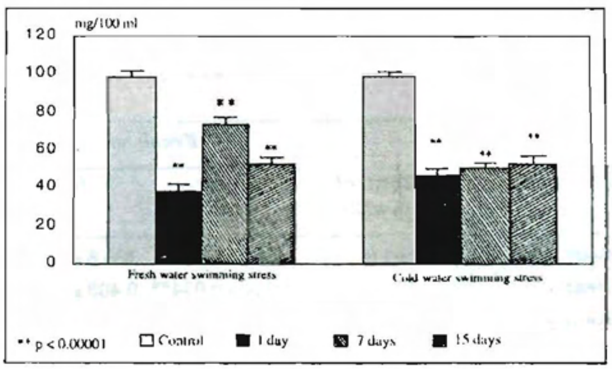

Fig. 2: Cholesterol level in fresh water swimming and cold water swimming stress.

of CWS stress exposure and one day of FWS stress. After one day stress exposure, FWS stress resulted in significant hypoglycemia than in CWS stress for the identical duration of stress exposure.

Total serum cholesterol level decreased significantly ($F = 197.04$, $P < 0.00001$) in all the three subgroups of both FWS stress and CWS stress (Fig 2). More significant reduction in the cholesterol level was observed after one day stress exposure in both the types of stress. Cholesterol level decreased significantly more after 7 days of CWS stress when compared to FWS stress for the same duration.

DISCUSSION

The whole body weight decreased significantly after 7 days of stress. The effect of stress on the body weight appeared to be more marked in the initial period of exposure to the stress. This decrease in body weight, when animals were exposed to stress, agrees with the various other reports (12, 13) available. The decrease in body mass could be due to the decreased food intake in the animals under the influence of stress (14). After 15 days of exposure to the stress, the body weight gradually recovered back to the initial body weight. This could be due to the adaptation to the stressful situation when the stress period was prolonged (5).

The observed cardiac hypertrophy after CWS stress and FWS stress is in accordance with the previous study by Henry and Stephens (15) who reported an increase in the heart weight after exposing the animals to psychological stressors. Forced swimming being a physical stress increases the work load on the heart and thereby increasing heart weight (16).

Increased kidney weight after FWS and CWS stress supports the earlier reports by Chang et al (16) who observed an increase in the kidney weight after exposing the animals to exercise stress. Forced swimming might have increased the work load on the kidney as there was every chance for the animals to have more water intake during swimming. Changes in the homeostatic mechanism such as increase in cardiac output and blood pressure during stress might have contributed to increase in kidney weight in stress.

Stress induced adrenal hypertrophy is a well established observation (14, 17, 18). Forced swimming stress causes greater release of adrenaline and greater increase in the weight of adrenal gland (19). Under the influence of stress, there is strong stimulation of adrenal gland leading to hyperplasia and hypertrophy of cells (20).

The leucopenic response observed in this study might be attributed to the action of adrenal steroids (21, 22). Jensen and Rasmussen (23) have shown the involvement of adrenal glands on the white cell number. Absolute eosinophil count decreased significantly in both CWS stress and FWS stress. This observation confirms already published reports (24, 25). The eosinopenia observed after forced swimming stress could be due to the corticosteroids released during stress. A significant neutropenia observed after swimming stress was in contrast to the published reports (21, 24). This decreased neutrophil count could be due to the abnormal distribution due to local chemotaxis causing cell retention in certain organs. Under the influence of stress neutrophils might exit from the circulation as there could have been increased chance of local tissue damage leading to increased capillary permeability after swimming stress.

Blood sugar response to stress reported in various study was highly contradictory (26). In the present study it was observed that stress produced severe hypoglycemia rather than the much anticipated glycemic response. This observation confirms the previous reports by Rodnick et al (27) who had observed a decrease in blood glucose level after physical training by either swimming or running. It is well known that the sensitivity to the effect of insulin which decreases blood glucose is enhanced after physical exercise. It is possible that the observed hypoglycemia after stress is probably due to the increased sensitivity to insulin (28).

Serum cholesterol level decreased significantly after forced swimming. This confirms the earlier observations by Tsopanakis and Tesseromatis (29), who found a decline in total cholesterol level when rats were exposed to cold swim stress for a period of 20 and 60 days. But hypocholesterolemia after stress is in contradiction to other report (30, 31). Cholesterol response to stress is rather contradictory and may depend on situational, environmental and inter individual factors. The physiological mechanism of stress induced changes in cholesterol remain largely unelucidated. It appears likely that the Hypothalamic - Pituitary - Adrenal (HPA) axis contributes to the stress induced cholesterol changes (32).

There was maximum response to forced swimming after 7 days of exposure to stress period in the various physiological parameters studied, both in fresh water swimming and cold water swimming. When the stress period was prolonged to 15 days time, there was gradual recovery back to normal. This reduced response of the animals to prolonged stress could be because of the habituation or adaptation of the HPA axis (5, 33). In this study no habituation or adaptation was observed in the biochemical parameters (blood sugar and cholesterol) after forced swimming stress.

From the foregoing it can be concluded that stress in the form of freshwater swimming and cold water swimming increased the weight of the heart, kidney and adrenal glands. There was a significant hypocholesterolemia and hypoglycemia which was maintained even when the stress period was prolonged. It was observed that short single stress like one day forced swimming stress was as effective as prolonged stressor in bringing about the stress induced alterations in the body.

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