Original Article

Effect of different forms of acute stress in the generation of reactive oxygen species in albino wistar rats

K. K. Smitha* and J. K. Mukkadan

Little Flower Medical Research Centre, Angamaly – 683 572

Abstract

Stress is common for all organisms either in the form of eustress (beneficial) or as distress (harmful). Stress is highly diverse in present world and its effects in organisms are well studied. This study is a comparative effect of stress in the generation of reactive oxygen species in albino Wistar rats, which are seldom reported. Here animals were grouped into four and the test animals of each group were administered with any one of the following stress, namely forced swimming induced stress (FS), and noise induced stress (NS), immobilization stress (IS) and overcrowding stress (OS). After stress administration, serum cortisol was estimated as a bio marker of stress in the albino rats, and the liver homogenate were used to estimate superoxide dismutase (SOD) by using rat Elisa kit in the spectrophotometer. The data were processed with unpaired 't' test. The cortisol levels were found to be increased in a highly significant level (P<0.001) in all the groups as compared to the normal control. And the stress level was found to be maximum in the FS group in comparison with other groups. The mean cortisol level in different stress groups such as FS, NS, IS and OS were found to be 4.15, 3.7, 3.63, 3.62 μ gm/dl respectively. Among all the stressed groups, the SOD level in the FS group were found to be increased in a highly significant level (P<0.001) in comparison with normal control group. The SOD level in FS group was (30.75 U/mgm. protein) followed by OS (28.96), noise (28.88) and IS (28.77).

Introduction

Stress is a common effect faced by all organism. The vulnerability of stress is different in different organisms; human being is never an exception (1). Stress is an ability to withstand a definite amount of strain and it disturbs the homeostasis of the living body. Stress causes biological changes, and if sufficiently sustained, they may adversely affect the organisms’ well being (2). It occurs during major life events, trauma and abuse; it can also be related to the environment in home, workplace or neighborhood. An organism responds to stress with certain adaptive responses. According to the intensity and duration of stress these adaptive responses may lead to pathological changes. Though the positive effect of stress (eustress) and negative effect of stress (distress) are equally discussed, the pathological effect is more concerned issue (3). Stress has an important role in the causation and precipitation of the major lifestyle diseases such as hypertension, diabetes, acid peptic disease, obesity etc.

A free radical can be defined as any molecular species capable of independent existence that...
contains an unpaired electron in an atomic orbital. The presence of an unpaired electron, results in certain common properties that are shared by most radicals. Many radicals are unstable and highly reactive. They can either donate an electron or accept an electron from other molecules, therefore behaving as oxidants or reductants (4). The exposure to stress can stimulate numerous pathways, leading to increased production of oxygen free radicals (5). Reactive oxygen species (ROS) are produced intracellularly through multiple mechanisms, the major sources being mitochondria, peroxisomes, endoplasmic reticulum, and the NADPH oxidase (NOX) complex in cell membranes (6). The amount and type of free radical generation, due to stress is related to the type of stress and duration of stress. So a study of different psycho physical stress in the generation of reactive oxygen species was planned.

Materials and Methods

Animals

The present research experiment was carried out in 423 adult male Wistar rats, weighing in the range of 180±30 g, which were 60 days of age when considered for the experiment. Out of the 500 rats considered, only 423 of them were included in different groups and results of only these rats were accepted, and the remaining of them were either discarded from experiments due to physical disabilities or due to immature death. The rats were bred and maintained at the central animal research facility (Rodent house Register number: 496/01/a/CPCSEA) of the Little Flower Medical Research Centre (LFMRC), Angamaly. Rats were housed in polypropylene cages (30 x 22 x 14 cm). Paddy husk was used as bedding material, which was changed on alternate days. The colony was maintained in a well aerated room with exhaust and ceiling fans. The rats were maintained at 12 hrs light-dark cycle. The room temperature was 28±4°C. Three to four rats were housed in each cage. Four experimental methods were planned separately for four types of stress. They are forced swimming stress, immobilization stress, overcrowding stress and noise stress. Each stressed animal groups were kept against almost equal number of normal control without giving any stress.

Application of Stress methods in animals

Forced swimming (FS) induced stress was induced in rats by forcing them to swim in a cylinder (30 cm diameter and filled to a height of 20 cm with 15 cm of space above the head of the rat) for 2-h session a day for five consecutive days for acute stress (7). The experiments were done from 9 AM – 11 AM to minimize the circadian variability. Noise stress (NS) in rats were produced by loudspeakers (15 W), driven by a white noise generator (0-26 kHz), installed 30-cm above the cage. Thus a noise level was set at 100 dB or above uniformly throughout the cage and was monitored by a sound level meter. Each animal was exposed to noise stress for 4 h/day for 15 days. For immobilization stress (IS) animals were kept with its limbs stretched on a board and immobilized with adhesive tape. Movement of head was restricted by keeping the head in a metal loop coiled around the neck. The rats were kept immobilized from 10 am to 12 noon for 7 days to produce acute stress (8). The major advantage of using immobilization as a model of stress is that it produces an inescapable physical and mental stress to which adaptation is seldom exhibited (9). The over crowding of the animal was performed according to Nagaraja et.al (10). Group of animals were kept in small polypropylene cages [25 cm x 20 cm x 15 cm] in such a way that minimum movement was possible for rats inside their cages. This overcrowding condition itself was a stress for the rats. Overcrowding stress (OS) was given for 15 days. For each day overcrowding was given from morning 10 AM to 12 noon.

Hypercortisolism is one of the most consistent biological finding in the stress (11). In this experiment, thus serum cortisol level was taken as a bio marker of stress in animals. The serum cortisol was estimated by the Rat cortisol ELISA kit purchased from Bio Compare Company India. Estimation of cortisol was estimated by using the spectrophotometer. The concentration of cortisol in the samples is then determined by comparing the O.D. of the samples to the standard curve. There is compelling evidence that superoxide dismutase (SOD) are essential for biological defense against superoxide anions (12). The SOD was estimated by the method based on the reduction of Nitro blue
tetrazolium (NBT) to water insoluble blue formazan. Liver homogenate (0.5 ml) was taken, and 1 ml of 50 mM sodium carbonate, 0.4 ml of 24 μM NBT, and 0.2 ml of 0.1 mM EDTA were added. The reaction was initiated by adding 0.4 ml of 1 mM hydroxylamine hydrochloride. Zero time absorbance was taken at 560 nm followed by 5 min at 25°. The control was simultaneously run without liver homogenate. Units of SOD activity were expressed as the amount of enzyme required to inhibit the reduction of NBT by 50%. The specific activity was expressed in terms of units/mg of protein (13).

Results

The cortisol levels were found to be increased in a highly significant level (P<0.001) in all the groups as compared to the normal control. And the stress level was found to be maximum in the FS group in comparison with other groups. The mean cortisol level in different stress groups such as FS, NS, IS and OS were found to be 4.15, 3.7, 3.63, 3.62 μg/ml respectively. Of all the stressed groups, FS shows maximum cortisol production (4.15 Mean μg/ml) with high significance (P<0.001) followed by NS (3.70), IS (3.63) and OS (3.62) (Table I). Of all the stressed groups, the SOD level in the FS group were found to be increased in a highly significant level (P<0.001) in comparison with normal control group. The SOD level in FS group was (30.75 U/mg protein) followed by OS (28.96), noise (28.88) and IS (28.77) (Table II).

Discussion

In this study the rats were grouped and subjected to different psychophysical stress namely FS, NS, IS and OS. The aim of the study was to assess the extent of ROS produced by these stress and its comparison among the different stress groups. Several studies have revealed the generation of ROS by the stress. However a comparative analysis of the aforesaid stress is seldom worked though it is highly relevant in the current fast moving society. Different types of stress causes various impacts in the systems of organism beyond our speculations and imaginations especially in the enzymatic and hormonal levels (14).

Several biological parameters can be taken as markers of stress in both albino rats and human being. Of these important ones are glucose, cholesterol, epinephrine, ACTH and cortisol (15). Most researchers rely the results of cortisol as the best stress marker in the albino rats. Glucocorticoids are characteristic mediators of stress responses, and the detrimental effects of chronic stress on health have been proposed to be attributed to their immunosuppressive properties (16). Based on the severity of stress the values of cortisol vary. According to this study FS causes maximum stress followed by the NS. This may be because of the strenuous nature of forced swimming, which causes both physical and mental stress. Though the amount of cortisol level was less in OS as compared to FS and NS, the OS issues can’t be considered as least important one, as the variation in the cortisol level between the test group and standard group was more in this group than the other two groups. Overcrowding is one of the major problems faced by the world today. The authorities in different countries had put forward many plans and budgets for rectifying or even shrink this problem.

Superoxide dismutase is a direct indication of ROS in an organism (17). SOD levels were found to be

<table>
<thead>
<tr>
<th>Stress</th>
<th>Normal</th>
<th>Stressed</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NS</td>
<td>52</td>
<td>2.16±0.42</td>
<td>54</td>
</tr>
<tr>
<td>FS</td>
<td>50</td>
<td>2.17±0.42</td>
<td>53</td>
</tr>
<tr>
<td>IS</td>
<td>54</td>
<td>1.97±0.63</td>
<td>54</td>
</tr>
<tr>
<td>OS</td>
<td>53</td>
<td>2.13±0.34</td>
<td>53</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Stress</th>
<th>Normal</th>
<th>Stressed</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NS</td>
<td>52</td>
<td>23.64±1.24</td>
<td>54</td>
</tr>
<tr>
<td>FS</td>
<td>50</td>
<td>23.79±1.71</td>
<td>53</td>
</tr>
<tr>
<td>IS</td>
<td>54</td>
<td>23.61±1.17</td>
<td>54</td>
</tr>
<tr>
<td>OS</td>
<td>53</td>
<td>23.73±1.43</td>
<td>53</td>
</tr>
</tbody>
</table>
high in FS group. This means that more ROS are produced by FS in comparison with other stress. In normal conditions, there is a steady state balance between the ROS production and their scavenging by the cellular antioxidant system. More muscular exercise is needed in FS, thus resulting in a fatigue stage. This condition will increase the mitochondrial respiration in FS which in turn produce more ROS (18). The order of stress production based on cortisol level, was as follows FS, NS, IS, OS, but the order of SOD production was in a different order such as FS, OS, NS, IS, which shows that stress production and free radical generation are not proportional in the case of OS, which might be due to the increased variation shown between the test group and standard group, as compared to the other stress groups. This study doesn’t measured total anti oxidant status in these animals because SOD is one of the body’s primary internal anti-oxidant defenses, and plays a critical role in reducing the oxidative stress (19). The antioxidant enzymes serve as the body’s most potent defense against free radicals. They include glutathione peroxidase, catalase, and perhaps the most important internally generated antioxidant is superoxide dismutase (SOD). It is present both inside and outside cell membranes.

Conclusion

Conclusions made from this study are that, forced swimming, immobilization, noise and overcrowding were sufficient to produce a significant amount of stress in albino rats. All these stress produced ROS in a statistically significant level. Among the following acute stress, forced swimming, immobilization, noise and overcrowding; FS is causing maximum stress in albino rats. And out of four types of stresses, FS caused more reactive oxygen production and can be evaluated by the SOD activity. Also it was noted that among all these stress least harmful stress is produced by overcrowding whereas least production of ROS was in immobilization stress.

References