Chronic stress induced tissue malondialdehyde level in amygdala nucleus lesioned wistar rat

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Abstract

Chronic stress has significant adverse effects on health and is a risk factor for many illnesses. The idea that the brain categorizes stressors and uses response pathways that vary according to the category has gained significant support in the recent years. The present study was designed to elucidate the possible role of amygdala nucleus on chronic physical and chronic psychological stress induced tissue lipid peroxidation level. Adult Wistar albino rats were divided into three major groups normal, sham lesioned and amygdala lesioned. Each group was further subdivided into control groups and stress groups. In each group ten animals were taken. The amygdala lesion was performed according to the stereotoxic coordinates by Paxinos and Watson. The stressed group of rats were subjected to chronic swimming (Physical) and chronic immobilization (Psychological) stress for 60 days. Tissue MDA levels (nanomoles of MDA/g wet tissue) was estimated by Kartha and Krishnamurthy method. MDA levels of heart, liver and kidneys were estimated. The stress induced increase in MDA level in amygdala lesioned rats was significantly more (P<0.001) during immobilization stress than swimming stress. The data of the present study support the hypothesis that the brain recognizes at least two major categories of stressor, which has been referred as physical and psychological. Further, our study also provides the supportive evidence that the response of a psychological stress to a greater extent is regulated by amygdala nucleus.

Keywords: Amygdala, Lesion, MDA, Immobilization, Swimming, stress.

1. Introduction

In the modern era, stress has undoubtedly become an integral part of human life. Literature survey shows that stressful conditions alter physiological homeostasis leading of variety diseases. Experimental models are required to better understand the role of brain nucleus in stress and elaborate new therapy. Oxidative stress due to physical as well as psychological forms of stress has become the focus of interest due to its deleterious effects on physiological systems. Forebrain structures are known to influence stress responses [1]. Among them, the amygdala has attracted continued interest because of its central role in processing emotional information [2]. Literature survey shows the involvement of amygdala in autonomic responses to stress [3, 4]. The role of specific brain nucleus in response to the type of stressors has remained unanswered to this date. It is still a matter of debate that whether the brain recognizes two major categories of stressor, which were referred as ‘physical’ and ‘psychological’ based on the type of nucleus which is activated. Elevated levels of malondialdehyde (MDA) one of the by-products of lipid peroxidation has been reported in the cardiovascular, neurological and in other diseases [5]. There is a lack of information available in literature on the precise influence of the amygdala on chronic stress-induced formation of lipid peroxidation level in different tissues. The present study was an attempt to elucidate the comparative analysis of regulatory role of amygdala nuclei in regulating physical and psychological stress.

2. Materials and methods [6]: All procedures in this present study were performed in accordance with the guidelines established by the Institutional Animal Ethics Committee and of the Society for Neuroscience Policy on the Use of Animals in Research. Adult albino rats (150 to 250 g) of Wistar strain were used in the present study. The rats were procured from the central animal breeding center at our university. Animals were housed individually in polypropylene cages (29 cms x 22 cms x 14 cms) during the experimental period at 28±2 °C temperature and 50±5% humidity. The rats were maintained under standard laboratory conditions with 12 h light: 12 h dark cycle. Rats were anesthetized (Pentobarbital sodium, 40 mg/kg, i. p.) and then sacrificed by giving the lethal dose of Pentobarbital sodium. The amygdala lesion was performed according to the stereotoxic coordinates prescribed in the Paxinos and Watson rat stereotoxic atlas [7]. The
procedure was repeated on either side to produce bilateral lesions. The surgical procedures performed in the sham lesioned control group were the same, except for the passing of the DC. The animals were divided into three major group. Each group was further subdivided into three subgroups as control group and two experimental stress groups. Each subgroup contained ten animals. The stressed groups of rats were subjected to swimming stress and immobilization stress. All the stress experiments were done between 10 AM to 12 Noon to minimize circadian variability.

2.1 Chronic Stress Procedure

**Chronic Immobilization stress** [8, 9]: The immobilization chambers used in this study were plastic tubes of varying sizes to accommodate all sizes of rats (15 cms long and 4 cms diameter, 16 cms long and 5cms diameter, 17 cms long with 6 cms diameter). The tubes had a conical head at one end. The conical head area contained numerous perforations which served as breathing holes. The rat was placed inside the tube with head in the conical end. The rats were totally restrained by packing the rear end of the tube and closing it firmly with a stopper. Rats were exposed to chronic stress in the form of immobilization for 2 hour per day for a period of 60 days.

**Chronic swimming stress** [9]: The rats were allowed to swim in the plastic tubs containing tap water maintained at room temperature. The water level in the plastic tub was always kept at 30 cms from the bottom. Rats were forced to swim in this tub until exhaustion. The point at which the animals became unable to stay at surface and showed signs of sinking was considered to be the point of exhaustion. After the stress session, the rats were towel dried and then placed back in their respective cages where food and water were available ad libitum. Animals were subjected to force swimming daily for 60 days.

2.2 Grouping of Animals

**Normal Control (NC)** - This subgroup of normal rats was not subjected to any kind of stress

**Normal chronic Swimming stress- (N-CS)**- This subgroup of normal rats was subjected to chronic swimming stress daily for sixty days.

**Normal chronic Immobilization stress- (N-Cl)** - This subgroup of normal rats was subjected to chronic immobilization stress daily for sixty days.

**Amygdala sham lesioned control (ASL-C)** - This group of rats received the same surgical procedure for lesioning of amygdala as mentioned above except for passing of the DC (direct) current and these animals were not subjected to any kind of stress.

**Amygdala sham-lesioned chronic swimming stress (ASL-SS)** - This group of rats was subjected to sham lesion at amygdaloid nucleus and underwent chronic swimming stress daily for sixty days.

**Amygdala sham-lesioned chronic immobilization stress (ASL-IS)** - This group of rats was sham lesioned at amygdaloid nucleus and underwent chronic immobilization stress daily for sixty days.

**Amygdala lesioned control (AL-C)** - This subgroup of amygdala lesioned rats was not subjected to experimental stress.

**Amygdala lesioned chronic swimming stress (AL-SS)** - This subgroup of rats was lesioned at amygdalal nucleus and subjected to chronic swimming stress for sixty days.

**Amygdala lesioned chronic immobilization stress (AL-IS)** - This subgroup of rats was lesioned at amygdalal nucleus were subjected to chronic immobilization stress one hour per day for sixty days.

2.3. Preparation of the tissue homogenate: At the end of experiment, all rats were anaesthetized with sodium pentobarbital (40 mg/kg body weight), heart, liver, kidney, were quickly removed. Each of these tissues was separately transferred to a glass homogenizer containing 10 ml of 10 mM cold phosphate buffer saline (PBS - pH 7.4). The tissues were homogenized using an electrical homogenizer (Remi 8000 RPM). The unbroken cells and cell debris were removed by centrifugation at 3000 RPM for 10 minutes using Remi C 24 refrigerated centrifuge (-4 °C). The obtained supernatant was used for estimation of Lipid Peroxidation:

2.4. Estimation of Lipid Peroxidation: Lipid peroxidation was estimated according to the method of Kartha and Krishnamurthy [11]. This assay is based upon the reaction of TBA with malondialdehyde (MDA) which is one of the aldehyde products of lipid peroxidation. Five ml of the homogenate (freshly prepared) was incubated in 50 ml conical flask at 38-39 °C for 30 minutes along with “the blank” in a water bath in separate conical flasks. After incubation, 1ml of aliquots was added to the tube containing 1.5 ml of 20% cold trichloroacetic acid (TCA) and then centrifuged for 10 minutes. After centrifugation, 2 ml of supernatant fluid was taken in a test tube and to it was added 2 ml of 0.7% thiobarbituric acid (TBA) and kept in the boiling water bath for 10 minutes. The development of pink color was measured at 535 nm by using Spectronic D-20 Spectrophotometer. TBA reactive material was expressed in terms of nanomoles of malondialdehyde (MDA)/g wet tissue, taking molar extinction coefficient of malondialdehyde (MDA) as 1.56 X 105.

2.5. Histological Analysis: On completion of the experimental procedures, all the rats were sacrificed and their brains, after transcardial formol saline injection, were dissected and processed for histological study. Serial sections 5 µ thickness were taken and stained with Haematoxylin and eosin (H&E) [12] (Fig. 1). These sections were examined under dissection microscope to confirm the lesion and the lesion sites were again magnified under power 10X for a detailed view.

2.6. Statistical Analysis: The datas were summarized using mean ± SEM or median and interquartile range depending on the skewness. For normally distributed data one way ANOVA was used and Kruskal Wallis test was used for skewed data. This was followed by multiple comparison tests for significant F value in ANOVA. The data of pre and post lesion was analyzed using two ways ANOVA followed by post hoc tests in case of significant F value of ANOVA. P<0.05 were considered as statistically significant.
Table 1: Swimming stress and Immobilization stress induced changes on tissue MDA following swimming stress and  immobilization stress. Values are expressed as mean±SEM; nanomoles of MDA/g wet tissue.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Heart</th>
<th>Liver</th>
<th>Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>9.586±0.788</td>
<td>20.187±0.514</td>
<td>14.291±0.439</td>
</tr>
<tr>
<td>N-SS</td>
<td>20.22±0.86**</td>
<td>39.26±0.20**</td>
<td>20.12±0.88**</td>
</tr>
<tr>
<td>N-IS</td>
<td>18.80±0.22**</td>
<td>40.22±1.20**</td>
<td>22.91±0.54**</td>
</tr>
<tr>
<td>ASL-C</td>
<td>9.20±0.30NS</td>
<td>21.01±0.20NS</td>
<td>14.12±0.22NS</td>
</tr>
<tr>
<td>ASL-SS</td>
<td>21.438±1.61‡‡</td>
<td>41.425±0.703‡‡</td>
<td>21.291±0.633‡‡</td>
</tr>
<tr>
<td>ASL-IS</td>
<td>18.865±0.49‡‡</td>
<td>40.789±1.30‡‡</td>
<td>18.134±0.84‡‡</td>
</tr>
<tr>
<td>AL-C</td>
<td>10.244±0.36</td>
<td>19.70±2.27‡‡</td>
<td>18.134±0.84‡‡</td>
</tr>
<tr>
<td>AL-SS</td>
<td>19.70±2.27‡‡</td>
<td>40.88±0.55‡‡</td>
<td>20.134±0.84‡‡</td>
</tr>
<tr>
<td>AL-IS</td>
<td>23.620±0.487‡‡</td>
<td>49.290±0.98‡‡</td>
<td>30.010±0.56‡‡</td>
</tr>
</tbody>
</table>

n= number of rats

NC versus ASL-C – Non significant (NS)
** P < 0.001; compared between NC versus N-SS and N-IS
‡‡ P < 0.001; compared between ASL-C versus ASL-SS and ASL-IS
§§ P < 0.001; compared between AL-C versus AL-SS and AL-IS
### P< 0.001; AL-SS versus AL-IS

3. Results: It is evident from the table I that the MDA level of the heart, liver and kidney did not so any significant changes in between the NC and ASL-C groups. Exposure to chronic stressors significantly (P<0.001) increased the tissue MDA level in the normal group, sham lesioned groups and lesioned groups when compared to their respective control group. Further, Exposure to immobilization stress in amygdala lesioned groups showed a significant increase (P<0.001) in the MDA level of heart, liver and kidneys when compared to amygdala lesioned swimming stress groups.

4. Discussion: The biochemical manipulations in the body are good markers that can be of great use to understand, target and manage stress induced etiologies. The available literature suggests that the stress regulatory circuits are activated by a particular stressor which is crucially dependent on stimulus attributes [13]. The central component of stress responses have been recently redefined and presumed that various categories of stressors have been handled to some extent in different ways [14]. Recent reviews have suggested the existence of two generalized stress pathways namely limbic sensitive and limbic insensitive [15, 16]. Although the stressors trigger a core set of endocrine, autonomic and behavioral responses, the brain pathways that drive these responses might differ according to the stressor category. The nature of the stressor itself is important in the pattern of expression of stress responses. The core element of the physiologic responses is broadly similar and it appears that they are generated by the neural pathways that may vary according to the stressor category. A number of pathological conditions have been implicated due to free radical formation [17]. Therefore, lipid peroxidation was estimated in different brain tissues in order to evaluate their relative role in stress induced regulatory mechanisms. Limbic and hypothalamic brain structures integrate emotional, neuroendocrine, and autonomic inputs that determine the magnitude, neural and hormonal response to stressful experiences [18]. Studies have also showed that amygdala nucleus is the “window of limbic system” through which exteriorization of emotional manifestation takes place, plays an important role in the organization of stress response [19, 20]. In the present study, no significant difference was observed in any of the parameters in between the sham lesioned groups and the normal groups. This
finding confirms that surgical procedure did not affect the studied parameters of the study. Exposure to stress situations can stimulate numerous pathways leading to increased production of free radicals [21]. It is well known that free radicals generate a cascade producing lipid peroxidation, protein oxidation, and DNA damage and cell death [22]. Stress induced oxidative metabolism on the body depends upon the involvement of various tissues. Hence, the estimation of these oxidative metabolic products on certain tissues was also included in this study. In the present study, MDA level of the tissues were estimated by TBARS (thiobarbituric acid reactive species) assay to evaluate lipid peroxidation level in heart, liver and kidneys, following exposure to two different models of acute and chronic stress. This assay is the easiest method used to study the effects of different treatments on lipid peroxidation and can be applied to crude biological extracts [23, 24, 25]. The results of the present study showed that swimming stress significantly increased the MDA level in the heart, liver and kidneys in normal groups and sham lesioned groups and lesioned groups. Swimming stress elevates oxygen consumption and metabolic activity due to increased muscle contraction [26]. Elevated oxygen consumption during increased metabolic activity increases the electron leakage from the mitochondrial transport system and causes an increase in oxidative stress, lipid peroxidation and generation of free radicals, which can be harmful to the cell [27]. The results of the present study is in agreement with the previous reported studies [28, 29] as swimming stress significantly increased oxidative free radical formation. Although the effect of acute physical stress on free radical production and antioxidant system in rats have been intensively investigated, the precise effect of repeated physical stress on the different organs is yet to be confirmed as the reported studies have shown contradictory results by the investigators [29, 30]. In recent years it has been shown that psychological stress may change the balance between pro-oxidants and antioxidant factors inducing oxidative damage [30]. Immobilization stress which is widely employed to provoke psychological stress has also been reported to be associated with oxidative damage in rats [30]. In the present study, repeated immobilization stress increased the tissue lipid peroxidation level as observed in the previous studies [30, 31]. Repeated psychological stress on daily basis might lead to oxidative damage by changing the balance between oxidant and antioxidants factors. The results of the present study suggest the involvement of amygdala nucleus in minimizing the stress induced formation of free radicals. Exposure of stress, increased the tissue MDA level. Literature survey show that the AVP (Arginine –Vasopressin) released within the amygdala seems to be involved in the generation of passive coping strategies in stressful situation during stress [32]. Due to the loss of stress coping strategies, stress might have caused increased formation of free radicals. The present study supports the definite role amygdala in stress tolerance. This shows that amygdala nucleus appears to play a potent role in minimizing the stress induced formation of lipid peroxidation during an exposure to the psychological type of stress rather than a physical type of stress like swimming. The present study provides the supportive evidence that the response of a psychological stressor to a larger extent is regulated by the amygdala. There might be the presence of an intricate web of reciprocal independent connections of amygdala nucleus to the brain areas regulating these responses designed for the psychological stress. However, the precise role of these nuclei and their interaction among themselves and other brain areas in regulating homeostasis warrants further study.

5. References


