IMMOBILIZATION STRESS-INDUCED CHANGES IN BRAIN ACETYLCHOLINESTERASE ACTIVITY AND COGNITIVE FUNCTION IN MICE

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In the present study, the effect of acute and chronic immobilization stress on brain acetylcholinesterase (AChE) enzyme activity and cognitive function in mice was investigated. Mice were immobilized by strapping for 150 min. One group of mice were only immobilized once (acute stress) while in another group mice were immobilized (150 min) daily for 5 consecutive days (chronic stress). Specific AChE enzyme activity (µmol min⁻¹ mg⁻¹) was estimated by a spectrophotometric method in the whole brain of mice subjected to acute and chronic stress. In the acute stress group, AChE activity (0.24922 ± 0.011) in the detergent-soluble fraction was found to be significantly decreased in comparison to the control group (0.33561 ± 0.022). Chronic stress did not cause any significant change in AChE activity in the detergent-soluble fraction. In the salt-soluble fraction, AChE activity was significantly decreased only in the chronic stress group (0.08791 ± 0.011) as compared to the control group (0.12051 ± 0.011). A passive avoidance test was used to assess cognitive function. The transfer latency time (TLT) from a light to dark chamber was recorded in the control and acute stress groups (30 min after immobilization is over) on day 1 (Trial I) and the following day (Trial II). The acute stress group showed an increase (178%) in TLT from Trial I to Trial II, which was significantly higher than that of the non-stress control group (75%). In the chronic stress group, Trial I was undertaken 30 min after the last immobilization, i.e. on day 5 and 24 hr later, Trial II. However, the chronically stressed mice showed an increase (70%) in TLT similar to the control group. Thus this study shows that acute immobilization stress may enhance cognitive function in mice which may be attributed to a decrease in AChE activity leading to an increase in cholinergic activity in the brain.

KEY WORDS: immobilization, stress, brain, acetylcholinesterase, passive avoidance.

INTRODUCTION

Stress is known to induce alterations in various physiological responses even leading to pathological states [1]. The stress-induced effects are supposed to be an outcome of altered activity of different mechanisms such as central neurotransmitters, neurohormonal factors, particularly those linked with the pituitary–adrenal axis, and free radical generation [2–4]. The majority of studies relating stress with neurotransmitters concerns the biogenic amines [5]. The cholinergic system, which has been proven to play an important role in the regulation of several central functions, however, has received less attention. There are some studies linking stress with cholinergic activity. The reported changes in levels of brain acetylcholine (ACh), activity of its synthesizing enzyme choline acetyltransferase (ChAT) and metabolizing enzyme acetylcholinesterase (AChE), choline reuptake and receptor activity following stress are inconsistent and varied. Depending upon the kind of stress applied, ACh concentration in the brain of mice was reported to decrease, increase or remain unchanged [6]. The dynamics of ACh was found to be altered by immobilization stress [7]. In addition, there are reports indicating strain-dependent stress-induced changes in inbred Wistar–Kyoto rats, which are behaviorally more reactive to stress than Brown–Norway rats, in their hippocampal cholinergic system [8]. These findings do suggest changes in basal level activity of ACh in the brain after stress. However, in those studies, correlation of the biochemical changes in the cholinergic system with behavioral responses was not attempted.

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The fact that ACH is the most important neurotransmitter implicated in cognition [9] and the possibility that stress may influence cognitive activity [10] prompted us to investigate the status of AChE activity (in brain) and cognitive responses in mice subjected to acute as well as chronic stress. Moreover, in this study, AChE activity has been analysed on the basis of different molecular forms, which have not received attention in earlier studies.

MATERIALS AND METHODS

This study was conducted on male Swiss mice (25–30 g). The animals were kept in standard housing conditions with a 12 h light and dark cycle. Food and water were available ad libitum.

Immobilization stress

Mice were subjected to immobilization stress in a prone position for 150 min. One group of mice (n = 10) were only immobilized once (acute stress) while in another group, mice (n = 10) were immobilized (150 min) daily for 5 consecutive days (chronic stress) [7]. The groups of acutely and chronically stressed mice was further subdivided into two subgroups (n = 5). One subgroup was used to study AChE-specific activity in brain and the another one was investigated for cognitive functions. AChE activity was also measured on the second, third and fourth day of chronic stress in separate groups (n = 5).

Acetyl cholinesterase (AChE) assay in brain

(1) Tissue preparation. Immediately after stress (acute and chronic), mice were given mild anesthesia with anaesthetic ether. The whole intact brain was then removed carefully and placed on the petridish, over ice. The brain was washed with ice-chilled normal saline repeatedly to clean. A 10% (w/v) homogenate of brain samples was prepared first by homogenizing in an Ultra-Turrax T25 homogenizer at a speed of 9500 rpm with three intervals of a few seconds in between the runs, using a sodium phosphate buffer (30 mmol l⁻¹, pH 7.0). Sodium phosphate buffer was taken in a volume sufficient volume to make the final volume for 10% homogenate. The half volume was separated and used as the salt-soluble fraction for AChE assay and estimation of protein concentration in the samples.

One per cent Triton X-100 (1% w/v in 30 mmol l⁻¹). Sodium phosphate buffer (pH 7.0) was then added in sufficient volume to make the final volume for 10% homogenate, which was added slowly while stirring the homogenate on ice; this fraction was used as the detergent-soluble fraction.

All the homogenates were centrifuged at 100 000 g at 4°C in a Beckman Ultracentrifuge (LE 80) using a fixed angle rotor (80 Ti) for 60 min. The supernatant was collected and stored at 4°C. Aliquots of this supernatant were diluted in the ratio of 1 : 10 and used as a source of enzyme for the assay.

(2) Enzyme assay. The assay of AChE in the above-mentioned supernatant was performed by the modified Ellman’s method [11] using acetylthiocholine iodide as substrate at a final concentration of 1 mmol l⁻¹. A kinetic profile of the enzyme activity was studied spectrophotometrically at 412 nm at an interval of 15 s. The assay for each sample was run in duplicate and each experiment was performed three times.

Protein was estimated in the range 0.01–0.1 mg ml⁻¹ in the brain samples by the Folin–Lowry method [12] and the modified method of Lowry [13] in the salt-soluble and detergent-soluble fractions, respectively, using bovine serum albumin (BSA) as standard at a concentration of 1 mg ml⁻¹.

The bio-chemicals used in the assay; acetylthiocholine iodide, DTNB and BSA, were purchased from Sigma Chemicals, USA.

Passive avoidance test

The mice were subjected to single-trial passive avoidance test as described by Brioni et al. [14]. The passive avoidance test was studied by a computerized shuttle box (Columbus Instruments, Ohio, USA) provided with the software program PACS 30. The shuttle box is comprised of two compartments. An automated door was used to isolate the compartments. After an exploration period of 30 s for acclimatization, the animal was subjected to a trial of 270 s. Each mouse was placed in the bright (light intensity 10) compartment and on transfer to the dark compartment it was given an electric shock (0.5 mA for 5 s) through the floor grid. The computerized door was set to close upon transfer, subjecting the mouse to the full duration of the electric shock. Infrared sensors monitored the transfer from one compartment to another, which was recorded as transfer latency time (TLT) in seconds. TLT was recorded in the control and acute stress groups (30 min after immobilization) on day 1 (Trial I) and the following day (Trial II). In the chronic stress group, Trial I was given 30 min after the last immobilization (day 5) and Trial II 24 h later. The criterion for improved cognitive activity was taken as an increase in the TLT on Trial II as compared to Trial I.

Behavioral activity

In a separate group of mice, a profile of behavioral activity of each mouse was recorded 30 min after acute and chronic stress for a period of 10 min by a computerized Digiscan animal activity monitoring system (Omnitech Instrument Inc. Ohio, USA).

Mean values and standard error means (SEMs) were calculated for the specific activity of AChE in the brain and TLT (passive avoidance test) of each group. The significance of any difference between the values of the two groups was determined by Student’s t test.
RESULTS

Effect of stress on AChE activity (Fig. 1)

AChE-specific activity in µmole min⁻¹ mg⁻¹ of protein in the acute stress group was significantly low (0.24922 ± 0.011) as compared to the control (0.33561 ± 0.022) and chronic stress (0.29417 ± 0.01) groups in the detergent-solubilized fraction. The specific activities of AChE in the detergent fractions on the second-, third- and fourth-day stress groups were not significantly different from that of the control group. In the salt-soluble (non-detergent) fraction, the AChE-specific activities in the control and acute stress groups were 0.12051 ± 0.011 and 0.10975 ± 0.007. There was no significant difference between these values, whereas from second-day to fifth-day (chronic) stress group a significant decrease was observed in the salt-soluble fraction of AChE-specific activity.

Effect of stress on the passive avoidance test (Fig. 2)

In the control, acute and chronic stress groups, there was a significant increase in the TLT from Trial I and Trial II. The TLT values (mean ± SEM) on Trial I for control, acute and chronic stress groups were: 98.6 ± 14.5, 85.4 ± 18.2 and 105.1 ± 11.6 s, and Trial II were: 171.2 ± 21.4, 238.6 ± 13.1 and 178.7 ± 24.9 s, respectively. The TLT in the acute stress group on Trial II was significantly higher as compared to those of control and chronic groups.

Effect of stress on behavioral activity

No significant difference was observed on the behavioral locomotor activity profile between the control and stressed mice at 30 min. The horizontal activity counts (mean ± SEM) for control, acute stress and chronic stress were 143 ± 21, 136 ± 18, and 126 ± 27, respectively.

DISCUSSION

As immobilization stress is believed to be the most severe type of stress in rodent models and has a comparative effect in humans, this type of stress was used in the present study [15]. The immobilization stress was found to affect the activity of different fractions of AChE in the brain depending upon the manner by which mice were subjected to the stress. There was a significant decrease in AChE activity in the detergent-soluble fraction only when

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**Fig. 1.** A histogram showing the effect of immobilization stress on the specific activity of AChE activity in mice brain. The stress on day 1 and day 5 is considered as acute and chronic stress, respectively. The AChE activity for each group is denoted by the mean ± SEM values of six observations. *P < 0.05, significant difference from control.

**Fig. 2.** A histogram showing the effect of acute and chronic immobilization stress on transfer latency time (TLT) in a passive avoidance test. *P < 0.001, significant difference from Trial I; @, P < 0.01, significant difference from control group.
the stress was applied on an acute basis whereas there was no change in the chronic stress group, as compared to the control group. In contrast, chronic stress showed a significant decrease in AChE activity in the salt-soluble fraction.

The AChE enzyme is well reported to occur in different molecular isoforms [16]. In addition, there is a differential localization of these isoforms in neuronal cells. The two major isoforms are globular monomer (G1) protein and a globular tetramer of the same monomer subunit (G4). The G1 isoform is reported to be present in the soluble cytoplasm of the neuronal cells whereas the G4 isoform is predominantly a membrane-bound enzyme [17]. The experimental procedure followed in the present study leads to extraction of different isoforms in different fractions, i.e. the salt-soluble fraction consists of mainly the G1 form of AChE whereas the detergent-soluble fraction consists of both G1 and G4 isoforms but predominantly the G4 form of AChE [18]. The decrease in AChE activity in the acute stress group can thus be well accounted for by the decrease in the G4 isoform of AChE. Chronic stress affects mainly the G1 isoform of AChE.

Some workers have also demonstrated that the cholinergic system is altered due to stress. Gilad et al. [8] studied the cholinergic changes in the hippocampus of inbred Wistar–Kyoto (WKY) rats that are behaviorally more reactive to stress than Brown–Norway (BN) rats. The immobilization stress, acute and chronic, caused a decrease in accumulation of choline and an increase in QNB binding as part of an adaptive process. Finkelstein et al. [7] investigated the dynamics of cholinergic synaptic mechanisms in female rats subjected to acute and chronic immobilization stress. They observed rapid activation of presynaptic hippocampal cholinergic terminals by stressful stimuli (acute) expressed by an increase in choline uptake and newly synthesized ACh release. After prolonged periods of stress (chronic), adaptive changes in the cholinergic terminals expressed by a reduction in choline uptake and elevation in the number of muscarinic binding sites were also obtained in that study. The septo-hippocampal cholinergic system was suggested as an integral part of the adaptive response to stress. In the present study, an adaptive process suggested by Finkelstein et al. [7] has also been seen in AChE activity. On first exposure to stress (acute), a significant decrease in AChE activity was seen but the next application of stress on second-day activity of AChE showed recovery and remained unchanged on subsequent daily stress up to the fifth day (chronic). Fatranska et al. [6] reported activation of the cholinergic system in rat brain areas, mainly in the hippocampus and hypothalamus, under certain stressful conditions. A significant decrease of ACh concentration in these areas was found following cold stress for 1 or 24 h. However, the change in AChE activity differed in the two areas. Activity of AChE was elevated in hippocampus after 1 and 24 h exposure to cold while a decrease in the activity in the hypothalamus was obtained only after 24 h exposure in their study.

The results of those studies may not indicate a definite uniform pattern for biochemical changes in the cholinergic system in rat brain areas, which may be due to differences in strains and nature of stressors. However, there is no point of difference with regard to the conclusions of those studies in rats brain areas and the present one performed in mice whole brain that stress exerts a definite significant effect on the central cholinergic system. However, one very relevant question of whether the stress-induced biochemical changes in the cholinergic system were enough to cause alteration in behavioral responses was not addressed in those studies. In the present study we made an attempt to answer it by exploring correlation between stress-induced changes in AChE activity and cognitive behavior.

The central cholinergic system is considered to be the most important neurotransmitter involved in regulation of cognitive functions. Cholinergic neuronal loss in the hippocampal area is the major feature of Alzheimer’s disease and to enhance central cholinergic activity by use of anticholinesterase, at present, is the mainstay of the pharmacotherapy of senile dementia of Alzheimer type [19,20]. There are reports that cognitive functions may be affected adversely, particularly in elderly subjects, due to stress [10]. In this study, a passive avoidance test was employed because this test has been suggested to be based more on cognitive function [21]. It is pertinent to mention here that in the present study the passive avoidance test was conducted after stress at the time at which behavioral locomotor activity was not significantly altered. The TLT is considered to be a parameter proportionately linked to cognitive functions, i.e. an increase in TLT indicates improvement and vice versa, a decrease means cognitive deficit. There was an increase in TLT from the first trial (I) to the second trial (II) in control, acute stress and chronic stress groups. The magnitude of increase in TLT was more pronounced in the acute stress group (178%) as compared to that of the control group (75%) and chronic group (70%). The results suggest that acutely stressed mice showed better cognitive activity than control and chronically stressed mice. This observation can be explained very well by the changes seen in AChE activity in these groups. It appears that a decrease in the G4 isoform (detergent-soluble fraction) is principally involved in the effect of acute stress on cognitive function. The low AChE activity in the acute stress group may increase cholinergic activity by raising the level of ACh leading to improved cognitive action. The chronic stress did not cause changes in AChE activity (detergent-soluble fraction) significantly different from that of the control group, which may be due to adaptive mechanisms and henceforth cognitive behavior remained similar to the control group. However, chronic stress caused a significant decrease in the G1 isoform of AChE (salt-soluble fraction) but failed to produce any change in passive avoidance task performance. This indicates
that the G1 isoform of AChE may not have a significant role in the immobilization stress-induced effect on cognition. Thus, it may be concluded from this study that acute immobilization stress improve cognitive functions in adult mice by suppressing brain AChE activity. On repeated subjection to stress (chronic) AChE activity normalizes, which may be attributed to an adaptive process developed for the cholinergic system during chronic stress. It appears that, possibly, the G4 isoform of AChE is predominantly affected by immobilization stress.

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REFERENCES