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Effects of Acute Footshock Stress on Antioxidant Enzyme Activities in the Adolescent Rat Brain

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Summary

In a previous study we demonstrated that acute footshock stress increased glutathione peroxidase activity in the prefrontal cortex and striatum of adult male rats. Adolescents may respond differently to stress as life stressors may be greater than at other ages. The present study examined the effects of the acute footshock stress on superoxide dismutase (SOD) and glutathione peroxidase (GPx) enzyme activities and thiobarbituric acid reactive substances (TBARS) levels in adolescent male and female rat brains. We demonstrated that acute footshock stress increased SOD activity in the prefrontal cortex, and increased GPx activity in the hippocampus in female rats. In males, acute footshock stress increased GPx activity in the prefrontal cortex and hippocampus. Footshock stress did not change TBARS levels. These results indicate a strong role of gender in the response of adolescent subjects to various aspects of stress.

Key words

Footshock stress • Dopamine • Prefrontal cortex • Hippocampus • Striatum • Superoxide dismutase

Introduction

The effects of early stress are linked to a number of neuropsychiatric disorders (Spear 2000). Preclinical studies suggest that stress in early life can promote long-term changes in multiple neurotransmitter systems and brain structures and functions implicated in the etiology of anxiety, depression, drug abuse and schizophrenia in the mouse, rat and primates (Graber *et al.* 1991, Spear 2000). Many disorders among humans often begins during adolescence (Spear 2000). Many adolescent-specific neurobehavioral alterations observed in humans are also seen in rats of comparable age. Spear and Brake (1983) defined periadolescence as the age period around the time of sexual maturation, when age-specific behavioral and psychopharmacological discontinuities are evident. Based on this criterion, the age period of approximately P30 to P42 was designated as periadolescence in rats.

In humans and other animals (Graber *et al.* 1991) physical growth is generally related to growth of mental abilities (Spear 2000) with increases in most cognitive capabilities occurring during adolescence. One brain region prominently altered during adolescence across a variety of species is the prefrontal cortex (PFC), an area thought to subserve higher cognitive abilities (Diamond 1996). Maturational changes during adolescence are also evident in other brain regions such as the hippocampus and striatum of rodents (Teicher *et al.* 1995, Wolfer and Lipp 1995).

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Dopaminergic systems undergo substantial reorganization during adolescence. Developmental events may alter the relative balance of dopaminergic activity between the PFC and striatal or mesolimbic terminal regions. The dopamine input to the PFC in nonhuman primates and rats increases during adolescence to peak at levels well above those seen earlier or later in life (Kalsbeek *et al.* 1988, Teicher *et al.* 1995).

The actual incidence of life stressors is possibly greater in adolescence than at other ages. If the stressors are selectively given during adolescence, they might activate many of the neural systems undergoing developmental change (Laviola *et al.* 1999), including mesocorticolimbic dopaminergic projections (Spear 2000). Stress-induced elevations in corticosterone may play a critical role in activation of dopamine transmission and may interact with mesocorticolimbic brain regions (Imperato *et al.* 1989). During adolescence, females may be exceptionally vulnerable to stress. They are not only more susceptible than males but at this developmental stage they also perceive events to be more stressful than at other ages (Wagner and Compas 1990).

Dopamine is metabolized by monoamine oxidase with hydrogen peroxide produced as a product. Hence, increased turnover of dopamine evokes oxidative stress derived from the increased production of hydrogen peroxide. Generation of reactive oxygen species (ROS) can be a major component of decreased cell function and eventual death (Halliwell 1992). Various types of stress such as mild electric footshock, restraint, food deprivation and anticipation stress cause a selective increase in dopamine metabolism (Dunn 1988).

Glucocorticoids (GCs) are hormones secreted by the adrenals in response to both physical and psychological types of stress. GCs increase the toxicity of oxygen radical generators, and may increase the basal level of ROS in the cells (McIntosh *et al.* 1998).

In a previous study, we have demonstrated that very mild (0.2 mA) footshock stress does not change glutathione peroxidase (GPx) activity in the adult male rat prefrontal cortex and striatum, while more intense (1.6 mA) footshock stress increased glutathione peroxidase activity in the prefrontal cortex and striatum (Gonenc *et al.* 2000). However, antioxidant enzyme activities and lipid peroxidation levels induced by stress differ in the adolescent male and female rat brains, but this topic has not been examined. Therefore, the aim of this study was to investigate the effects of acute footshock stress on superoxide dismutase (SOD) and GPx enzyme activities, and TBARS levels, as an indicator of lipid peroxidation in the hippocampus, prefrontal cortex and striatum areas of adolescent male and female rats.

Material and Methods

Animals

Six groups of Wistar female and male rats aged 38 days of age (n=7 for each group) were used. The animals were maintained under standard colony conditions with a 12 h light/dark cycle (lights on at 07:00 h) at constant room temperature (23±2 °C), and humidity (60 %) and *ad libitum* food and water throughout the experiments. Experiments were carried out between 9:00-12:00 h in a sound-attenuated and air-regulated experimental room. All experiments were performed in accordance with the guidelines provided by the Experimental Animal Laboratory and approved by the Animal Care and Use Committee of the Dokuz Eylul University, School of Medicine.

Experimental design and tissue preparation

The animals were divided into three groups: control group and animals subjected to electric footshocks of 0.2 mA intensity and to electric footshock intensity of 1.6 mA. Each of the three groups consisted of seven males and seven females. Rats were exposed to electric footshocks of 160 ms duration with a 160 ms interval for 20 min.

In a previous study we showed that the optimum time for elevation of enzyme activities appears within 30 min following footshock stress (Gonenc *et al.* 2000). Therefore, 30 min after footshock stress the rats were killed by cervical dislocation under ether anesthesia. PFC, striatum and hippocampus tissues were separated on an ice-cold surface. Tissue homogenates and supernatants were prepared as described by Carrillo *et al.* (1991). Aliquots of the homogenate and supernatant was stored at –85 °C until determination of TBARS levels, and SOD and GPx enzyme activities. All measurements were made within 10 days.

Determination of SOD activity

SOD activity was determined using a RANSOD kit (Randox Labs, Crumlin, UK) (Delmas-Beauvieux *et al.* 1995). Xanthine and xanthine oxidase were used to generate superoxide radicals that react with 2-4-iodophenyl-3-4-nitrophenol-5 phenyl tetrazolium chloride (INT) to form a red formazan dye. Substrate

concentrations were 0.075 μmol/l for xanthine and 0.037 μmol/l for INT. SOD inhibits this reaction by converting the superoxide radical to oxygen. An SOD unit inhibits the rate of reduction of INT by 50 % in a complex system with xanthine/xanthine oxidase. Due to the small linearity range of the test, the sample must be diluted so that the percentage of inhibition falls between 30 % and 60 %. A standard curve was prepared using the standard provided in the kit, and the value for the supernatant was read from this curve. SOD activity in the supernatant was measured at 505 nm on a Shimadzu UV-1201V spectrometer. Results were expressed as SOD U/mg protein.

Determination of GPx activity

GPx was determined using a RANSEL kit (Randox Labs, Crumlin, UK). GPx catalyses the oxidation of glutathione (at a concentration of 5 μmol/l) by cumene hydroperoxide according to the method of Paglia and Valentine (1967). In the presence of glutathione reductase (at a concentration $\geq 0.75.\ 10^{-3}$ U/l) and 0.35 μmol/l of NADPH, the oxidized glutathione is immediately converted to the reduced form with a concomitant oxidation of NADPH to $NADP^{+}$. The decrease in absorbance at 340 nm is then measured at 37 °C. The assay was performed on a supernatant. The GPx unit was defined as the enzyme activity able to convert 1 μmol of NADPH to NADP in 1 min. Results were expressed as GPx U/mg protein.

Determination of TBARS level

TBARS level was estimated according to the method of Rehncrona *et al.* (1980). 0.5 ml of homogenates were extracted with 0.5 ml of trichloroacetic acid (20 % wt/vol). After centrifugation, 0.9 ml of the supernatant were added to 1 ml of thiobarbituric acid (0.67 % wt/vol). The samples were heated in boiling water for 10 min. After cooling the absorbance at 532 nm was recorded. A standard curve was prepared using 1, 1, 3, 3-tetraethoxypropane and the value for the homogenate was read from this curve. The results were expressed in nmol/mg protein.

Determination of protein concentration

Protein contents of the supernatant and homogenate were determined using a U/CSF protein kit (Roche Diagnostics, Germany). The sample is preincubated in an alkaline solution containing EDTA, which denatures the protein and eliminates interference of magnesium ions. Benzethonium chloride was then added, producing a turbidity that is read at 505 nm. U/CSF protein determinations can be performed as an endpoint assay with a sample blank or as a rate assay.

Statistics

Results are presented as means ± S.E.M. All data were analyzed by one-way analysis of variance followed by the Scheffe test.

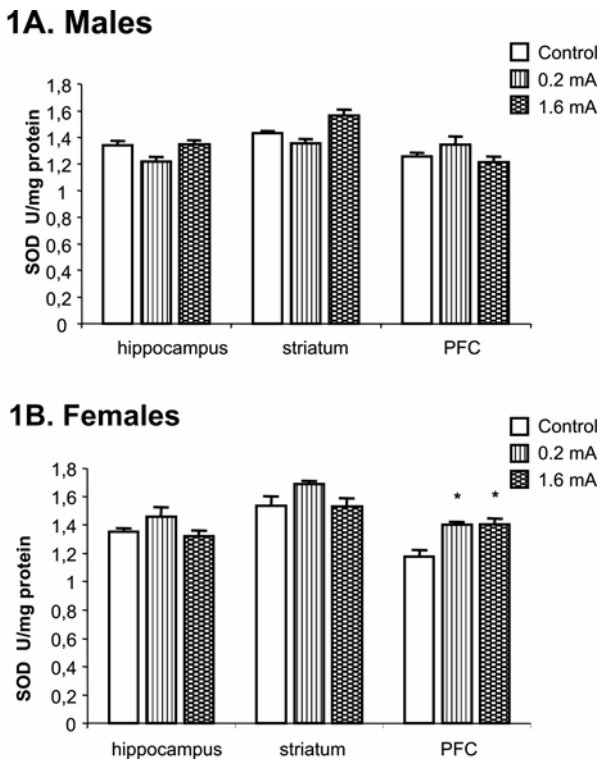

Fig. 1. Effects of footshock stress for 20 min on SOD activity in the prefrontal cortex (PFC), striatum and hippocampus of adolescent male (1A) and female rats (1B). Values are means ± S.E.M. *p<0.05 compared with the unstressed control. PFC:

Results

The SOD activity was significantly increased by footshock stress (at both intensities) only in the PFC in females (Fig. 1). In PFC, footshock stress (both of 0.2 and 1.6 mA) caused a significant increase in GPx activity in males (Fig. 2A), but not in females (Fig. 2B). In the hippocampus, footshock stress (at both intensities) caused a significant increase in GPx activity in females (Fig. 2B), while the more intense (1.6 mA) footshock stress increased GPx activity in males (Fig. 2A). Footshock stress did not change TBARS levels in any group.

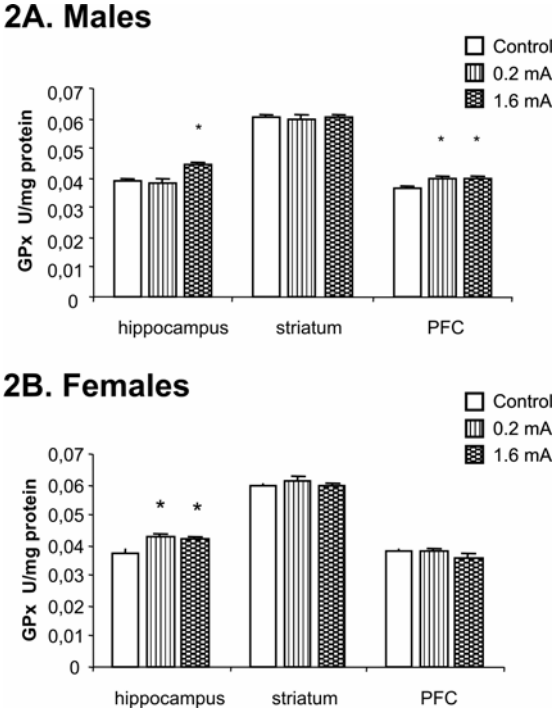

Fig. 2. Effects of footshock stress for 20 min on GPx activity in the prefrontal cortex (PFC), striatum and hippocampus of adolescent male (2A) and female rats (2B). Values are means ± S.E.M. *p<0.05 compared with the unstressed control. PFC.

Discussion

We showed that acute stress (at both intensities of 0.2 mA and 1.6 mA) increased GPx activity in the prefrontal cortex in adolescent males. Several studies revealed that stressful events increase dopamine metabolism in the PFC (Pani *et al.* 2000). The increase in GPx activity may occur in order to scavenge elevated levels of hydrogen peroxide that are generated by dopamine metabolism. Our previous study has shown that only 1.6 mA acute stress increased GPx activity in the PFC of adult males (Gonenc *et al.* 2000). As for neurobiological correlates, pubertal rats are known to differ from adult ones. PFC is prominently remodeled during adolescence with the volume of PFC declining around adolescence. Dopamine input to PFC increases during adolescence in nonhuman primates to peak at levels notably higher than those seen earlier or later in life (Spear 2000).

In this study, acute footshock stress did not change enzyme activities in the striatum of adolescent males. Our previous study has shown that only 1.6 mA acute stress increased GPx activity in the striatum of adult males. Brain areas such as the striatum are thought to mature at a different pace, and the neural organization attained during adolescence is markedly different from that of the adults. Basal dopamine synthesis in the striatum of rats is lower in adolescence than in adults, as dopamine turnover is likewise lower in adolescent rats than in adults (Spear 2000).

The hippocampus has the highest density of glucocorticoid receptors in the brain and is involved in the regulation of the corticoid and behavioral responses to stress. Stress increases glutamate concentration in the hippocampal synapse. Activation of glutamate receptors increases the production of ROS (McIntosh *et al.* 1998). This increase can cause a compensatory increase in the expression of GPx. Glucocorticoid receptor binding in hippocampus peaks in adolescent rats at higher levels than levels in adulthood. The corticoid response to stressors is also more pronounced during adolescence (Spear 2000).

The present study showed that acute stress (both of 0.2 mA and 1.6 mA) increased GPx activity in the hippocampus and SOD activity in the PFC of adolescent females. On the contrary, in adolescent males, footshock stress (at both intensities) caused a significant increase in GPx activity in the PFC, and only more intense (1.6 mA) footshock stress increased GPx activity in the hippocampus. These differences may be due to differences in stress response between female and male brains. Adult female rats also show a greater glucocorticoid response after various forms of stress (Laviola *et al.* 2002). However, results from previous studies about the effect of stress response of adolescent female rats are conflicting. During adolescence, females may be more vulnerable to stress inducing events than at other ages or than in the males (Wagner and Compas 1990). In contrast to this study, Laviola *et al.* (2002) showed that periadolescent females exhibited a much lower corticosterone response to the acute stress challenge when compared to the adults. Moreover, recent studies in rodents strongly indicated that estrogen increases the dopamine turnover rate and presynaptic dopaminergic activity in female rats. The density of dopaminergic innervation is also higher in female rats (Walker *et al.* 2000). The PFC and hippocampus also have higher levels of dopamine activity in females (Beck and Luine 2002).

Footshock stress for 20 minutes did not induce any significant changes in TBARS levels. However, prolonged stress may cause excess ROS and TBARS levels. Liu *et al.* (1996) have shown that stress for 8 hours causes lipid peroxidation in the cerebral cortex but

not in the striatum. Matsumoto *et al.* (1999) have demonstrated that stress for 2–16 h significantly increased TBARS levels in the whole brain. Additional studies exploring various durations of stress are needed to explain whether stress causes lipid peroxidation in the rat brain.

In conclusion, the present results suggest that acute footshock stress increases SOD activity in the PFC and GPx activity in the hippocampus in female rats. In males, acute footshock stress enhanced GPx activity in the PFC and hippocampus. To our knowledge, this is the first study to demonstrate the effects of footshock stress on oxidative stress in adolescent male and female rat brains. However, additional research focusing on sex-specific differences is necessary. These results indicate the gender plays a role in the response of adolescent subjects to various aspects of stress.

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