Pak. J. Biochem. Mol. Biol., 2022, 55(2):122-127

Research paper

Chronic administration of tyrosine improves behavioral deficits and enhances blood glucose levels in clinically healthy rats following immobilized stress

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ABSTRACT

Tyrosine, a metabolite for the synthesis of several depression and anxiety-affecting neurotransmitters helps to alleviate physical performance decrements in rats under conditions of high psychological stress. There has been a correlation between depression with the perturbation of cerebral levels of neurotransmitters. The study was designed to find out that tyrosine supplementation mitigates stress-induced decrements in behavioral activities in healthy rats under immobilized stress. 18 rats were taken and divided into three groups control, stress, and stress + tyrosine. Immobilized stress was given to the animals by the restrainer. Anxiety functions and depression-like symptoms were monitored by elevated plus maze and force swimming test respectively. The biochemical assessment was done by estimating plasma glucose levels. Administration of tyrosine produced anxiolytic effects and decreased depression-like symptoms that can attribute to increased brain norepinephrine concentrations. Blood glucose levels were also significantly increased in both test groups following tyrosine administration. It is concluded that tyrosine in immobilized stressed rats significantly improved behavioral deficits by increasing glucose levels in the blood.

KEYWORDS: Tyrosine, Depression, Anxiety, Norepinephrine, Immobilized stress

INTRODUCTION

Tyrosine is classified as a neutral amino acid and is commonly found in a protein-rich diet and is starting compound for the synthesis of several neurotransmitters and other metabolites including dopamine, epinephrine, catecholamine, and norepinephrine [1]. A large number of cellular processes and several signal transduction pathways are conducted by enzymes such as protein-tyrosine phosphatases and protein-tyrosine kinases [2,3]. These two enzymes are highly functional in the central nervous system [3], which is coherent in describing the essentiality of neuronal functions of the phosphorylation of tyrosine [4,5]. The catecholamine synthesis is controlled by the enzyme tyrosine hydroxylase and is considered the first step for the biosynthesis of catecholamine in nervous systems [6]. Studies reported that numerous functions are altered due to acute stress because of neurons of the hypothalamus and locus cerulus, whereas such neurons are thought to be of noradrenergic type [7]. It has been suggested by several studies that depression is managed by precursors of catecholamine (phenylalanine, tyrosine) and of serotonin (tryptophan, 5-hydroxytryptophan) [8]. In order to evaluate depression-like behavior in rats, the immobilization stress model is used in which restrainers are utilized for a

certain time period to restrict the movement of animals and thus induce depression in their behavior [9].

Snyder and Myerhoff, [10] explained that tryptophan and tyrosine are involved as a nutrient and a metabolite of several neurotransmitters. Moreover, the pathophysiology of many psychological disturbances such as attention deficit disorder (ADD) without or maybe with hyperactivity are concerned with the disruption in the release or production of such neurotransmitters. Scientists suggested that the importance of food effects on the ratios of phenylalanine and tyrosine in plasma in the human brain catecholamine production and release is under the influence of varying levels of tyrosine in the brain. Tyrosine administration results in the elevation of brain tyrosine and the effect on the hydroxylation of tyrosine to dopamine [11]. Studies suggested that tyrosine, being an essential amino acid improves physical functions, mental processes and gives pleasant feelings.

The present study is designed to evaluate the administration of tyrosine on the behavioral and biochemical functions following immobilized stress.

MATERIALS AND METHODS

18 Albino Wister rats were used in this study. They all were locally bred (150-190 g) and obtained from Aga Khan Hospital Karachi. After bringing they were caged separately in a calm room under a 12 h light-dark cycle and maintained surrounding temperature (22℃ ± 2). Initially, animals were habituated, and experimentation started after 3 days of habituation. Rats were randomly divided into 3 groups, the control is given saline while one test group is exposed to immobilized stress and the second test group is given intraperitoneally tyrosine (200mg/kg) and immobilized stress. All experiments were conducted after approval of the institutional review board (IRB) of Federal Urdu University, Karachi, and performed in strict accordance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (No. 85-23, revised 2011).

2.1 Behavioral Tests

2.1.1 Restrain Stress

For Immobilized stress, test animals were daily restrained for 3 hr/day. The procedure was repeated continuously for about 15 days in well-ventilated Plexiglas tubes. During stress, they have no access to food and water.

2.1.2. Forced Swimming Test:

This apparatus is designed in a glass tank. Specifications and experimentation are explained [12]. During the testing phase, the swimming behavior of animals was observed (that which animals were able to swim throughout the swim chamber). The experiment is based on monitoring the immobility time. So when the animal is showing immobile status it will only keep its head above the water and attempt no effort to swim.

2.1.3 Elevated plus Maze Test:

The elevated plus maze test is commonly used to assess anxiety functions. Detail experimentation is described [12]. Rats placed at end of the open arm and time taken to enter in the closed arm is noted.

2.2 Biochemical Tests

Plasma blood glucose levels were estimated by collecting plasma with the help of kit methods using Micro lab 300.

3. STATISTICAL ANALYSIS

One-way ANOVA with Tukey's post-hoc test was used for the analysis of depression-like symptoms, anxiety-like effects, and

glucose levels using SPSS version 20. *p* values less than 0.05 were considered significant.

RESULTS

Behavioral Test:

Fig. 1 shows tyrosine-induced behavioral effects/changes in restraint-stressed rats, on activities in the force swimming test (immobility time). Analysis of data using one-way ANOVA showed that the effects of restraint and restraint-tyrosine were both significant. Post hoc analysis through TUKEY'S test exhibited increased ($F(2,15)=30.09$ $p<0.01$) immobility time in the restraint group. While the time of immobilization of restraint-tyrosine-treated rats was reduced ($p<0.01$). Tyrosine-induced behavioral changes in restraint-stressed rats, on activities in the elevated plus maze (time spent in the open arm), are shown in figure 2. Data analysis through one-way ANOVA exhibited that the effects of restraint and restraint-tyrosine were both significant. Post hoc analysis through TUKEY'S test showed reduced ($F(2,15)=11.09$ $p<0.01$) time spent in the open arm in the restraint group. While activities of restraint-tyrosine-treated rats increased ($p<0.01$).

Biochemical Tests

Figure 3 shows the tyrosine-induced increase in blood glucose levels in restraint-stressed rats. Data analysis by one-way ANOVA showed that the effects of restraint and restraint-tyrosine were both significant. Post hoc analysis through TUKEY'S test exhibited increased glucose ($F(2,15)=31.09$ $p<0.01$). While activities of restraint-tyrosine-treated rats were also increased ($p<0.01$).

DISCUSSION

The present study showed that tyrosine attenuated depression-like symptoms and produces anxiolytic effects in rats significantly since immobilization stress is considered the severest stress type while using animal models and having relative effects in humans. Different studies reported that stress is an important risk factor for HPA axis dysfunction and is associated with an increased risk for anxiety disorders [13]. Further reported by Mario et al. [13], the dysfunction of the HPA axis is partially attributable to an imbalance between glucocorticoid and mineralocorticoid receptors because a number of glucocorticoid receptors are involved in anxiety disorders after the malfunctioning of receptors. As in the current study, stress produces depression-like symptoms and anxiety-like effects which are attenuated by tyrosine supplementation. We can suggest the association of tyrosine with HPA axis stimulations that results in decreasing depression-like symptoms and anxiety as well in animals. In some previous studies, scientists reported the same effect as the decrease in anxiety-like behavior in rodents in the elevated plus maze because of tyrosine administration [14].

Soyong et al. [15] reported that stress leads to some endocrinological changes that produce anxiety because anxiety and other stress-related endocrinological changes are related to glucocorticoid and sphingolipid metabolites. Exposure to stress damages the brain regions and results in behavioral deficits. Such deficits improved tyrosine administration [15]. Some early studies also suggested that tyrosine is the starting metabolite for the production of noradrenaline, and dopamine while influencing depression. The current study showed that restraint-tyrosine-treated rats exhibited anxiolytic behavior. This effect can be suggested by the release of the above-stated neurotransmitter following tyrosine administration. Richard et al. [16] reported that tyrosine and tryptophan may participate in producing antidepressant effects just like drugs by showing their

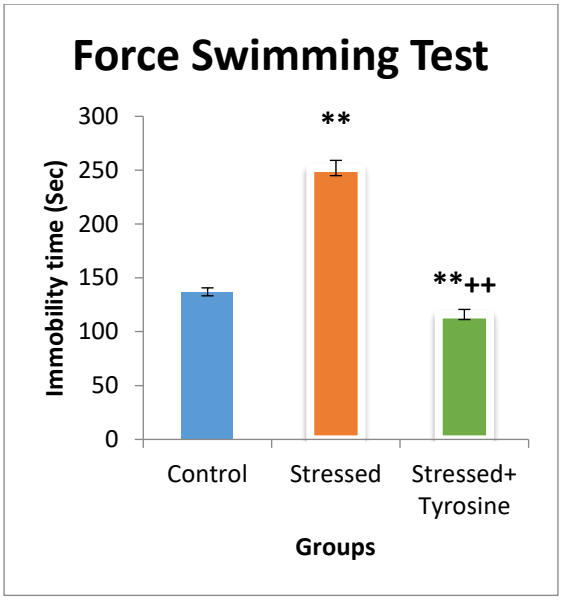

Figure 1: Representing values are means ± S.D (n=6), behavioral data were analyzed by One way ANOVA. Post Hoc analysis done by Tukey's **P<0.01 vs Control, ++P<0.01 stressed was considered significant.

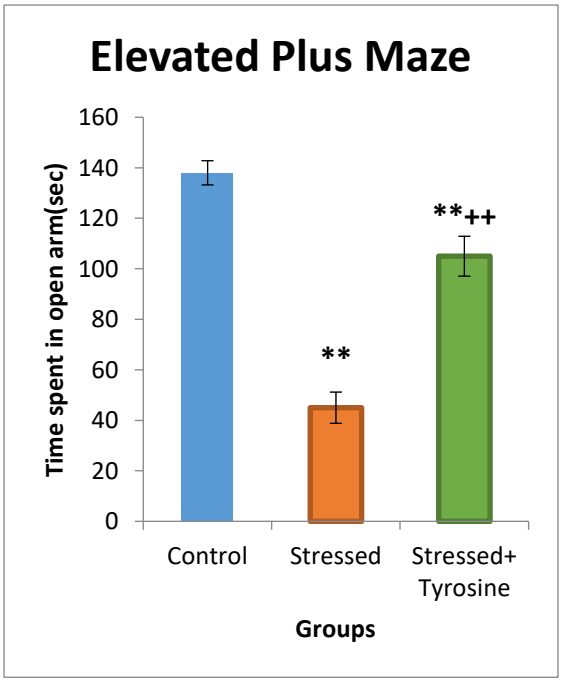

Figure 2: Values are mean ± S.D (n=6), and behavioral data were analyzed by One way ANOVA. Post Hoc analysis done by Tukey's **P<0.01vs Control, ++P<0.01vs Stressed was considered significant.

noradrenergic activity [16]. The present study also showed a decrease in depression-like symptoms that can be attributed to the involvement of hormone imbalance due to exposure to immobilized stress.

In previous studies, it has been found that tyrosine was a strong predictor of diabetes among people [12]. It has been concerned that type 2 diabetes mellitus is linked strongly with several amino acids in plasma, yet tyrosine's association with type 2 diabetes occurrences is the strongest, regardless of obesity [12]. Research shows that tyrosine has a role in the transportation of glucose and also in gluconeogenesis. Its catabolism is very fast causing elevated gluconeogenesis and much slowed down blood glucose clearance, thereby forming 3-nitrotyrosine, by combining free radical with free tyrosine, which may have a damaging effect on β-cells of pancreases [17]. Current results show that stress increases glucose levels and after administration of tyrosine levels were further increased. Increase in glucose could be attributed with the release of hormones after exposure to stress. Scientists suggested the mechanism of hyperglycemia due to stress is the up regulation of α2-adrenergic receptors [18]. Previous experimental studies show the inhibition of absorption of glucose from intestinal region following stress exposure [19]. Insulin resistance has been reported to be associated with metabolism of tyrosine in several studies. Therefore signaling pathway of insulin could be inhibited and also resistance because of already present insulin be exaggerated by increased levels of tyrosine [20]. Furthermore, sufficient levels of phenylalanine could trigger tyrosine synthesis, thereby stimulating insulin release [10]. The present study also showed that tyrosine treatment has an impact on biochemical parameters as significantly increased glucose levels. The advantageous aspects are coherent on familiar neuro-chemical changes while

administrating animals with tyrosine supplementation [19].

In conclusion administration of tyrosine at 200mg/kg dose produced anxiolytic effects and decreased depression-like symptoms as well as increased glucose levels following immobilized stress. Administration of tyrosine is one way or another involved in neurotransmitter activation and hormonal metabolism that resulted in the improvement of behavioral deficits.

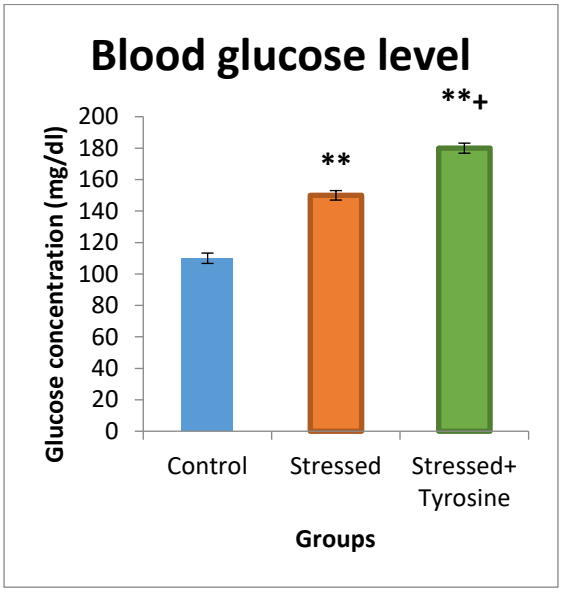

Figure 3: Values in mean ± S.D (n=6), behavioral data were analyzed by One way ANOVA. Post Hoc analysis done by Tukey's **P<0.01 vs Control, +P<0.05> vs Stressed was considered significant.

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