

The Effect of L-Arginine on The Brain Tissue of Stressed Rats

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ABSTRACT

Introduction: This study was conducted to determine the possible beneficial results of L-arginine on prefrontal cortex of rats which impressed by immobilization stress to define the synchronous impression of stress and nitric oxide (NO) on evolution of prefrontal cortex of rats after birth.

Methods: Forty-eight one month, male Wistar rats were divided into two groups: stressed and non-stressed. L-Arginine (200 mg/kg) as a NO synthase (NOS) inducer and L-NAME (20 mg/kg) were injected intraperitoneally (IP) and 7-nitroindazole (25 mg/kg) as non-specific was injected subcutaneously (S.C.) for 4 weeks. The kind of stress was immobilization for 4 weeks, every other day. The brain was removed after this period and each brain divided into two parts in a coronal section manner. Anterior part used for histological studies with H&E staining and posterior part used for measurement of NO production using spectrophotometer at 540 nm wavelength.

Results: Statistical analysis of microscopic and light microscopic finding showed that thickness of prefrontal cortex and NO production were significantly decreased in stressed rats and especially in groups which received 7- nitroindazole and L-NAME and L-arginine could reverse these results.

Discussion: According to this research, we could say that L-arginine decreases the cortical damages in stressed rats and 7-nitroindazole and L-NAME increase this damage in non-stressed group. Although in non stressed groups, L-arginine, L-NAME and 7- nitroindazole were all non-protective and damaging.

1. Introduction

L-arginine has powerful health properties and is referred to by scientists as the miracle molecule, because it has been identified as the natural substrate of nitric oxide synthase and is now recognized as a major player in the regulation of biological function of brain. There are many factors that they can prevent the natural Development of brain's cortex after birth. One of the most important of these factors is stress which can produce a lot of free radicals which cause lipid peroxidation reaction and this reaction cause structural and functional

damages to cell membrane (1). Brain is so sensitive to existence of free radicals because there are a lot of fat acids in brain which easily can peroxide because there isn't so much anti oxide enzymes in brain (2-8).

Ischemia, hypoxia, physical stress and of course mental stress such as immobilization can product free radicals (7-12). One of the most well known of free radicals is NO which produced by different cells of mammals and has two different roles in body as neurotransmitter and citotoxic factor (13). NO is synthesized from O₂, l-arginine and nitric oxide synthase (NOS) enzyme by endothelium of vessels. NOS have some isomers: type

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I (n NOS), type II (I NOS), type III (e NOS). On other hand, some materials such as L-NAME and 7-nitroindazole can inhibit production of NO. In this research we studied on prefrontal cortex of brain because it has important role on character and judgment and treatment. Stresses after birth have a lot of damages on this area so can exist so much characterized disorders in human.

2. Methods

In this study, we used 48 one-month aged male Wistar rats, weighing 80-100 g at 23-27 °C, 12/12 light/dark cycle, with free access to food and water. L-arginine (200 mg/kg) injected IP, one day apart. L-NAME (20 mg/kg) injected IP, two days a week and 7-nitroindazole (25 mg/kg) injected S.C. synchronously with heating two days a week.

The rats divided into two groups: with stress and without stress then each group divided into 4 groups with 6 rats in each group as follows:

1. Control I (receiver of 2 cc of normal saline with stress)
2. Control II (receiver of 2 cc of normal saline without stress)
3. Experimental A1 (receiver of L-arginine 200 mg/kg with stress)
4. Experimental A2 (receiver of L-arginine 200 mg/kg without stress)
5. Experimental B1 (receiver of L-NAME 20 mg/kg with stress)
6. Experimental B2 (receiver of L-NAME 20 mg/kg without stress)
7. Experimental C1 (receiver of 7-nitroindazole 25 mg/kg with stress)
8. Experimental C2 (receiver of 7-nitroindazole 25 mg/kg without stress)

For induction of stress, the rats keep immobile for 6 hours (8-2 P.M.) with special tools named wire wrap. After 30 days rats was anesthetized by xylazine (90 mg/kg) and ketamine (10 mg/kg), then the brains divided in two parts by a coronal section manner.

Anterior part fixed by formalin and tissue processing was done using rotary microtome, 10 serial cross sections were obtained and stained with H & E.

Posterior part used for measurement of NO. NO is an unstable molecule and immediately transform to nitrite and nitrate (12- 21), so we measured the level of nitrite in the brain. First we prepared solch solution by mixing chloroform and methanol in the proportion of 2:1, then

posterior part homogenized with solch solution then put it in the -20 °C freezer. After one hour extract the substrate with centrifuging at 11000 rpm and measured the nitrite level with Griess reagent in spectrophotometer at 540 nm. At last we convert the results to a standard curve which shows the amount of nitrite and level of light absorption. This curve prepared from determining the level of light absorption of 1 mol, 2 mol, 3 mol, 4 mol of nitrite sodium solution.

2.1. Data and Statistical Analysis

All data are expressed as mean \pm SD. One-way ANOVA was used for data analysis, followed by the Tukey test for post hoc analysis. A p value $<$ 0.05 was considered to be statistically significant.

3. Results

The thickness of prefrontal cortex comparison: the thickness of the prefrontal cortex between with stress and without stress groups shows: 1- A significantly decrease of thickness in prefrontal cortex of rats which were under stress in all groups (Table 1), 2- A significantly decrease of thickness in groups which received L-NAME, 7-nitroindazole in comparison with control groups (Table 1), 3- L-arginine can decrease stress damages on brain but not significantly (Figures 1-2).

3.1. Measurement of the Level of the No in Brains Tissue

Table 2 shows the level of the production of NO in all groups. Stress groups had a decreased level of the NO in comparison with groups which were without stress. While the groups which received NO inhibitors (L-NAME, 7-nitroindazole) showed a lower level of nitrite in comparison with control group and the groups which received L-arginine showed upper level of nitrite in comparison with control groups.

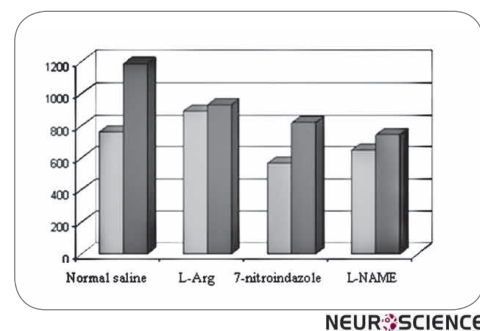
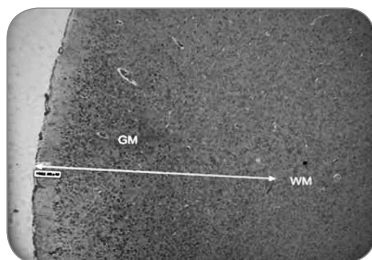
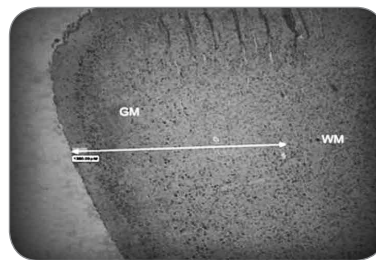


Figure 1. Comparison of thickness of prefrontal cortex in different groups with stress and without stress groups. (Blue: with stress. Red: without stress)



Picture 1. Coronal section of prefrontal cortex of rat. group: EXP A 2, mag: 100, stainin: H & E
GM: Gery Matter
WM: White Matter



Picture 2. Coronal section of prefrontal cortex of rat. group: EXP A 1, mag: 100, stainin: H & E
GM: Gery Matter
WM: White Matter

Table 1. Comparison of production of NO in different groups with stress and without stress groups.

Groups	With Stress/mol	Without Stress/mol
Normal Saline	2.35	2.63
L- Arginine	2.75	3.6
L- NAME	1.9	2.2
7-Nitroindazole	1.5	2

NEUROSCIENCE

4. Discussion

L-arginine is an essential amino acid found in foods like milk, cheese, yogurt, meat, and other proteins. It is a precursor for the synthesis of nitric oxide (NO) and stimulates growth hormone for anti aging benefits. Useful sources of NO for study in animal models are its essential amino acid substrate L-arginine and pharmacological donors. NO plays a significant role in the Brain's development in human after birth until 10 and even 14 years old (22, 24). Obviously this period is the most sensitive time against external pressures and mental stress which can interrupt this process.

About the relation between stress and prefrontal cortex and NO, we should mention that effects of NO are dose dependent. At normal dose it is a synaptogene which cause growth of neuron's processes and in this way increase their relationship with other cells which is necessary for increasing long life of cells. In this order, NO increases cell numbers and neuron's process, so NO increases thickness of cortex. At higher dose, NO acts as a toxic material which interacts with O₂ and forms peroxynitrate. Peroxynitrate can prevent the acts of NGF. NGF is responsible for saving size and numbers of cells and antioxidant enzymes activity so any disorders happen in function of NGF is followed by neurodegenerative disease, nerve membrane damages and disorder in regulation of cell numbers (23). We should mention that oxidative or physiologic stresses can produce abundant

amounts of free radicals such as NO which can prevent the normal function of NGF at higher dose and in this way stresses can decrease the nerve cells size and cell numbers and the thickness of cortex.

In groups which received L-arginine as NO precursor with stress in comparison with other groups which were under stress decreasing of the thickness of cortex is almost compensated but not significantly. Perhaps with using of higher dose of L-arginine or increasing the number of samples can help to have a significant difference. In groups influenced by stress which received L-NAME and 7-nitroindazole, the thickness of cortex is lower than the groups which received normal saline and L-arginine. It indicates that lack of nitric oxide as neurotransmitters in stressful occasions is destructive.

It is worthy to mention that reduction of thickness of prefrontal cortex in group which received L-arginine without stress derives from tissue damages because of extraordinary production of NO and peroxynitrate.

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