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The Study of the Effect of Sodium Nitroprusside in Anxiety-Like Behavior in Mice in Comparison With Diazepam

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Abstract

Anxiety has become a highly paramount field of research attention in psychopharmacology today. Sundry studies have shown a nitric oxide role in the regulation of anxiety. The goal of the study was to investigate sodium nitroprusside ability to affect anxiety-like behavior in mice and to compare this effect with the standard anxiolytic drug, diazepam, using both plus maze test and light/dark box test. The results revealed that sodium nitroprusside at a dose of 1 mg/kg had a significant effect on the behavior in both of the elevated plus maze test and light/dark test. However, at higher dose (3 mg/kg), it has significantly increased the anxiogenic-like effect in the light/dark box test. Diazepam at a dose of 2 mg/kg increased the time spent in open arms in elevated plus maze test and that in light chambers of light/dark test. These outcomes suggest that a nitric oxide pathway seems to play an important role in anxiety. Furthermore, sodium nitroprusside at a dose of 1 mg/kg showed a nearly anxiolytic ability, when compared with diazepam.

Keywords: anxiety; elevated plus maze; light/dark test; mice; nitric oxide; sodium nitroprusside.

دراسة تأثير صوديوم نايتروبروساید في السلوك المماثل للقلق في الفئران بالمقارنة مع االدايازيبام

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الخلاصة

أصبح القلق مجالًا بالغ الأهمية في الابحاث المهتمة في علم الأدوية النفسي اليوم. أظهرت دراسات المتنوعة دور أوكسيد النيتريك في تنظيم القلق. قد كان الهدف من هذه الدراسة هو البحث عن قدرة صوديوم نايتروبروساید للتأثير على السلوك الشبيه بالقلق لدى الفئران ومقارنة هذا التأثير مع العلاج القياسي للقلق الديازيبام و ذلك باستخدام اختبار المتاهة الزائدية المرتفعة واختبار الصندوق المضيء / المظلمعلى الفئران. أظهرت النتائج أن صوديوم نايتروبروساید بجرعة 1 مغ / كغ كان له تأثير معنوي على السلوك في كلاالاختبارين:اختبار المتاهة المرتفة وإختبار الصندوقالمضيء / المظلم ولكن عند تناول جرعة أعلى (3 ملغ / كغ) ، فقد كانت الزيادة في السلوك المماثل للقلق كبيرة في اختبار الصندوقالمضيء / المظلم. علاج الديازيبام بجرعة 2 ملغ / كغ قد كان تأثيره أن زاد من الوقت الذي تقضيه الفئران في الأذرع المفتوحة في اختبار المتاهة الزائديةالمرتفعة وكذلك في الحجرة المضيئة من صندوق الاختبار المضيء/المظلم. تشير هذه

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النتائج إلى أن مسار أوكسيد النيتريك يلعب دورًا مهمًا في القلق. علاوة على ذلك ، أظهر صوديوم نايتروبروسايد بجرعة 1 مغ / كغ بأن لهالقدرة على التخلص منالقلق وذلك بالمقارنة مع الديازيبام.

Introduction

Symptoms of anxiety are prevalent in the society and its disturbances are one of the most common problems of mental health [1]. They usually continue for a years, and are associated with considerable personal tribulation, starting commonly early in human life, increasing both morbidity and mortality, and constituting an economic burden [2].

To date, forms of anxiety have been treated with agents that work on γ-aminobutyric acid neurotransmission, such as benzodiazepines, and on serotonergic neurotransmission, such as buspirone that acts as a partial agonist of the 5-HT**1**A receptor, as well as medications that act as selective serotonin reuptake inhibitors. However, resistant to these treatments by some disorders of anxiety were presented [3, 4]. Thus, there is an urgent need to evolve another strategy or pathway for anxiety treatment [5].

Interestingly, several previous scientific articles showed that the l-arginine/nitric oxide/cGMP track is involved in experimental anxiety. Therefore, the effect of sodium nitroprusside was selected in this study.

Sodium nitroprusside is one of the agents of nitric oxide donors [6]and its therapeutic traits were described firstly at the end of 1800s when it was widely used in clinical and pharmacological studies as a potent vasodilator [7, 8]. This medication has been in use clinically for severe hypertension since 1929 [9]. It is actually available for intravenous route administration.

Nitric oxide is a short-lived, soluble gas with high diffusion in the brain that acts as an inter- and intra-cellular messenger [10]. It is synthesized from L-arginine by nitric oxide synthase enzymes [11].

Additionally it was considered as a relaxing factor derived from the endothelium and involved in relaxation of blood vessels [12]. It acts in various body physiology aspects, including vascular tone [13], cellular immunity [14], and neurotransmission [10]. Notably, in the central nervous system, nitric oxide is an important mediator. Also some data suggest that nitric oxide has a significant role in the modulation of benzodiazepines effects [15].

The localization of nitric oxide synthase in all parts of the brain especially in the amygdala, hypothalamus and hypocampus[16] explains that nitric oxide is involved in the control of a diversity of brain functions including neurotoxicity, locomotion, processing of spinal pain, and anxiety [17].

Experiments on animal models enable scientists to obtain insights into the normal or abnormal behavior of humans for investigating relations of brain behavior and if this behavior is underlying processes related to neuropsychobiology[18].

The development of suitable models of animals has markedly assisted to clarify the role of pharmacological molecules in circuits of brain that are related to anxiety. These anxiety models examine the natural behavioral manners of rodents to ethologically develop dependent on behavioral patterns [19]. These comprise tasks of "approach avoidance" [20] in which rodents are exposed to an environment of aversion or threatening such as elevated open arms in the elevated plus-maze, open field, and light/dark box tests, with anxiety-like behavior in each case is concluded from rising of avoidance behavior.

The elevated plus-maze test, one of the most popular anxiety tests designed for rodents, depends on the aversion of rats or mice naturally for open spaces in elevated plus maze [21, 22]. This test is sensitive to the effects of both anxiolytic and anxiogenic agents.

Additionally, light/dark test has been highly used to investigate anxiety-like behavior in adult rodents, and it may be a useful model to assess neural systems [23].

The aim of the present study was to examine the involvement of nitric oxide in the anxiety process by the assessment of the effects of both 1 mg/kg and 3mg/kg of sodium nitroprusside (nitric oxide donor) in two exploratory models of anxiety in mice, namely elevated plus maze test and light/dark box test.

Materials and method

Animals

Twenty four mice aged 5-6 weeks (19–25 g) were used in the present study. All animals were housed as four groups of six mice per cage (cage size 46×27×14 cm) in a room with a temperature of 24 ± 2°C and 12 hours light per 12 hours dark cycle(lights at 7 a.m. to 7 p.m.). The animals were

allowed for free access to food and water before the experiments. All procedures were accomplished in Pharmacology lab/College of Pharmacy/University of Thi-Qar.

Drugs

Diazepam ampoule (10 mg/2ml) from Watson Pharmaceuticals, India was used. Two different doses (1 mg and 3 mg/kg) of sodium nitroprusside were tested in the present study. These drugs were diluted with 0.9% saline. All solutions were prepared freshly on the test day and were injected intraperitoneally (i.p) 30 min before the testing. Saline was used as a vehicle.

Experimental protocol

Twenty four male and female Swiss albino mice were involved in the present study and divided into four groups (n=6 in each group):

Group I: saline-treated mice served as a negative control group.

Group II: diazepam (2 mg/kg, i.p.) treated mice were considered as a positive control group.

Group III: sodium nitroprusside (1 mg/kg) served as the first tested group [24].

Group IV: sodium nitroprusside (3 mg/ kg) served as the second tested group [24].

Assessment of anxiolytic activity

Elevated Plus Maze

The elevated plus-maze consisted of two closed arms (30×5×15 cm) and two opened arms (30×5 cm) extended from a central stand (5× 5 cm). The two arms of each pair were identical arms and were opposite to each other. The elevation of the entire apparatus was to a height of 50 cm away from the floor to observe the anxiolytic effect of agents on the behavior of mice. At the beginning, the mouse was positioned at the center of the maze with its head toward the open arm and allowed to be in this session for five minutes. The mouse behavior on the maze was recorded as: (1) the number of entries into the open or closed arms and (2) time spent by the mouse in each of the arms. Entering into an arm was noted only when all paws had crossed out the central area.

Light and Dark BOX

This device was of an open-top wooden box and comprised two different chambers; a black chamber (20×30×35 cm) painted with black color, whereas the second chamber was painted with white color. A small opened doorway (7.5×5 cm) was the link between the two chambers. Each mouse was placed in the center of the light compartment individually, and then followed up for the next five minutes for the number of crossings between these two compartments and the time spent in the dark and light compartments. After receiving treatment or saline, mice were returned to their cages. The period of the test was five minutes, during which the number of transitions and time spent in the light and dark compartments were registered.

Statistics

The results were expressed as the mean ± SEM. Data were analyzed using one–way analysis of variance (ANOVA) to determine statistical significance among groups of mice. Post hoc comparisons among group means were made using Tukey's test. Statistical analysis was performed using a software package (SPSS 16). The level of significance was defined as $p \leq 0.05$**.**

Results

Elevated plus-maze

Diazepam at a dose of 2 mg/kg significantly increased the open arm entries and the time spent in open arms in comparison with the saline group. Sodium nitroprusside at a dose of 3 mg/kg had no significant effect on the behavior in the plus-maze test, but at a dose of 1 mg/kg it showed significantly increased entries and time spent in open arms ($p \leq 0.05$) [Table- 1].

Table 1-Effects of diazepam and sodium nitroprusside on the behavior of mice in elevated plus maze test.

Groups	Time spent in an arm (sec.)		No. of entries (n)	
	Open arm	Closed arm	Open arm	Closed arm
Saline group	8.7±2.7	22.3±4.7	17	44
Diazepam (2 mg/kg)	12.2±1.5	8.7±2.1	70	34
Sod. Nitroprusside (1mg/kg)	14.2±1.6	10.8±1.3*	63	45
Sod. Nitroprusside (3mg/kg)	8.9±2.0	24.6±5.7	20	43

*****Level of significance in comparison to sodium nitroprusside 3mg/kg (significant, *p* value ≤ 0.05).

Light and Dark Box

Diazepam-treated mice increased the time spent in the light area in comparison with the time spent in the dark area of the same group, with a reduced number of transition between compartments. Sodium nitroprusside (1 mg/kg)-treated mice showed a significant increase in the time spent in light compartment in comparison to that of diazepam-treated animals. Higher dose of sodium nitroprusside (3 mg/kg) significantly increased the duration of presentation in the dark compartment as compared to that of the diazepam group. Number of mice transition between the chambers at the two assessed doses of sodium nitroprusside was not significantly different [Table-2].

Table 2-Effects of diazepam and sodium nitroprusside on the behavior of mice in the light and dark exploration test.

Groups	Time spent in the compartment (sec.)		Number of transitions (n)
	Light	Dark	
Saline group	15.2±1.5	22.9±4.1	46
Diazepam (2mg/kg)	16.8±4.3	8.0±0.8	30
Sod. Nitroprusside (1mg/kg)	22.1±7.0*****	19.6±4.1	35
Sod. Nitroprusside (3mg/kg)	10.9±1.6	37.7±7.2*****	37

* Level of significance in comparison to diazepam group (significant, *p* value ≤0.05).

Discussion

Anxiety can be considered as a defensive mechanism in an organism that is exhibited in response to novelty. It is generally characterized by the negative feelings and apprehension [25].

The two most popular behavioral tests, namely the light/dark exploration and elevated plus maze tests, can effectively assess the behavior related to anxiety in rodents [26, 27].

In earlier studies, sodium nitroprusside has been administered to experimental animals and found to produce either anxiogenic [24, 28]or anxiolytic effect [29].

The present study evaluated the effects of two doses of sodium nitroprusside on anxiety in both elevated plus-maze and light/dark tests.

The elevated plus maze has been frequently used to assess and evaluate anxiolytic- or anxiogenic-like influences of drugs. It is detected based on the natural fear of rodents to the rising and open fields. Rats and mice tend to avert high areas and, therefore, evasion of the open arms in elevated plus maze test is a reflection of anxiety behavior [30]. The time spent in the open arms was used for the evaluation of drug impact on anxiety.

In agreement with previous reports [31, 32], diazepam increased the time spent in the two open arms of the maze compared to the time spent in the two closed arms, confirming its anxiolytic effects.

In this study, sodium nitroprusside (1 mg/kg) significantly increased the time spent in the open arms. In contrast, sodium nitroprusside revealed anxiogenic effects at a higher dose (3 mg/kg), which might be explained by increasing the time spent in the closed area in the plus-maze test, which is in accordance with previous findings [33].

The decreased avoidance to the open arms is a result of an anxiolytic effect expressed by an increased number of open arm entries and time duration, along with decreased number of closed arm entries and time spent in the elevated plus maze.

The light/dark box test is based on the nature of rodents to avoid initially lighter areas [34]. In this paradigm, agents that decrease anxiety work to increase the amount of time spent in the light compartment. The number of transitions between compartments serves as an indicator of locomotor activity and the impact of the drug on locomotor behavior. Generally, it is acknowledged that the time spent in the light compartment is a more sensitive indicator of the anxiolytic effects of agents as compared to the transitions' number [35, 36].

The present results show that a lower dose of sodium nitroprusside (1 mg/kg) significantly increased the amount of time spent by animals in the light compartment but not the number of transitions between the compartments. It should be taken into account that the decreased number of transitions reflects the sedative effect of the drug. These results are consistent with a previous study that revealed the anxiolytic effect of sodium nitroprusside (1 mg/kg) [29]. On the other hand, the

current study's findings are in disagreement with those of the same previous study which investigated the sedative effect of a higher dose of sodium nitroprusside (3 mg/kg) in the light/dark exploration test. These results show that sodium nitroprusside possessed anxiolytic effects at a lower dose (1 mg/kg).

As a nitric oxide donor, sodium nitroprusside has been utilized in two doses to determine the direct effects of nitric oxide on the behavioral function by generation of exogenous nitric oxide in the brain. However, the mechanism behind the potential anxiogenic or anxiolytic actions of nitric oxide donors has yet to be explained.

Some studies have suggested that the NO/cGMP pathway in the brain is responsible for behavior alternation [37] but there still some arguments of the specified role of nitric oxide in both anxiogenesis and anxiolysis.

Conclusion

It remains difficult to evaluate nitric oxide donors in terms of anxiety. Our results suggest that sodium nitroprusside has an anxiolytic effect at a lower dose (1mg/kg), while at a higher dose (3mg/kg) it tends to increase anxiety. These findings shed the light on the involvement of nitrergic systems in anxiety. Moreover, the anxiolytic effect of the lower dose of sodium nitroprusside (1 mg/kg) seems to be approximate or similar to that produced via the anxiolytic diazepam (2 mg/kg).

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