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The effect of L-arginine and its antagonist L-NAME and Methylene blue on sensory and cognitive function in mice

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Summary

The present study was done to focus light on possible enhancement of the functional performance of male mice and female in neuronal behaviors by using L-arginine as a precursor of nitric oxide (NO). The results showed increase of latency period to reach the novel object in L-arginine treated groups and decrease in both L-NAME and methylene blue treated groups in both periods of treatment (15 and 30) days and were more prominent in male than in female mice as compared with control groups. Similar results were observed in passive avoidance latency period to enter the dark compartment. There was a reduction in latency period to reach the alternative arm of T-maze test in L-arginine treated groups and increase in both L-NAME and methylene blue treated groups in both periods of treatment (15 and 30) days in both genders. It could be concluded that L-arginine-NO pathway plays an important role in improving memory in male more than female mice.

Key words:- L-NAME, Methylene blue, sensory, cognitive

تأثيرات ال-ارجنين ومضاداته L-NAME وMethylene blue على الوظائف التحسسية والإدراكية في الفئران

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الخلاصة

أنجزت الدراسة الحالية لتسليط الضوء على إمكانية تسريع الإنجاز الوظيفي لذكور وإناث الفئران في السلوكيات العصبية باستخدام ال-أرجنين كواهب لأوكسيد النتريت (NO).أظهرت النتائج زيادة في الوقت الفعال للوصول إلى الهدف الغريب غير المألوف في المجاميع المعالجة بال-أرجنين ونقصان في كلا مجاميع المعالجة بالـL-NAME والـmethylene blue في كلا فترتي المعالجة (15 و30) يوم، وكانت أكثر جلياً في الذكور مقارنة في إناث الفئران مقارنة بمجاميع السيطرة. لوحظت نفس النتائج في الفترة الفعالة للتجنب السلبي passive avoidance للدخول للحجيرة المعتمة. هناك اختزال في الفترة الفعالة للوصول إلى الذراع البديل في اختبار T-maze في المجاميع المعالجة بال-أرجنين وزيادة في كلا المجاميع المعالجة بالـL-NAME و methylene blue في كلا فترتي المعالجة (15 و30) يوم وفي كلا الجنسين. من الممكن أن نستخلص أن مسلك ال-أرجنين-أوكسيد النتريت ذو فرط حيوية في تحسين الذاكرة في الذكور أكثر منه في إناث الفئران.

كلمات مفتاحية : ال-ارجنين, التحسسية, الإدراكية.

Introduction

Behavior is the adjustment of the animal to its environment under specified conditions. It simply means what animal does, how it does, and usually in response to stimuli from the environments (1 and 2). There were different pharmacological remedies used to treat the behavioral disorders such as cognitive impairment. L-arginine is one of modifiers of behaviors. It acts as neuromodulator in the central and peripheral nervous systems from which nitric oxide (NO) is derived that acts as a retrograde messenger in the central nervous system (3). Nitric oxide (NO) is diffusible molecules endowed with inter cellular messenger properties in several biological

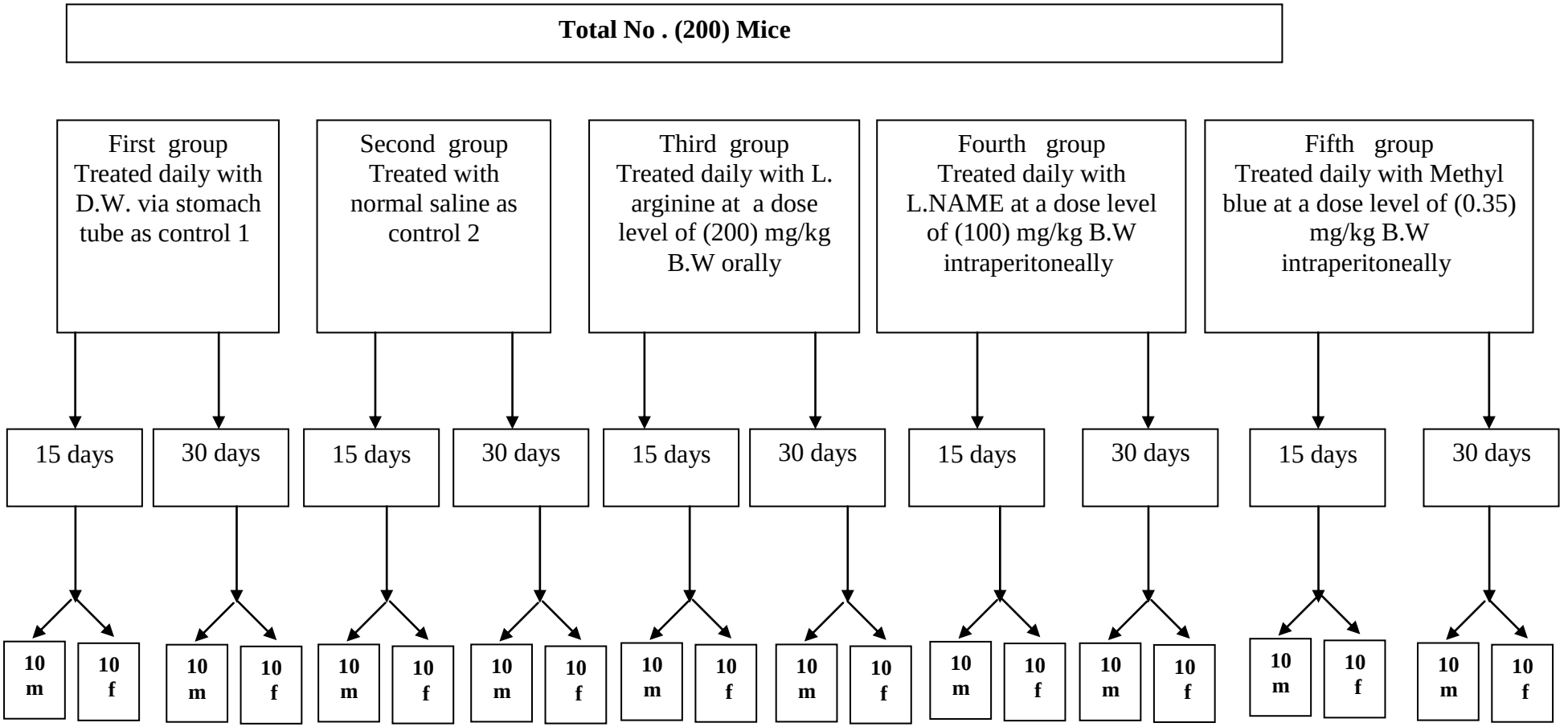
systems including the brain (4). This molecule plays an important role in learning and memory (5). Learning and memory field has focused on hippocampus, amygdale and cerebral cortex as critical sites in the brain where the plasticity underlying learning and memory occurs (6). Many reports suggested that L-arginine-NO system is part of the mechanisms underlying learning and memory. Long-term potentiation (LTP) in the hippocampus and long-term depression (LTD) in the cerebellum to be involved with learning and memory (7and 8). Because calcium-dependent activation of Ras-pathway of neural activity-dependent long-term changes in nervous system, (NO) may be a key mediator linking activity to gene expression and long-term plasticity (9). In mice, the formation of long-term memory (LTM) requires an increase in intracellular cyclic adenine monophosphate (cAMP) and requirement of the cAMP-dependent protein-kinase (PKA) that phosphorylates the transcription factor, cAMP - response element- binding protein (CREB) (10). The roles of (cAMP) pathway in the formation of long-term memory (LTM) are often supplemented by other signaling pathways, most notably by the (NO-cGMP) signaling pathway (11and12). The objective of this study was done to focus light on possible enhancement of the functional performance of male mice and female in neuronal behaviors by using L-arginine as a precursor of nitric oxide (NO).

Materials and Methods

One hundred male and 100 female white albino mice, weighing 25-30 gm. with an average of 27.5±0.02 gm. were used. They were kept under suitable environmental conditions of 20-25 ^{o}C. in an air conditioned room, (12) hours light and nourished *ad libitum*. Ten animals of each sex were given 200 mg/kg B.W. of L-arginine orally daily for 15 or 30 days. Other groups of ten mice of both sexes were likewise treated with 100 mg/kg B.W. of L-NAME and methylene blue 0.3 mg/kg B.W. intraperitoneally (13). Similar groups were given D.W. and normal saline, which served as control groups (Figure 1). The study consists of three experiments; the first one is object recognition test which is non spatial memory task based on spontaneously exploratory activity which used to test the novel object recognition capabilities of a mouse. In this task, mice are presented during a sample phase (learning) with either one or two identical objects and after a variable retention interval, animals are returned to the open-field and exposed to a novel object along side an identical copy of the object they had explored previously that is now familiar (14). The second experiment is passive avoidance task which described as a mouse has to choose between responding to obtain appositive reinforcement (entering to a dark compartment) and not responding to avoid an electric shock (not entering the dark compartment). The latency to refrain from entering into the dark compartment serves as an index of conditioned suppression and the ability to avoid, and allows memory to be assessed while the third experiment T-maze test is a spatial task in which animal learn to alternate between arms based on their memory of the previously visited arms. The latency to visit the correct choice (visited alternate arm) was the measure recorded (14).

Data were analyzed by using completely Randomized Design in factorial experimental (Two-way) ANOVA. In any experiment used two or three factors according to type of experiment. For calculation the effect of factors on dependent traits using (SPSS package 2008). To compare between treatments used Duncan, (1955) for multiple ranges. All the data were analyzed by using the procedures of (15). A probability of ($P< 0.05$) was considered as significant differences.

Figure (1): Experimental Design of Sensory and Cognitive Function



m = male

f = female

Results and Discussion

The effects of L-arginine and its antagonist L-NAME (NO synthase inhibitor) and methylene blue (soluble guanylyl cyclase inhibitor) on the object recognition time latency to reach the novel object table (1) and on the passive avoidance test latency period to enter the dark compartment table (2) for both genders, revealed significant differences at ($P<0.05$) between treated groups (15 and 30) days and control groups, which displayed increase in L-arginine and decrease in both L-NAME and methylene blue.

The results of T-maze test table (3) revealed significant decrease at ($P<0.05$) in the latency period to reach the alternative arm in L-arginine treated groups and increase in L-NAME and methylene blue treated groups as compared with control groups.

The results of novel object recognition which depended on the hippocampus and cortex brain areas (16) might be attributed to the role of L-arginine-NO pathway on cognition through enhancing and improving learning and memory due to, increase in synaptic transmission after stimulation of an excitatory pathway in the cortex and hippocampus called as long-term potentiation (LTP) by L-arginine-NO pathway and found to increase long-term potentiation by activating soluble guanylyl cyclase (sGC) and ultimately cyclic guanosine monophosphate (cGMP) which inturn increased nitric oxide (NO) formation in the cortex and hippocampus (7,17and18). Furthermore, L-arginine-NO-pathway might be involved increase release of excitatory neurotransmitters glutamate and acetylcholine through (cGMP)-dependent mechanism in the brain cortex and hippocampus which inturn supports synaptic plasticity through the critical role of glutamate, acetylcholine and nitric oxide in pathways associated with long-term potentiation, therefore this effect of L-arginine-NO pathway on these excitatory neurotransmitters might be involved in improving memory performance and enhanced cognitive function (19,12and 21). The passive avoidance test was used to evaluate emotional, learning and memory, L-arginine-NO pathway was modulated latency period time to enter the dark area. These results presumably due to the important role of L-arginine-NO pathway on cognitive through increasing nitric oxide (NO) concentrations, due to increase the activity of nitric oxide synthase (NOS) in the cortex, amygdala and hippocampus brain areas of adult mice. On other wise, L-arginine-NO-pathway might be stimulate release of argininevasopressin (AVP), neurohypophysial hormone, which facilitates memory, and nitric oxide (NO) synthesis evoked the vasodilatation caused by (AVP), and improvement of learning and memory due to at an angiotensin II (22), which increases regional cerebral blood flow by dilating cerebral arteries in rabbit and rodents (18 and 22). Although angiotensin II stimulate mostly the acquisition, while argininevasopressin stimulate consolidation of memory processes, both peptides have been found to facilitate recall of information in a passive avoidance test (24 and 25). In the T-maze test the simple capital (T) shape design incorporates a single choice point with only two alternatives, in which mice model was used to alternate between arms based on their memory of the previously visited arms. T-maze test and other behavioral tests of learning and memory are visuospatial tests of cognitive function and animals with impaired visual acuity may perform poorly on these tasks because of poor vision (26). The results in this test might be attributed to the effect of L-arginine-NO pathway on cognitive due to, provoked visual responses through L-arginine-NO pathway by modulation N-methyl-D-aspartate (NMDA) receptor-mediated excitation within the dorsal lateral genicular nucleus (dLGN), a major target of output from retina, which involved in visually specific pathways beside modulatory pathway which influence the activity of (dLGN) cells in arousal states (27,28 and 29). On other wise, the highly diffusible gas

nitric oxide (NO) may act in the proximity of the synaptic area of the parabrachial terminals of the brain stem, diffusing to act on retinogeniculate or other synapses utilizing (NMDA) receptors, thus release of nitric oxide may facilitate visual transmission in the thalamus affecting the functional activity of neuronal population in an extended volume of tissue, (29). Furthermore, the gender differences in learning and memory might be attributed to levels and activities of nitrate and nitrite (NO metabolites) which found higher in adult male brain areas of cortex, hippocampus, midbrain and cerebellum than female rodents (30).

In conclusion, the present data show that L-arginine-NO pathway is more prominent in enhancing and improving learning and memory in male than female mice.

Table (1): The Object Recognition time (minute) in L-arginine treated orally male and female mice, L-NAME intraperitoneally and with Methyl blue intraperitoneally daily (Object Recognition Test).

Periods of treatment and sex / Groups	15 days		30 days	
	Male	Female	Male	Female
D.W. as control 1.	3.52±0.20 Aa	1.25±0.25 Ba	3.01±0.19 Aa	1.35±0.20 Ba
Normal saline as control 2.	3.00±0.20 Aa	1.20±0.25 Ba	3.21±0.19 Aa	1.21±0.25 Ba
L-arginine (200)mg/ kg B.W.	6.31±0.84 Ab	4.00±0.47 Bb	6.00±0.62 Ab	4.33±0.29 Bb
L-NAME (100)mg/ kg B.W.	1.38±0.16 Ac	0.44±0.21 Bc	1.30±0.12 Ac	0.40±0.14 Bc
methyl blue (0.35)mg/ kg B.W.	1.33±0.18 Ac	0.35±0.23 Bc	1.24±0.10 Ac	0.43±0.20 Bc

Values are presented as Mean ±SE
Small letters denoted to (P<0.05) different between treated groups of certain sex.
Capital letters denoted to (P<0.05) gender differences.
Number=10mice/group

Table (2): The Passive Avoidance latency period (minute) in L-arginine treated orally male and female mice, L-NAME intraperitoneally and Methylblue intraperitoneally daily (Passive Avoidance Test).

Periods of treatment and sex / Groups	Male mice						Female mice					
	15 days			30 days			15 days			30 days		
	Habituation day	Training day	Testing day	Habituation day	Training day	Testing day	Habituation day	Training day	Testing day	Habituation day	Training day	Testing day
D.W as control 1	5.26 ± 0.60^{Ba}	3.16 ± 0.48^{Ca}	18.20 ± 3.20^{Aa}	5.00 ± 0.60^{Ba}	3.00 ± 0.46^{Ca}	18.61 ± 3.20^{Aa}	5.00 ± 0.25^{Ba}	3.20 ± 0.20^{Ca}	19.00 ± 1.83^{Aa}	4.62 ± 0.25^{Ba}	2.34 ± 0.20^{Ca}	18.81 ± 1.83^{Aa}
Normal saline as control 2.	5.20 ± 0.60^{Ba}	3.20 ± 0.48^{Ca}	18.80 ± 3.20^{Aa}	5.26 ± 0.60^{Ba}	3.00 ± 0.48^{Ca}	18.81 ± 3.20^{Aa}	4.62 ± 0.25^{Ba}	3.00 ± 0.20^{Ca}	19.21 ± 1.83^{Aa}	4.82 ± 0.25^{Ba}	3.16 ± 0.20^{Ca}	18.61 ± 1.83^{Aa}
L-arginine (200)mg/ kg B.W.	1.69 ± 0.20^{Bb}	1.04 ± 0.26^{Bb}	26.55 ± 3.80^{Ab}	1.00 ± 0.22^{Bb}	1.00 ± 0.21^{Bb}	26.14 ± 2.50^{Ab}	2.00 ± 0.13^{Bb}	1.21 ± 0.18^{Bb}	26.00 ± 1.80^{Ab}	1.66 ± 0.12^{Bb}	1.32 ± 0.20^{Bb}	25.52 ± 1.84^{Ab}
L-NAME (100)mg/ kg B.W.	7.60 ± 1.50^{Bc}	5.21 ± 1.40^{Cc}	3.23 ± 0.22^{Ac}	7.00 ± 1.05^{Bc}	5.61 ± 1.30^{Cc}	3.16 ± 0.27^{Ac}	7.02 ± 1.03^{Bc}	5.00 ± 1.18^{Cc}	3.00 ± 0.22^{Ac}	6.14 ± 0.92^{Bc}	6.05 ± 1.92^{Bc}	3.24 ± 0.92^{Ac}
Methyl blue(0.35)mg/ kg B.W.	7.00 ± 1.50^{Bc}	5.00 ± 1.40^{Cc}	3.00 ± 0.22^{Ac}	6.70 ± 1.05^{Bc}	5.00 ± 1.07^{Cc}	3.05 ± 0.27^{Ac}	7.39 ± 0.92^{Bc}	5.17 ± 0.91^{Cc}	3.20 ± 0.24^{Ac}	7.00 ± 1.08^{Bc}	6.17 ± 0.94^{Bc}	3.00 ± 1.06^{Ac}

Values are presented as Mean ± SE.
Small letters denoted to ($P < 0.05$) different between treated groups.
Capital letters denoted to ($P < 0.05$) different between experimental days of certain period of treatment.
Number = 10 mice / group.

Table (3): The T-maze latency period (second) in L-arginine treated orally male and female mice, intraperitoneally with L-NAME and intraperitoneally with Methyl blue daily (T-Maze Test)

Periods of treatment and sex / Groups	15 days		30 days	
	Male	Female	Male	Female
D.W. as control 1.	8.60 ±1.40[a]	8.48 ±1.10[a]	8.70 ±1.40[a]	8.46 ±1.10[a]
Normal saline as control 2	8.62 ±1.40[a]	8.44 ±1.12[a]	8.70 ±1.44[a]	8.62±1.20[a]
L-arginine (200)mg/ kg B.W.	2.66 ±0.40[b]	2.60 ±0.46[b]	2.70 ±0.33[b]	2.64 ±0.40[b]
L-NAME (100)mg/ kg B.W.	17.30 ±2.40[c]	17.27 ±2.18[c]	17.32 ±2.40[c]	16.93 ±2.60[c]
Methyl blue (0.35)mg/ kg B.W.	17.39 ±2.60[c]	16.97 ±0.80[c]	17.00 ±2.33[c]	17.20 ±2.76[c]

Values are presented as Mean ±SE
Small letters denoted to (P<0.05) different between treated groups of certain sex.
Number = 10 mice/group

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