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Influence of L-arginine and molsidomine on ambulatory locomotor activity in the Y-maze test in mice

Nitric oxide (NO) is an atypical neurotransmitter/neuromodulator that is synthesized from L-arginine (L-ARG) by the enzymes NO synthases (NOS; 6). Manipulation of NO formation modifies many brain physiological, pathological and biochemical conditions (2, 8). Accumulating evidence indicates that NO plays an important role in the control of motor behaviour and locomotion in animals. Previously, it has been found that NOS inhibitors reduced spontaneous locomotor activity in rodents (4, 5, 9). For instance, N^G-nitro-L-arginine (NNA – a nonspecific NOS inhibitor) decreased open arm exploration in the plus maze (3, 4). Similarly, N^G-nitro-L-arginine methyl ester (L-NAME) reduced spontaneous locomotion in mice and rats (1, 9). Moreover, 7-nitroindazole (7-NI – a preferential neuronal NOS inhibitor) reduced locomotor activity in rats and mice (1, 10). The same situation has been observed after administration of 3-bromo-7-nitroindazole, 1-(2-trifluoromethylphenyl)-imidazole and S-methyl-L-thiocitrulline (more potent neuronal NOS inhibitors) that reduced significantly locomotion in rodents (5). Moreover, it has been found experimentally that L-ARG (a natural precursor of NO) attenuated significantly 7-NI- and L-NAME-induced locomotor deficits in rodents (1).

Since NOS inhibitors (7-NI, NNA, and others) decreased spontaneous and exploratory locomotor activity in rodents, one can suggest that NO is involved in locomotor activity and behaviour control in animals. Considering the above-mentioned facts, it was of some pivotal importance to determine the influence of L-ARG (a natural precursor of NO) and molsidomine (MOL – a donor of NO) on ambulatory locomotor activity in mice subjected to the Y-maze test.

MATERIAL AND METHODS

Animals. All experiments were performed on male Swiss mice, kept in colony cages with free access to food and tap water, under standardized housing conditions. The animals were randomly assigned to experimental groups consisting of 8 mice. Each mouse participated only in one experiment. All tests were performed between 9.00 a.m. and 2.00 p.m. to minimize confounding effects of circadian rhythms. All experimental procedures described hereupon were approved by the Board for Supervising Ethics in Medical Experiments at the Medical University of Lublin.

Drugs. MOL (Polfa Warszawa, Poland) and L-ARG (Research Biochemicals International, Natick, MA, USA) were suspended in 1% solution of Tween 80 (Sigma, St. Louis, MO, USA) in saline and administered intraperitoneally (i.p.) in a volume of 5 ml/kg body weight: L-ARG – at 60 min, and MOL – at 30 min before the test.

Ambulatory locomotor activity evaluation in the Y-maze test. The evaluation of ambulatory locomotor activity of animals in the Y-maze test is based on rodents' innate curiosity to explore novel areas and to enter arms of the Y-maze. The Y-maze apparatus consisted of three identical arms (three compartments of 15 x 15 x 10 cm) with connector (10 x 6 x 10 cm) radiating out from the centre. The walls of each arm of the Y-maze test were made of a diversely coloured plastic: the first arm was black, the second – white, and the third was striped in black-and-white vertical patterning. The floor of each arm was covered by paper towels, which were changed at each testing in order to eliminate olfactory stimuli. To minimize stress occurring when animals are placed in new environmental conditions, the maze was placed in a sound-attenuated room under dim illumination (70 lx). Mice were placed separately at the end of one of the arms (starting arm), their head pointing away from the center of the maze, and they were allowed to traverse freely the apparatus for 5 min. The entrance of the animal was considered to be complete when the hind paws of the mouse had entirely entered the arm. The series of arm entries were manually recorded by an investigator who was not aware of the treatments. The total number of arm entries within 5 min reflects inquisitive behaviour of animals (locomotor activity of animals at the rudimentary level). In the present study, the Y-maze was used only for locomotor and general exploration measurement as presented earlier (7). Ambulatory locomotor activity of animals is expressed as total number of arm entries within the 5 min of observation time as means ± SE of at least 8 determinations.

Statistics. Ambulatory locomotor activity (general exploration) of the animals evaluated in the Y-maze was analyzed by one-way ANOVA followed by the *post-hoc* Bonferroni's test for significant treatment effects relative to control means.

RESULTS

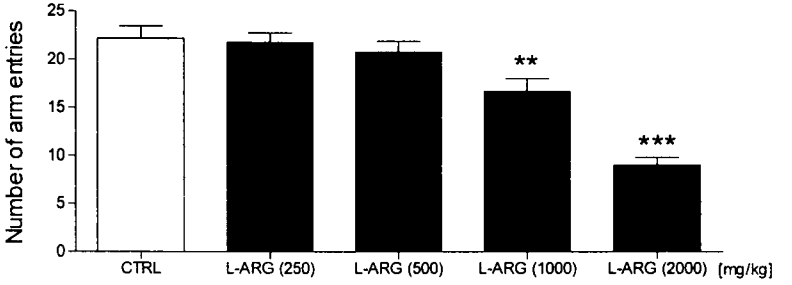
EFFECT OF L-ARG ON AMBULATORY ACTIVITY TESTING

In the Y-maze test, L-ARG administered alone (i.p., 60 min. before the test) reduced in a dose dependent manner locomotor activity of animals. One-way ANOVA revealed that mean number of arm entries of animals following L-ARG administration differed significantly from that observed by a chance [$F(4, 45) = 27.2$; $p<0.0001$]. The mean number of arm entries within the 5-min observation period for control (vehicle-treated) animals was 22.2 ± 1.27 and did not differ significantly from that for animals administered with L-ARG at doses of 250 and 500 mg/kg, which was 21.8 ± 0.96, and 20.8 ± 1.10, respectively (Fig. 1). In contrast, with the *post-hoc* Bonferroni's test, the mean number of arm entries for the animals injected with L-ARG at doses of 1000 and 2000 mg/kg was significantly reduced from 22.2 ± 1.27 (control) to 16.7 ± 1.33, and 9.0 ± 0.87, respectively (at $p<0.01$ and $p<0.001$ vs. control; Fig. 1).

EFFECT OF MOL ON AMBULATORY ACTIVITY TESTING

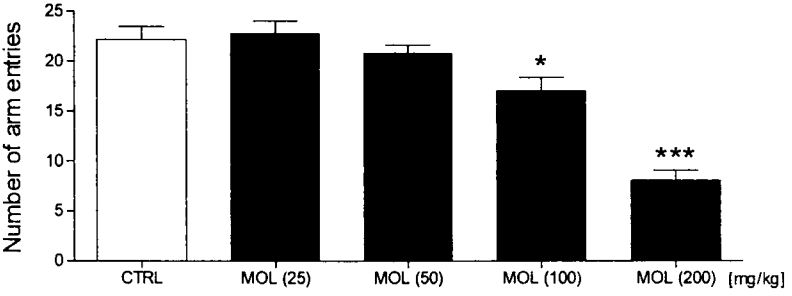
MOL administered separately (i.p., 30 min. before the test) reduced dose-dependently ambulatory locomotor activity of animals challenged with the Y-maze test. With one-way ANOVA, the mean number of arm entries of animals after MOL administration differed significantly from that observed by a chance [$F(4, 45) = 24.5$; $p<0.0001$]. The mean number of arm entries within the 5-min observation period for control (vehicle-treated) animals was 22.2 ± 1.27 and did not differ significantly from that for animals administered with MOL at doses of 25 and 50 mg/kg, which was 22.8 ± 1.25, and 20.8 ± 0.88, respectively (Fig. 2). In contrast, with the *post-hoc* Bonferroni's test,

the mean number of arm entries for the animals injected with MOL at doses of 100 and 200 mg/kg was significantly reduced from 22.2 ± 1.27 (control) to 17.1 ± 1.34, and 8.10 ± 0.99, respectively (at $p<0.05$ and $p<0.001$ vs. control; Fig. 2).



Columns represent the mean number of arm entries of the animals in the Y-maze test ± SE as the error bars. L-Arginine (L-ARG) was administered systemically (i.p.) 60 min before the testing session. The observation time of ambulatory activity in the Y-maze test was set up to 5 min. Statistical analysis of the data was performed with one-way ANOVA followed by the *post-hoc* Bonferroni's test **p<0.01 and ***p<0.001 vs. control group

Fig. 1. Influence of L-arginine (a natural precursor of NO) on ambulatory locomotor activity in the Y-maze test in mice



Columns represent the mean number of arm entries of the animals in the Y-maze test ± SE as the error bars. Molsidomine (MOL) was administered systemically (i.p.) 30 min before the testing session. The observation time of ambulatory activity in the Y-maze test was set up to 5 min. Statistical analysis of data was performed with one-way ANOVA followed by the *post-hoc* Bonferroni's test *p<0.05 and ***p<0.001 vs. control group

Fig. 2. Influence of molsidomine (a donor of NO) on ambulatory locomotor activity in the Y-maze test in mice

DISCUSSION

The effects produced by L-ARG and MOL (two agents increasing NO content in the brain) with respect to impairment of ambulatory locomotor activity in mice were assessed in the Y-maze test. Results indicated that L-ARG (a natural precursor of NO, administered at doses of 1000 and

2000 mg/kg) and MOL (a donor of NO – at 100 and 200 mg/kg) significantly decreased ambulatory locomotor activity of animals challenged with the Y-maze test.

As already mentioned, it has been documented that 7-NI (a preferential neuronal NOS inhibitor) administered at a dose of 120 mg/kg, significantly suppressed locomotor activity in mice subjected to the electronically measured open-field test (5). The impaired locomotor activity has also been observed in animals administered with 7-NI at doses of 20 and 50 mg/kg (11). In this case, the NOS inhibitor significantly reduced total distance travelled by animals in the electronically measured open-field test in mice (11). Likewise, 7-NI at doses of 40, 80 and 160 mg/kg produced (in a dose--dependent manner) locomotor deficits in the pole test in mice (1). Moreover, the reduced locomotor activity has been observed in rats administered with 7-NI at a dose of 120 mg/kg and challenged with the open-field and elevated plus-maze tests (12). Similarly, NNA (at doses ranged between 10–120 mg/kg) decreased dose-dependently open arm exploration in the plus maze (3, 4), and L-NAME (at 80 mg/kg) considerably reduced spontaneous locomotion in mice and rats (1, 9). Moreover, it has been observed that L-ARG (at 300 mg/kg) partially reversed 7-NI- and L-NAME-induced locomotor deficits in rodents (1).

Our results, indicating reduction of locomotor activity after administration of L-ARG and MOL, are quite similar to those reporting the decrease in ambulatory locomotor activity of animals following the administration of 7-NI and NNA. Considering molecular mechanisms of action of MOL, L-ARG, 7-NI and NNA, one can ascertain that both, increase and decrease in NO content in the brain produced locomotor activity impairment. Thus, one can indirectly ascertain that only normal physiological concentrations of NO in the brain are responsible for normal ambulatory activity in animals. In contrast, any substantial changes in NO concentrations related to the reduction or increase in NO content in the brain impaired locomotor activity in mice.

Another fact is worthy of mentioning while interpreting the results of this study. It is widely accepted that NO has vasodilatatory properties in living organisms and thus, the administration of MOL and L-ARG increases NO concentration not only in the brain of experimental animals, but also in the whole body. In such cases, the decrease in blood pressure as a result to NO-related vasodilatation in peripheral tissues can be responsible for the decreased locomotor ambulatory activity in experimental animals subjected to the Y-maze test.

CONCLUSIONS

1. In this study, a close relationship between increasing doses of L-ARG and MOL and their resultant changes in locomotor activity was observed.

2. In case of a separate and/or combined use of MOL and L-ARG (at high doses), the reduction of ambulatory locomotor activity in animals is expected in preclinical studies.

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SUMMARY

The aim of this study was to determine the effect of L-arginine (L-ARG – a natural precursor of nitric oxide [NO]) and molsidomine (MOL – a donor of NO) on the ambulatory locomotor activity in the Y-maze test in mice. Results indicated that L-ARG (administered i.p., 60 min before the test) at doses of 1000 and 2000 mg/kg significantly impaired ambulatory locomotor activity by reducing the total number of arm entries by animals within 5 min of observation period from 22.2 (control) to 16.7 ($p<0.01$), and 9.0 ($p<0.001$), respectively. In contrast, L-ARG at 250 and 500 mg/kg did not alter significantly ambulatory locomotion in animals. MOL (administered i.p., 30 min. before the test) at doses of 100 and 200 mg/kg considerably reduced ambulatory locomotor activity by decreasing the total number of arm entries by animals from 22.2 (control) to 17.1 ($p<0.05$), and 8.1 ($p<0.001$), respectively. Only, MOL at doses of 25 and 50 mg/kg produced no significant changes in locomotor activity in mice as observed within 5 min of testing. Based on this preclinical study, one can conclude that L-ARG and MOL administered at high doses may exert locomotor activity deficits in humans.

Wpływ L-argininy i molsidominy na aktywność ruchową w teście labiryntu Y u myszy

Celem pracy była ocena wpływu L-argininy (L-ARG – naturalnego prekursora tlenku azotu [NO]) i molsidominy (MOL – donora NO) na aktywność ruchową w teście labiryntu Y u myszy. Wyniki wykazały, że L-ARG (podana i.p., 60 min., przed testem) w dawkach 1000 i 2000 mg/kg istotnie zaburzała aktywność ruchową poprzez zmniejszenie całkowitej liczby wejść do ramion labiryntu przez zwierzęta w ciągu 5 min. okresu obserwacji z 22,2 (grupa kontrolna) do odpowiednio 16,7 ($p<0,01$) i do 9 ($p<0,001$). Przeciwnie, L-ARG w dawkach 250 i 500 mg/kg nie zmieniała istotnie aktywności ruchowej u zwierząt. MOL (podana i.p., 30 min., przed testem) w dawkach 100 i 200 mg/kg istotnie zmniejszała aktywność ruchową poprzez zmniejszenie całkowitej liczby wejść do ramion labiryntu przez zwierzęta z 22,2 (grupa kontrolna) do odpowiednio 17,1 ($p<0,05$) i do 8,1 ($p<0,001$). Tylko MOL w dawkach 25 and 50 mg/kg nie wywierała istotnych zmian w aktywności

ruchowej u myszy podczas 5 min. obserwacji w teście. W oparciu o to przedkliniczne badanie można stwierdzić, że L-ARG i MOL podawana w wysokich dawkach może wywierać zaburzenia aktywności ruchowej u ludzi.