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Anticonvulsant action of 7-nitroindazole, a nitric oxide synthase inhibitor, in the maximal electroshock seizure threshold model in mice

Nitric oxide (NO) is a gaseous chemical molecule acting as a second messenger and atypical neurotransmitter in the brain (10). NO is synthesized from L-arginine by the enzyme NO synthase (NOS) and a co-product of L-cytrulline (11). The role of NO in the development and pathogenesis of seizures has been investigated by several authors, who have reported contradictory data, showing that NO exerted either anti-or pro-convulsant properties in various animal models of epilepsy (6, 15).

In pharmacological studies examining the role of NO in the brain, 7-nitroindazole (7NI) takes a special position as a preferential inhibitor of neuronal NOS (1). It has been found experimentally that 7NI delayed the onset of seizures evoked by N-methyl-D-aspartate in mice (5); suppressed sound-induced seizures in DBA/2 mice (3); protected the animals against pilocarpine-induced seizures in DBA/2 mice and genetically epilepsy-prone rats (16, 17), as well as attenuated the severity of kainic acid-, pentylenetetrazole-, and picrotoxin-induced seizures in rats (7, 13, 14). In biochemical studies, it has been documented that 7NI inhibits preferentially neuronal NOS, having simultaneously no impact on endothelial NOS *in vivo* and blood pressure in animals (12).

The purpose of this study was to evaluate the effect of 7NI, administered intraperitoneally (i.p.) at high doses (up to 200 mg/kg), on the threshold for electroconvulsions in mice. Noticeably, the maximal electroshock seizure threshold (MEST) test is considered as an experimental (animal) model of tonic-clonic seizures and, to a certain extent, of partial convulsions with or without secondary generalization in humans (9). Generally, this test allowed the evaluation of anticonvulsant properties of drugs or compounds effective against "*grand mal*" seizures in humans.

MATERIAL AND METHODS

A n i m a l s. The experiments were carried out on male Swiss mice weighing 20–25 g, purchased from a licensed breeder (T. Górzkowska, Warsaw, Poland). The animals were housed in colony cages with free access to food (chow pellets) and tap water. The temperature was 22°C ± 1°C and animals were on a natural light-dark cycle. Experimental groups (consisting of 8 mice) were randomly assigned. All experimental procedures were approved by Local Ethics Committee at the Medical University of Lublin (License no: 479/04/509/2004).

Drug. 7NI (Sigma, St. Louis, MO, USA) was suspended in a 1% solution of Tween 80 (Sigma, St. Louis, MO, USA) in distilled water and injected intraperitoneally (i.p.) in a volume of 10 ml/kg at 30 min. prior to the MEST test. This pretreatment time before testing of 7NI was based on information about its biological activity from the literature (2, 3, 15).

Maximal electroshock seizure threshold (MEST) test. Electroconvulsions were produced by means of an alternating current (0.2 s stimulus duration, 50 Hz) delivered via ear-clip electrodes by a generator (Rodent Shocker, Type 221, Hugo Sachs, Freiburg, Germany). The criterion for the occurrence of seizure activity was the tonic hindlimb extension (i.e., the hind limbs of animals outstretched 180° to the plane of the body axis). In order to evaluate the threshold for electroconvulsions, at least 4 groups of mice, consisting of 8 animals per group, were challenged with electroshocks of various intensities to yield 10–30%, 30–50%, 50–70%, and 70–90% of animals with seizures. Then, a current intensity-effect curve was constructed, according to log-probit method by Litchfield and Wilcoxon (8), from which a CS_{50} (median current strength in mA) was estimated. Each CS_{50} value represents the current intensity required to induce tonic hindlimb extension in 50% of the mice challenged. Again, after administration of a single dose of 7NI to 4 groups of animals, the mice were subjected to electroconvulsions (each group with a constant current intensity). The threshold for electroconvulsions was denoted for 4 different doses of 7NI, as follows: 50, 100, 150 and 200 mg/kg.

Statistics. The CS_{50} values for 7NI (with their respective 95% confidence limits) were calculated by computer log-probit analysis according to Litchfield and Wilcoxon (8). Subsequently, the 95% confidence limits were transformed into standard errors (S.E.). Statistical evaluation of the data was performed using one-way ANOVA followed by the *post-hoc* Bonferroni's multiple comparisons test.

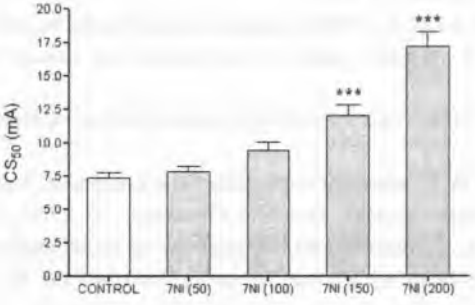
RESULTS

Results indicated that 7NI (administered i.p., at 30 min before the MEST test) increased the threshold for electroconvulsions in mice dose-dependently [F (4;131) = 32.77; $p<0.0001$]. The *post-hoc* Bonferroni's test revealed that 7NI (at 150 and 200 mg/kg) raised significantly the electroconvulsive threshold from 7.3 (6.5 – 8.3) mA to 12.0 (10.5 – 13.7) mA and 17.2 (15.2 – 19.6) mA, respectively ($p<0.001$; Fig. 1). In contrast, 7NI administered i.p., at lower doses of 50 and 100 mg/kg did not affect the electroconvulsive threshold in mice, although a tendency towards the increase in threshold was observed as the CS_{50} values were increased from 7.3 (6.5 – 8.3) mA to 7.8 (6.8–9.0) mA and 9.5 (8.2–11.0) mA, respectively (Fig. 1).

DISCUSSION

Here we showed that 7NI exerted a clear-cut anticonvulsant effect by increasing dose-dependently the threshold for electroconvulsions in mice. For the first time, it was found that 7NI at high doses of 150 and 200 mg/kg exerted the antiseizure influence in the MEST test in mice. Relatively recently, it has been documented that 7NI at 100 mg/kg potently inhibited picrotoxin-induced convulsions, although the drug did not alter NO concentration in the brain indicating that its anticonvulsant effect was devoid of involvement of NO (14). In contrast, 7NI at a higher dose of 200 mg/kg considerably reduced the NO content in the brain and thus, enhanced proconvulsant action in picrotoxin-induced seizures (14). Our findings indicated clearly that 7NI at 200 mg/kg increased the threshold for electroconvulsions, producing anticonvulsant properties in the MEST test. Obviously, the results reporting that 7NI at

200 mg/kg produced anticonvulsant properties are contradictory with those documented by Paul and Ekambaram (14). The observed discrepancy in the MEST test and picrotoxin-induced seizures can be explained by considering different seizure models and/or species used in both studies. Perhaps, picrotoxin as a chemical compound could pharmacokinetically modify the activity of 7NI and thus, produced proconvulsant effects in rats. Moreover, the existence of other mechanisms of action of 7NI, unrelated to NOS inhibition, which would be responsible for the increment in electroconvulsive threshold in mice, should be borne in mind. For instance, in neurochemical studies, it has been found that 7NI inhibited monoamine oxidase activity (4), and thereby the agent increased the content of monoamines in the brain. Noteworthy, monoamines exert the anticonvulsant influence *per se* and monoaminergic receptor-mediated events may contribute to the increased threshold for electroconvulsions in mice.



Columns represent median current strengths (CS_{50}s ± S.E. as the error bars) required to produce tonic hindlimb extension in 50% of the animals tested in the MEST test. 7NI was administered i.p., 30 min before the test. Statistical evaluation of the data was performed using log-probit method according to Litchfield and Wilcoxon (8) and one-way ANOVA followed by the *post-hoc* Bonferroni's test for multiple comparisons. ***p<0.001 vs. control (vehicle-treated) animals

Fig. 1. Influence of 7-nitroindazole (7NI) on the threshold for electroconvulsions in mice

CONCLUSIONS

Based on this preclinical study, one can ascertain that 7NI at the doses of 150 and 200 mg/kg exerted the anticonvulsant influence protecting the animals against electrically-induced seizures in the MEST test.

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SUMMARY

The objective of this study was to evaluate the effect of 7-nitroindazole (7NI; a nitric oxide synthase [NOS] inhibitor) in the maximal electroshock seizure threshold (MEST) test in mice. Results indicated that 7NI (administered intraperitoneally at doses of 150 and 200 mg/kg, 30 min. before the MEST test) significantly raised the electroconvulsive threshold in mice from 7.3 (6.5–8.3) mA to 12.0 (10.5–13.7) mA, and 17.2 (15.2–19.6) mA, respectively ($p<0.001$). In contrast, 7NI at low doses of 50 and 100 mg/kg did not affect significantly the electroconvulsive threshold, although the NOS inhibitor increased the threshold for electroconvulsions in mice. This preclinical study indicated that 7NI raised dose-dependently the threshold for electroconvulsions in mice.

Przeciwdrgawkowe działanie 7-nitroindazolu w teście progu maksymalnego wstrząsu elektrycznego u myszy

Celem pracy była ocena przeciwdrgawkowego działania 7-nitroindazolu (7NI, inhibitora syntazy tlenku azotu [NOS]) w teście progu maksymalnego wstrząsu elektrycznego (MEST) u myszy. Wyniki wykazały, że 7NI (podawany dootrzewnowo w dawkach 150 i 200 mg/kg, 30 min. przed testem MEST) istotnie statystycznie podnosił próg pobudliwości drgawkowej u myszy z 7,3 (6,5–8,3) mA do odpowiednio 12,0 (10,5–13,7) mA i 17,2 (15,2–19,6) mA ($p<0.001$). Przeciwnie 7NI w niższych dawkach 50 i 100 mg/kg nie wpływał istotnie na próg drgawkowy, chociaż ten NOS inhibitor zwiększał próg pobudliwości drgawkowej u myszy. To badanie przedkliniczne wykazało, iż 7NI w sposób zależny od dawki podnosił próg dla drgawek elektrycznych u myszy.