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No effect of 7-nitroindazole on the anticonvulsant action of vigabatrin and oxcarbazepine in pentylenetetrazole-induced seizures in mice

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Abstract: The aim of this study was to determine the effect of 7-nitroindazole (7-NI – a preferential neuronal nitric oxide synthase inhibitor) on the protective action of oxcarbazepine (OXC) and vigabatrin (VGB) – two newer antiepileptic drugs (AEDs) against pentylenetetrazole (PTZ)-induced seizures in mice. The clonic seizure activity in mice was evoked by subcutaneous injection of PTZ (100 mg/kg) and defined as clonus of the whole body lasting over 3 s with an accompanying loss of righting reflex in mice. The protective action of OXC and VGB against PTZ-induced clonic seizures was expressed as their median effective doses (ED_{50} values) suppressing clonic seizures in 50% of animals tested. The acute adverse-effect potentials of OXC and VGB in combination with 7-NI were evaluated in the chimney test (motor coordination) in mice. Results indicate that 7-NI administered intraperitoneally (50 mg/kg) did not significantly affect the anticonvulsant action of OXC or VGB against PTZ-induced clonic seizures. The ED_{50} values for OXC administered alone and in combination with 7-NI were 20.9 and 24.8 mg/kg. Similarly, the ED_{50} values for VGB administered alone and in combination with 7-NI were 595 and 622 mg/kg, respectively. Moreover, the examined combinations of 7-NI (50 mg/kg) with OXC (24.8 mg/kg) and VGB (622 mg/kg) did not affect motor coordination in the chimney test. In conclusion, 7-NI had no significant impact on the anticonvulsant activity of OXC and VGB in the mouse PTZ-induced seizure model, and did not affect motor coordination in animals subjected to the chimney test.

Key words: 7-Nitroindazole, nitric oxide, vigabatrin, oxcarbazepine, pentylenetetrazole-induced seizures, chimney test

INTRODUCTION

Nitric oxide (NO) is considered to be an endogenous neurotransmitter/ neuromodulator synthesized from the amino acid L-arginine by the enzyme NO synthase (NOS) [1-4]. The agent seems to play an essential role in numerous physiological and pathophysiological processes in the brain, including neuronal plasticity, cerebral blood-flow, cognitive and behavioural functions, as well as ischaemia and epilepsy [1-4]. Nevertheless, the exact role of NO in the pathophysiology of seizures has not been elucidated to date.

Experimental studies have revealed that 7-nitroindazole (7-NI – a preferential neuronal NOS inhibitor) exerts anticonvulsant properties by elevating the threshold for electroconvulsions and suppressing sound-induced seizures in DBA\2 mice [5-10]. 7-NI at a dose of 50 mg/kg completely inhibited the pentylenetetrazole (PTZ)-induced increase in NO levels in the hippocampus, but had no behavioural effect on PTZ-induced clonic convulsions in rats [11]. Thus, 7-NI suppressed the development of PTZ-induced kindling without affecting PTZ-induced seizures in rats [11]. Moreover, 7-NI administered systemically (i.p.) at a dose of 50 mg/kg significantly enhanced the antiseizure activity of clonazepam (CZP) and ethosuximide (ETS), but not that of phenobarbital (PB), valproate (VPA), tiagabine (TGB) or gabapentin (GBP) in PTZ-induced seizures in mice [12, 13]. Additionally, 7-NI potentiated the anti-electroshock action of PB, phenytoin (PHT), VPA, oxcarbazepine (OXC), and loreclezole (LCZ), but not that of carbamazepine (CBZ), topiramate (TPM), lamotrigine (LTG) and felbamate (FBM) in mice [7, 8, 14, 15]. In DBA\2 mice, 7-NI enhanced the antiseizure effects of PB, diazepam (DZP), VPA, CBZ, and to a lesser extent, those of PHT and LTG against audiogenic seizures [6]. Neurochemical studies have revealed that 7-NI at a dose of 50 mg/kg i.p. enhanced the action of GBP against picrotoxin-induced seizures in rats [16].

Considering the fact that 7-NI enhanced the anticonvulsant action of some conventional antiepileptic drugs (AEDs) against PTZ-induced seizures in mice, it was of pivotal importance to

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assess the influence of 7-NI on the antiseizure properties of two newer AEDs: OXC and vigabatrin (VGB) in PTZ-induced clonic seizures in mice. Generally, it is accepted that PTZ-induced seizures are thought to be an experimental animal model of myoclonic seizures in humans [17]. The potential adverse-effect profiles of OXC and VGB co-administered with 7-NI were determined in the chimney test, which allows assessment of the acute adverse effects produced by AEDs administered alone or in combination, regarding their ability to impair motor coordination in experimental animals [18].

MATERIAL AND METHODS

Animals and experimental conditions. All experiments were performed on adult male Swiss mice weighing 22-26 g. The mice were kept in colony cages with free access to food and tap water, under standardized housing conditions (natural light-dark cycle, temperature 21±1ºC, relative humidity 55±5%). After 7 days of adaptation to laboratory conditions, the animals were randomly assigned to experimental groups of 8 mice. Each mouse was used only once. All tests were performed between 09:00-14:00. Procedures involving animals and their care conformed with current European Community and Polish legislation on animal experimentation. Additionally, all efforts were made to minimize animal suffering and to use only the number of animals necessary to produce reliable scientific data. The experimental protocols and procedures listed below conformed to the *Guide for the Care and Use of Laboratory Animals* and approved by the Local Ethics Committee at the Medical University in Lublin.

Drugs. The following drugs were used in this study: 7-NI (Sigma, St. Louis, MO, USA), OXC (Trileptal – Novartis Pharma AG, Basle, Switzerland), and VGB (Sabril – Marion Merrell SA, Puteaux, France). All drugs were suspended in a 1% aqueous solution of Tween 80 (Sigma, St. Louis, MO, USA) and administered intraperitoneally (i.p.) in a volume of 0.005 ml/g of body weight. Fresh drug solutions were prepared on each day of experimentation and administered as follows: OXC and 7-NI – at 30 min., and VGB – at 240 min., before PTZ-induced seizures and the chimney test. These pretreatment times were based on their biological activity from the literature and confirmed in our previous experiments [7, 8, 13, 19]. The times of peak maximum anticonvulsant effects for the drugs were used as reference times in all experimental tests. PTZ (Sigma, St. Louis, MO, USA) was dissolved in distilled water and administered subcutaneously (s.c.) into a loose fold of skin in the midline of the neck in a volume of 0.005 ml/g of body weight.

Pentylenetetrazole (PTZ)-induced convulsions. The anticonvulsant activities of OXC and VGB administered alone and combined with 7-NI against PTZ-induced clonic seizures determined after s.c. administration of PTZ at its CD_{97} (100 mg/kg). The animals were treated with increasing doses of the AEDs, and the anticonvulsant activity of each drug was evaluated as its ED_{50} value (protecting 50% of mice against PTZ-induced clonic convulsions). To determine the ED_{50} values for the studied AEDs, OXC was administered at doses ranging between 15-30 mg/kg, whereas VGB was administered at doses ranging between 400-800 mg/kg. At least 4 groups of animals (8 mice per group) were used to estimate each ED_{50} value for OXC and VGB, calculated from the respective dose-response curves, according to Litchfield and Wilcoxon [20]. This experimental procedure has been described in more detail in our earlier studies [13, 19, 21-25].

Chimney test. The effect of combination of 7-NI with OXC and VGB (at doses corresponding to their ED_{50} values from the PTZ test) on motor coordination impairment were quantified with the chimney test of Boissier et al. [26]. In this test, the animals had to climb backwards up a plastic tube (3 cm inner diameter, 25 cm length). Motor impairment was indicated by the inability of the animals to climb backward up the transparent tube within 60 s. Data were presented as a percentage of animals that failed to perform the chimney test. This experimental procedure has been described in more detail in our earlier studies [14, 19, 21-23, 27].

Statistical analysis. The ED_{50} values with their 95% confidence limits were calculated by computer log-probit analysis according to Litchfield and Wilcoxon [20]. Qualitative variables from the chimney test were compared with Fisher's exact probability test. Differences among values were considered statistically significant if $P<0.05$.

RESULTS

Influence of 7-NI on the anticonvulsant activity of OXC and VGB against PTZ-induced clonic seizures. OXC and VGB administered singly displayed a definite anticonvulsant effect against PTZ-induced clonic seizures in mice (ED_{50} values presented in Table 1). The preferential NOS inhibitor 7-NI administered systemically (i.p.) at a dose of 50 mg/kg had no significant impact on the antiseizure activity of OXC in the PTZ test; the ED_{50} value for OXC in combination with 7-NI was 24.8 (21.9-28.2) mg/kg (Table 1). Similarly, 7-NI at a dose of 50 mg/kg did not significantly affect the antiseizure activity of VGB in the PTZ test; the ED_{50} value for VGB in combination with 7-NI was 622 (483-802) mg/kg (Table 1).

Table 1 Effect of 7-nitroindazole (7-NI) on anticonvulsant activity of oxcarbazepine (OXC) and vigabatrin (VGB) against pentylenetetrazole (PTZ)-induced clonic seizures in mice.

Treatment (mg/kg)	ED_{50} (mg/kg)	N	SE
OXC + vehicle	20.9 (17.9-24.4)	16	1.65
OXC + 7-NI (50)	24.8 (21.9-28.2)	32	1.61
VGB + vehicle	595 (458-774)	32	79.75
VGB + 7-NI (50)	622 (483-802)	32	80.54

Data are presented as median effective doses (ED_{50} values in mg/kg, with 95% confidence limits in parentheses) of OXC and VGB that protected 50% of animals against PTZ-induced clonic seizures. OXC was administered i.p. at 30 min.; VGB was given i.p. at 240 min. prior to the PTZ test. The PTZ-induced seizures were produced by s.c.-injection of PTZ at its CD_{97} (100 mg/kg). Statistical evaluation of data was performed with log-probit method according to Litchfield and Wilcoxon [20].
N – total number of animals used at those doses whose expected anticonvulsant effects ranged between 4 and 6 probits;
SE – standard error of ED_{50}.

Influence of 7-NI in combination with OXC and VGB on motor performance in the chimney test. The combination of 7-NI (50 mg/kg) with OXC (24.8 mg/kg) and VGB (622 mg/kg), both AEDs at doses being their ED_{50} values from the PTZ test, did not significantly impair motor coordination in animals subjected to the chimney test (results not shown).

DISCUSSION

Results presented in this study indicate that 7-NI administered i.p. at a dose of 50 mg/kg did not significantly alter the anticonvulsant action of OXC or VGB against PTZ-induced clonic seizures in mice. However, it was documented that 7-NI slightly reduced the antiseizure action of OXC and VGB by increasing their ED_{50} values against PTZ-induced seizures in mice, although statistical analysis of data did not reach significance. Thus, our findings are in agreement with those documented earlier, that 7-NI at the dose of 50 mg/kg did not affect the anticonvulsant activity of GBP and TGB against PTZ-induced clonic seizures in mice [13]. On the other hand, the same results are in contrast to those showing that N^G-nitro-L-arginine (NNA – a non-selective NOS inhibitor) applied i.p. at a dose of 40 mg/kg significantly attenuated the anticonvulsant action of OXC and VGB against PTZ-induced seizures in mice [19]. Moreover, it has been documented that NNA (administered i.p. at a dose of 40 mg/kg) attenuated the anticonvulsant action of ETS, but not that of CZP, PB or VPA in the PTZ test in mice [28]. In contrast, 7-NI (50 mg/kg) enhanced the antiseizure action of ETS and CZP, but not that of PB or VPA against PTZ-induced clonic seizures in mice [12]. Thus, considering the above-mentioned results one can ascertain that the apparent discrepancy between the anticonvulsant effects observed for the same AEDs in combination with various NOS inhibitors (NNA or 7-NI) resulted from these NOS inhibitors used experimentally. At present, it is difficult to elucidate the exact role of NO in seizure phenomena and its influence on the anticonvulsant action of conventional and newer AEDs in mice subjected to the PTZ-induced clonic seizures. Considering the fact that 7-NI is the preferential neuronal NOS inhibitor, one can ascertain that the reduction in NO content in the brain evoked by the application of 7-NI, slightly attenuated the anticonvulsant action of OXC and VGB. Thus, it might be hypothesized that NO could possess anticonvulsant properties in the PTZ test.

As already mentioned in the Introduction, 7-NI potentiated the anticonvulsant effects of LCZ, OXC, PB, PHT and VPA in the MES test in mice [7, 8, 14], as well as it enhanced the antiseizure effects of CZP and ETS in the PTZ test in mice [12]. In contrast, 7-NI did not affect the antiseizure activities of CBZ in the MES test, and had no impact on the antiseizure action of PB and VPA in the PTZ-induced seizures in mice [8, 12]. In the case of OXC, the drug synergistically interacted with GBP and exerted additive interaction with TGB in the PTZ test in mice [22]. With respect to VGB, the drug synergistically interacted with ETS, PB and TGB [23, 24, 29], as well as interacted additively with CZP and VPA in the PTZ test in mice [23].

Recently, the suggestion has been made that 7-NI is able to produce by itself the antiseizure effects in experimental models of epilepsy in rodents, and these effects seem to be independent on 7-NI-induced modulation of NO content in the brain [6-12]. If such is the case, the evaluation of the role of NO in seizure phenomena after pretreatment with 7-NI and newer AEDs might reflect not only the modulation of NO content in the brain, but also a direct antiseizure action exerted by 7-NI on PTZ-induced seizures in mice. Detailed discussion concerning the role of 7-NI in seizure phenomena and its influence on the antiseizure potential of conventional and newer AEDs has been presented elsewhere [4, 7-9].

The evaluation of acute adverse-effect potential for the combination of OXC and VGB with 7-NI revealed that the AEDs, 7-NI and their combination at doses from the PTZ test, had no impact on motor performance in the experimental animals.

Based on this preclinical study, one can ascertain that 7-NI had no impact on the antiseizure effects of OXC and VGB in the PTZ test, although a slight increase in the ED_{50} values of both AEDs against PTZ-induced seizures in mice was observed in this study. It seems that 7-NI was neutral when combined with OXC and VGB; therefore, one can indirectly ascertain that the modulation of NO content in the brain has no impact on the antiseizure properties of OXC and VGB in experimental animals.

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