THE EFFECT OF 7-NITRO INDAZOLE ON STRYCHNINE AND PENTYLENETETRAZOLE-INDUCED SEIZURES IN MICE*

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FARELERDE PENTİLENTETRAZOL VE STRİKNİN İLE OLUŞTURULAN KONVÜLSİYONLAR ÜZERİNDE 7- NİTRO İNDAZOLÜN ETKİSİ

ÖZET

Nitrik oksidin (NO) santral fizyolojik olaylarda, hücreler arası bir haberci olarak rolü olduğuna dair çeşitli kanıtlar mevcuttur. Çeşitli deneysel konvülsiyon modellerinde nitrik oksid etkisiz, prokonvülsan veya antikonvülsan olarak bildirilmektedir. Bu çalışmada beyindeki nitrik oksid sentaz (NOS) tipine spesifik bir inhibitör olan 7-nitro indazolün (7-Nİ), striknin (ST) ve pentilentetrazol (PTZ) konvülsiyonları üzerindeki etkisini araştırmayı planladık.

Subkütan (SK) 1.5 mg/kg ST veya 85 mg/kg PTZ verilerek; ilk miyoklonik spazm (İMS), toplam konvülsiyon süresi, ölüm zamanı ve ölüm oranları saptandı. 7 Nİ, AO veya serum fizyolojik (0.1 ml/ 25gr) ST veya PTZ uygulamasından 30 dk önce intraperitoneal olarak uygulandı. 7-Nİ intraperitoneal (i.p.) kullanım için yerfıstığı yağında (arachis oil, AO; peanut oil) çözüldü.

7-Nİ (30 mg/kg) her iki tip konvülsiyon modelindeki tüm parametreler üzerinde etkisiz bulundu. Ancak AO ve SF alan fareler karşılaştırıldığında AO, ST gruplarında tüm parametreleri, PTZ gruplarında ise sadece İMS'ı anlamlı olarak uzattı ($p < 0.05$). Bu sonuç muhtemelen i.p. verilen yerfıstığı yağının, daha sonra SK. verilen ST ve PTZ'ün absorpsiyon ve/veya dağılım özelliklerini etkilemiş olabileceğini akla getirmektedir. Sonuç olarak çalışmamızda kullanılan doz ve doz aralığında, 7-Nİ bu tip konvülsiyonlar üzerinde etkisiz görülmektedir.

***Anahtar Kelimeler**: Nitrik oksid, 7-nitro indazol, striknin, pentilentetrazol*

SUMMARY

There are some strong evidences about the role of nitric oxide (NO) as an intercellular messenger in the central physiological mechanisms. It is suggested that NO might be anticonvulsant, proconvulsant or ineffective on seizure activity in various experimental seizure models. In this study, the effects of 7-nitro indazole (7-NI), a selective inhibitor for the form of nitric oxide syntase enzyme found in the brain, on strychinine (ST) and pentylenetetrazole (PTZ) induced seizures in mice were investigated. ST (1.5 mg/kg) or PTZ (85 mg/kg) were administered subcutaneously (s.c.) to produce seizures. 7-NI, vehicle or saline (0.1 ml/25g, i.p.) were given 30 minutes prior to ST or PTZ to separate mice groups. 7-NI (30 mg/kg) was dissolved in arachis oil (AO; peanut oil) for intraperitoneal (i.p.) administration.

The first myoclonic jerk (FMJ), the total convulsion time (TCT), the survival time, and the rate of mortality were recorded. 7-NI had no effect on all parameters, both in ST and PTZ induced seizures. Interestingly AO compared to saline controls significantly delayed FMJ and survival time; increased TCT; decreased the rate of mortality induced by ST ($p < 0.05$). AO also significantly delayed FMJ induced by PTZ compared to saline controls. It is very possible that the i.p. injection of AO could have altered absorption and/or distribution of the subsequent s.c. injection of ST or PTZ. These observations suggested that 7-NI, at dose and time intervals used in this study, had no effect on seizure activity.

***Key Words**: Nitric oxide, 7-nitro indazole,* strychinine, pentylenetetrazole

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Although multipl mechanisms are known to be involved, the pathophysiology of epilepsy is not fully understood for any of the seizure type described. No single seizure type is explained by a single mechanism. One of the mechanisms involved in seizure termination might be the release of endogenous compounds such as nitric oxide (NO) (1) which has been proposed to modulate synaptic transmission (2,3) by activation of guanylate cyclase which leads to an increase in cyclic guanosine monophosphate (cGMP). Stimulation of N-methyl-D-aspartate (NMDA) receptors in brain causes seizures, releases NO, and leads to an increase in cGMP (4). The increase in cGMP is enhanced by L-arginine, a nitric oxide precursor, and blocked by nitric oxide syntase (NOS) inhibitors.

Strychinine (ST), a noncompetitive inhibitor of the glycine receptors, may exert its effect by blocking the inhibitory pathway of the Renshaw cells over the motor cells in the spinal cord resulting in increased stimulation by spinal reflexes of motor nerves (5). Pentylenetetrazole (PTZ) blocks γ-aminobutyric acid (GABA) mediated inhibition but other mechanisms may be also involved in PTZ induced seizures (6). In present study, the effects of 7-NI, a selective inhibitor of neuronal NOS, on ST and PTZ induced seizures in mice were investigated.

MATERIAL AND METHOD

Male Balb/c albino mice weighing 25-33 g were used. The animals were housed under standard laboratory conditions. They were allowed food and water ad libitum and kept in a room with 12 h light-dark cycle (21-24 ^{0}C). Ethical approval was granted by the Kocaeli University of Ethics Committee (Kocaeli, Turkey).

Experiments were performed on the mice having ability to remain at least 3 minutes on rotarod at the speed of 12 r.p.m. Mice were placed in plastic cylindrical cages to observe full view of the animal's motor responses to the seizures. Animals were randomly divided into control (saline + ST, n=17; AO + ST, n=18 or saline + PTZ, n=17; AO + PTZ, n=16) and 7-NI

Table 1. Effects of 7-NI (30 mg/kg,IP) and AO (0.1 ml/25 g,IP) on various parameters of ST (1.5 mg/kg,s.c.) and PTZ (85 mg/kg,s.c.) induced seizures (Mean ± S.E.M.).

	n	First Myoclonic Jerk (sec)	Total Convulsion Time (sec)	The Survival Time (sec)	The Rate of Mortality (%)
Saline + ST	17	193.9 ± 12.7	44.5 ± 9.4	277.6 ± 26.8	100
AO + ST	18	266.1 ± 13.8*	154.1± 50.8*	461.2 ± 68.3*	66.6*
7-NI + ST	15	254.8 ± 36.1	61.8 ± 19.5	406.6 ± 83.9	86.6
Saline + PTZ	17	97.5 ± 11.2	66.4 ± 8.1	509.6 ± 100.1	70.6
AO + PTZ	16	215 ± 16.8†	59.5 ± 10.8	691 ± 127.2	50
7-NI + PTZ	15	179.3 ± 36.5	41.3 ± 7.6	471.7 ± 90.6	80

* $p < 0.05$ compared to ST saline
† $p < 0.01$ compared to PTZ saline

groups (7-NI + ST, n=15 and 7-NI + PTZ, n=15). Four parameters of seizure severity were recorded: the first myoclonic jerk (FMJ, sec); the total convulsion time (TCT, sec); the survival time (sec); and the rate of mortality (%). Observation period was limited to 30 minutes. The experiments were carried out 10:00-12:00 a.m. in darkish and quiet laboratory conditions and in a blind manner. No animal was tested more than once.

Drugs and Chemicals: ST (1.5 mg/kg; Sigma, St Louis, USA) and PTZ (85 mg/kg; Sigma) were dissolved in saline and administered s.c. to produce seizure 30 min after 7-NI, AO or saline. 7-NI (30 mg/kg; Research Biochemicals, Natick, Mass.), a selective inhibitor of neuronal NOS, was dissolved in AO (peanut oil; Sigma) by sonication for i.p. administration. Injections were done in a volume of 0.1 ml/25g and at the same region of the body for each animal. Drugs were prepared fresh on the morning of each experiment. The dose and time intervals chosen was adapted from other studies that have examined the effect of 7-NI on the level of NOS inhibition (7), on antinociception (8), and on seizures (9,10) in vivo.

Statistics: Statistical comparisons were performed by using the Mann-Whitney U test and the difference between percents by Fisher's exact probability test.

RESULTS

The effects of 7-NI on seizures induced by ST and PTZ are shown in Table 1. Our findings suggest that, inhibition of NOS by 7-NI had no effect on all parameters investigated in ST or PTZ induced seizures in the dose and time interval used in this study. Interestingly AO (the vehicle for 7-NI) significantly delayed FMJ and survival time; increased TCT; decreased the rate of mortality in ST induced seizures ($p < 0.05$) compared to saline controls. It also significantly delayed FMJ in PTZ induced seizures ($p < 0.01$). AO is largely used in many edible products, including shortenings, margarines, and mayonnaise, as a cooking and frying oil, and a salad oil. No data was available on direct effect of the oil on central nervous system. But it also shortened the sleeping time in pentobarbital sleep (Erden et al., unpublished data). These surprising effects may be attributed to the alterating of absorption and/or distribution of the subsequent injections of PTZ or ST by AO. Although 7-NI increased the rate of mortality in ST or PTZ induced seizures, these results were statistically insignificant.

DISCUSSION

The discrepancies exist on the role of NO in various experimental seizure models. There is a complex participation of NO in central nervous system and it might play an anticonvulsant role, a proconvulsant role or no role in some cases. Literature findings suggest that NO and/or cGMP could modulate some ionic conductances which may result in an antiepileptic effect. NO produced in response to NMDA receptor activation leads to an enhancement of cGMP levels which induces the seizure activity termination (1). Inhibition of NOS by Nω-nitro-L-arginine methyl ester (L-NAME) increased the duration of NMDA induced seizures. It is reported that Nω-nitro-L-arginine (L-NA), a potent NOS inhibitor, enhanced seizure activity and increased mortality in kainate-treated mice, but 7-NI had no effect on this seizures (11). Therefore NO might be an endogenous anticonvulsant substance and NO donors may exert anticonvulsant activity by releasing NO at the cerebral levels. But the results in the present study disagree with these findings.

In some other studies, NO appears to contribute to the genesis of seizure activity. The facilitatory effects of L-arginine on seizure activity elicited by microinjection of both NMDA and kainate into the deep prepiriform cortex were dose-dependent and could be prevented by L-NAME (12,13). In other study, it has been shown that L-arginine possesses a proconvulsant effect and potentiates seizures induced by NMDA (14). Inhibition of NOS suppresses tonic-clonic seizures induced by PTZ (15). Moreover 7-NI attenuated the convulsions induced by kainic acid (16). Our results are in accordance with the previous findings that no remarkable enhancement of seizures was seen as a consequence of inhibiting NOS (17). NOS inhibitors, L-NAME and 7-NI, and a guanylate cyclase inhibitor methylene blue had no significant effect on the dentate activation seizure model (10). This suggests that an endogenous increase in cGMP is not involved in seizure termination in this model.

An explanation for our results is the possibility that 7-NI could not reach the target sites in high enough concentrations to be effective. Before any effect on seizure duration is evident, NO inhibition must exceed 95 % is postulated (18). 7-NI has been reported to cause an 80 % reduction in NOS activity after systemic administration (7).

The differences between these results may be attributed to the significant species difference, mode of seizure induction, different seizure termination mechanisms. The result of this study leads us to conclude that NO may not actively be involved in ST or PTZ induced seizure activity.

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