Mechanisms of Kindling Induced by Norbornan Intoxication

A. I. Golovko, M. B. Ivanov, O. A. Sviderskii, G. A. Sofronov, and V. V. Shilov

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 125, No. 6, pp. 653-656, June, 1998
Original article submitted May 28, 1997

Single injection of norbornan induced kindling by disordering postsynaptic GABAergic structures. Modulation of $GABA_A$ receptor chlorine ionic channels is most crucial, while disturbances in postsynaptic low-affinity $GABA_A$ receptors are less important for this phenomenon. It is unlikely that $GABA_B$, dopamine, and muscarinic neurotransmitter structures are involved into this process.

Key Words: *norbornan; kindling*

The development of convulsive syndrome is an important problem of modern neurobiology [4,6,9]. Understanding of the mechanisms of convulsions will provide the clue for creating new effective anti-convulsant drugs. Norbornan (NB, exo,cis-5,6-dichloro-2,2-dicyano-3,3-bis-(trifluoromethyl)-norbornane; bicycloheptane) is a useful tool to study the mechanisms of kindling [1,3]. Norbornan is a convulsant, an irreversible blocker of $GABA_A$ receptor chlorine channels [2,3]. It has been previously demonstrated that single injection of NB to albino mice led to sustained sensitization to picrotoxin and 3-mercaptopropionic acid toxicity [2].

Mechanisms by which single injection of NB causes kindling and the role of transmitter interaction in this phenomenon remain poorly understood were the subject of this study.

MATERIALS AND METHODS

Experiments were carried out on male albino mice weighing 22-25 g. Norbornan was dissolved in 50% dimethyl sulfoxide. Picrotoxin and haloperidol (both from Sigma) were suspended in physiological saline using Tween-80. Baclofen, muscimol, and oxotremorine (all from Sigma) were dissolved in physiological saline. Bicuculline (Sigma) were dissolved in 0.1 N HCl and adjusted to pH 5.5-6.0 with 1 N NaOH [12].

The drugs were injected intraperitoneally in a volume of 0.2 ml/10 g body weight (NB in a volume of 0.1 ml/10 g). Convulsant activity was assessed as described previously [5] by the latency of grade I-II and III-IV seizures and animals death. LD_{50} was calculated using the method of probit-analysis. The data were processed using the Student *t* test.

RESULTS

We previously demonstrated that single injection of NB in a dose equal to LD_{16} (0.095 mg/kg) to albino mice increases their sensitivity to picrotoxin and 3-mercaptopropionic acid toxicity [2]. These data led us to a conclusion on the crucial role of postsynaptic $GABA_A$-ergic structures in this phenomenon.

Since $GABA_A$ receptor is a complex multifunctional structure [8,10], it seems reasonable to evaluate the relationships between alterations in its components and the formation of kindling after single injection of NB. To this end, we studied convulsant activity of picrotoxin ($GABA_A$ receptor chlorine channel blocker) and bicuculline (antagonist of low-affinity $GABA_A$ receptors) 24 h after injection of NB in a dose equal to LD_{16} (Table 1).

Department of Military Toxicology and Medical Protections, Laboratory of Perfluorocarbons, Military Medical Academy, St. Petersburg

0007-4888/98/0006-0579$20.00

TABLE 1. Effect of Picrotoxin and Bicuculline against the Background of Single NB Injection (LD_{16}, 24 h before Experiment) in Albino Mice ($X \pm m$)

Agent	Control, mg/g	Effective doses (ED), mg/kg		Dose ratio		
				lethal doses	effective convulsant doses	
		grade I-II seizures	grade III-IV seizures		grade I-II seizures	grade III-IV seizures
				LD_{50} control/ LD_{50} experiment	ED_{50} control/ ED_{50} experiment	ED_{50} control/ ED_{50} experiment
Picrotoxin	6.27±0.63	4.19±0.62	5.37±0.58	2.00±0.35	3.77±0.57*	3.89±0.56**
Bicuculline	4.82±0.48	4.33±0.44	4.69±0.29	1.70±0.18	2.14±0.36	1.86±0.23

Note. *$p<0.02$, **$p<0.001$ compared with bicuculline-treated mice.

Single injection of NB considerably enhanced GABAlytic toxicity and kindling in experimental animals. The ratio between effective convulsant dose in the control to that in the experiment was significantly higher for picrotoxin than for the low-affinity $GABA_A$ ligand bicuculline [11]. These data suggest that the formation of kindling is associated with ionic channel alterations rather than with $GABA_A$ receptor changes.

In further experiments LD_{16} NB was injected 6 h prior to picrotoxin (4 mg/kg). This dose of picrotoxin induced grade I-II seizures in 20-40% mice and caused no grade III-IV seizures. In this experimental series we studied the role of different transmitter systems in the formation of kindling: muscimol and baclofen were used as $GABA_A$- and $GABA_B$-receptor probes, respectively; the contribution of dopaminergic and muscarinic systems to NB toxicity was evaluated using haloperidol and oxotremorin, respectively. We choose oxotremorine, since this drug similarly to other choline-positive agents reduces picrotoxin toxicity (Tables 2 and 3) [7,13].

Our experiments showed that baclofen had no effects on the latency of picrotoxin-induced seizures in experimental mice, while muscimol attenuated seizures but did not prevent animal death. These findings indicate a close involvement of $GABA_A$ergic transmitter systems into the formation of picrotoxin-induced kindling in animals pretreated with NB. As seen from Table 3, haloperidol and oxotremorine had no effect on picrotoxin toxicity in NB-preinjected mice, which argues against the involvement of dopamine and muscarinic neurotransmitter systems into this phenomenon.

Thus, kindling in albino mice induced by single injection of NB is probably associated with functional changes in the GABA/benzodiazepine/ionophore complex, changes in the ionophore being more im-

TABLE 2. Effects of Muscimol and Baclofen on Picrotoxin (4 mg/kg) Toxicity in Mice Injected with LD_{16} NB (0.095 mg/kg) ($M \pm m$)

Conditions	Latency of intoxication symptoms, min			Dead mice/total number per group
	grade I-II seizures	grade III-IV seizures	death	
Control 1(picrotoxin)	16.5±1.5 (2)	—	—	0/8
NB 6 h before+picrotoxin	6.6±0.7 (8)	13.5±2.5 (8)	20.0±3.6	7/8
Control 2 (muscimol, 1 mg/kg, 10 min prior to picrotoxin)	32.0±0.0 (1)	—	—	0/8
NB 6 h before+muscimol, 1 mg/kg, 10 min prior to picrotoxin	13.6±1.2** (10)	23.9±2.7* (10)	26.3±3.0	10/10
Control 1(picrotoxin)	23.7±3.7 (3)	—	—	0/8
NB 6 h before+picrotoxin	7.0±0.5 (10)	14.3±2.8 (7)	19.3±1.5	7/10
Control 2 (baclofen, 5 mg/kg, 10 min prior to picrotoxin)	28.0±5.5 (3)	—	—	0/8
NB 6 h before+baclofen, 5 mg/kg, 10 min prior to picrotoxin	8.2±0.8 (10)	18.8±3.2 (10)	22.5±2.7	10/10

Note. Here and in Table 3: *$p<0.02$, **$p<0.001$ compared with NB- and picrotoxin-treated mice.

TABLE 3. Effects of Haloperidol and Oxotremorine on Picrotoxin (4 mg/kg) Toxicity in Mice Injected with LD_{16} NB (0.095 mg/kg) (*M*±*m*)

Conditions	Latency of intoxication symptoms, min			Dead mice/total number per group
	grade I-II seizures	grade III-IV seizures	death	
Control 1 (picrotoxin)	26.0±4.0 (3)	—	—	0/8
NB 6 h before+picrotoxin	10.9±1.1 (12)	21.3±2.7 (11)	24.1±3.1	10/12
Control 2 (haloperidol, 0.5 mg/kg, 30 min prior to picrotoxin)	20.7±2.5 (3)	—	—	0/8
NB 6 h before+haloperidol, 0.5 mg/kg, 30 min prior to picrotoxin	9.4±1.0 (11)	18.7±2.6 (11)	23.1±2.2	10/11
Control 1(picrotoxin)	18.0±3.5 (3)	—	—	0/8
NB 6 h before+picrotoxin	6.6±0.7 (9)	15.3±2.8 (9)	21.3±1.5	8/9
Control 2 (oxotremorine, 75 mg/kg, 10 min prior to picrotoxin)	23.1±1.0 (2)	—	—	0/6
NB 6 h before+oxotremorine, 75 mg/kg, 10 min prior to picrotoxin	7.9±1.0 (7)	12.6±2.6 (7)	18.7±1.7	7/8

portant for this phenomenon than dysfunction of low-affinity $GABA_A$ receptors. It is unlikely that $GABA_B$, dopamine, and M-cholinergic neuro-transmitter structures are involved into this phenomenon.

REFERENCES

1. V. D. Gladkikh, N. A. Kolosova, and N. A. Loshadkin, *Toksikol. Vestn.*, No. 2, 10-14 (1995).
2. A. I. Golovko, M. B. Ivanov, T. V. Klyuntina, *et al., Byull. Eksp. Biol. Med.*, **123**, No. 1, 60-62 (1997).
3. A. I. Golovko, G. A. Sofronov, and T. V. Klyuntina, *Ibid.*, **121**, No. 4, 444-446 (1996).
4. G. N. Kryzhanovskii, M. N. Karpova, and O. Yu. Pankov, *Ibid.*, **111**, No. 4, 351-353 (1991).
5. G. N. Kryzhanovskii, A. A. Shandra, R. F. Makul'kin, and L. S. Godlevskii, *Ibid.*, **99**, No. 5, 527-532 (1985).
6. V. B. Prozorovskii and G. T. Frumin, *Farmakol. Toksikol.*, **54**, No. 1, 69-70 (1991).
7. V. P. Georgiev and N. D. Lambadjieva, *Neuropharmacology*, **20**, No. 9, 813-817 (1981).
8. E. R. Korpi, T. Kuner, and P. H. Seeburg, *Mol. Pharmacol.*, **47**, No. 2, 283-289 (1995).
9. C. J. Peterson, S. Vinayak, A. Pazos, and K. Gale, *Eur. J. Pharmacol.*, **221**, No. 1, 151-155 (1992).
10. E. Sanna, F. Garau, and R. A. Harris, *Mol. Pharmacol.*, **49**, No. 2, 213-217 (1995).
11. N. A. Sharif, *Int, Rev. Neurobiol.*, **26**, 85-150 (1985).
12. T. E. Sokrab, H. Kalimo, and B. B. Johansson, *Brain Res.*, **489**, No. 2, 231-236 (1989).
13. B. Sutor, J. J. Hablitz, *Brain Res. Dev. Brain Res.*, **46**, No. 1, 155-160 (1989).