GENERAL PATHOLOGY AND PATHOLOGICAL PHYSIOLOGY

Norbornane-Induced Changes in the Density of Chloride-Ion Channels in the Brain of Rodents

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In this study on rats and mice, the clinical course of poisoning in rats injected with norbornane [2,2-di-(trifluoromethyl)-3,3-dicyano-5,6-dichloronorbornene] in a dose equal to 2 LD_{50} was characterized by slow progression of the convulsive syndrome. Radioligand analysis revealed reduced densities of chloride-ion channels in the striatum and cerebellum of rats and in the whole brain of mice after norbornane administration. Picrotoxin in equitoxic doses did not alter the density of these channels. It is concluded that norbornane causes irreversible inhibition of chloride-ion channels.

Key Words: *norbornane; convulsions; chloride-ion channels; acylation*

A large group of convulsive poisons comprises agents which block the chloride-ion channels of $GABA_A$ receptors and which include picrotoxin, tetramethylenedisulfotetramine (TETS), 2,6,7-trioxabicyclo[2,2,2]octanes (bicyclophosphates, bicicloorthobenzoates, bicycloorthocarboxylates, organochlorine pesticides (polychlorocycloalkanes), and pharmaceutical preparations (penicillin, chlorpromazine, furosemide, and a number of others) [5,6,8]. A unique activity — irreversible inhibition of chloride-ion channels — is displayed by certain tert-butylbicycloorthobenzoate (TBOB) derivatives [7,9,10,13]. These compounds, referred to as acylating ligands, attract the close attention of those whose research interests extend to the GABA-benzodiazepine-ionophore complex viewed as a hypothetical entity with ill-defined morphofunctional characteristics [12].

In this *ex vivo* study an attempt was made to evaluate the reversibility of effects exerted by norbornane on the chloride-ion channels of $GABA_A$ receptors in the brains of rats and mice.

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MATERIALS AND METHODS

Rats (body weight 170-180 g) and mice (20-22 g) were used. Norbornane (NB) and picrotoxin (Serva) suspended in Tween-80-containing physiological saline were injected intraperitoneally. NB was injected in a dose of 0.35 mg/kg (which equals 2 LD_{50}) into rats and in a dose of 0.1, 0.4, or 0.5 mg/kg into mice; picrotoxin was administered only to mice in a dose of 0.5, 1.0, or 2.0 mg/kg. Convulsive activity was estimated as described by Kryzhanovskii *et al.* [3].

Radioligand analysis was performed as previously described [2,16], using ^{35}S-tert-butylbicyclophosphorothionate (NEN) (^{35}S-TBPS; 130 Ci/mmol, 2 nM) and ^{3}H-TBOB (Amersham) (31 Ci/mmol, 5 nM).

RESULTS

As shown in Fig. 1, NB and TETS were capable of blocking the specific ^{35}S-TBPS binding to synaptic membranes of whole rat brain (the IC_{50} was 3.8 nM for NB and 2.8 μM for TETS). These two agents

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may therefore be regarded as specific ligands of chloride-ion channels in $GABA_A$ receptors.

The intoxication in rats given NB at 2 LD_{50} was characterized by a relatively slow development of the convulsive syndrome (Fig. 2). Convulsive seizures were triggered by a loud noise or bright light. During interictal periods, the animals were adynamic and could be easily frightened. Convulsions progressively increased in intensity and ultimately led to the development of opisthotonos and to death approximately by the end of hour 6 after NB injection. This clinical course of NB poisoning differs from that observed with the "classical" chlorine-ion channel inhibitors, such as picrotoxin and 2,6,7-trioxabicyclo[2,2,2]octanes, which exert their toxic effects very rapidly [4,11,15]. Such differences are possibly due to the specific nature of NB interaction with the chloride-ion channel protein.

The density of chloride-ion channels in the brain of rats and mice at different times after NB and picrotoxin administration was evaluated using ^{3}H-TBOB (Tables 1 and 2). The recorded significant reduction of specific ^{3}H-TBOB in the striatum and cerebellum of rats given NB at 2 LD_{50} attests to irreversible inhibition of chloride-ion channels in $GABA_A$ receptors by this compound (Table 1). In the frontal cortex, the chloride-ion channel density showed little change, which may be a reflection of functional differences between chloride-ion channels of the frontal cortex and striatal and cerebellar channels. In our previous study [1], the convulsion-inducing activity of GABA-lytics also had little effect on the specific ^{35}S-TBPS binding in the frontal cortex while exerting marked effects in the striatum and cerebellum.

The systemically administered NB also caused irreversible inhibition of chloride-ion channels in the whole brain of mice (Table 2). The channel density was found to have decreased after NB

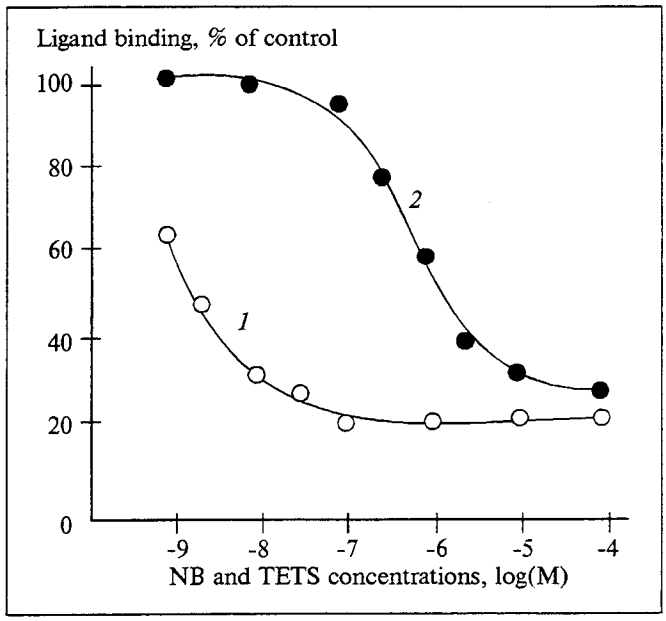

Fig. 1. Effects of NB (*1*) and TETS (*2*) on ^{35}S-TBPS (2 nM) binding with synaptic membranes of whole rat brain. Each value represents the results of three separate tests run in triple replicates.

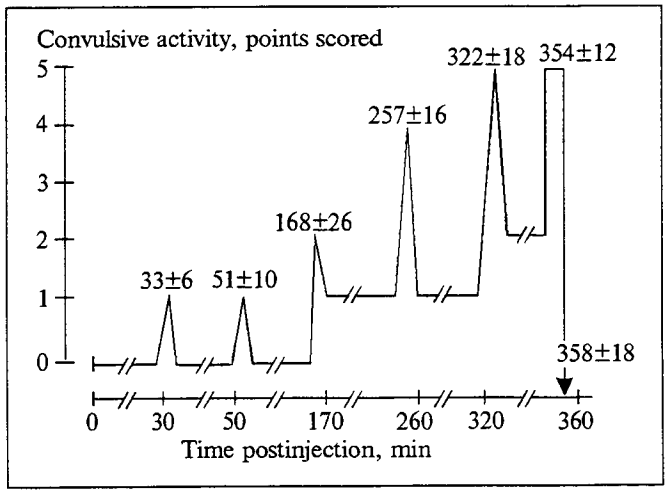

Fig. 2. Graphic representation of the time-course of the convulsive syndrome in rats after their injection with NB at 0.35 mg/kg (2 LD_{50}). Peaks correspond to the times of highest convulsive activity; figures above the peaks are the mean times (in min) of onset of convulsive seizures ($X \pm m$). The arrow marks the mean time of death.

TABLE 1. ^{3}H-TBOB Binding (% of Control Values) in Rats Injected with Norbornane (NB) at Two LD_{50}

Brain structure	Time after NB injection, min		
	15	120	onset of fourth-degree convulsive activity*
Frontal cortex	94±7	100±5	108±12
Striatum	86±4**	70±10**	82±8**
Cerebellum	93±8	75±15**	83±9**

Note. Radioligand concentration = 5 nM. *Fourth-degree convulsive activity signifies repeated clonic-tonic convulsions with the animal falling on its side [3], which in this case was not observed to occur before the 4th hour postinjection. **$p<0.05$ in comparison with the control group.

dose of 0.1 mg/kg which did not elicit convulsions during the observation period (30 min). After the doses of 0.4 and 0.5 mg/kg, some mice developed fourth-degree convulsive activity (repeated clonic-tonic convulsions causing the animal to fall on its side), and the chloride-ion channel density decreased by 49% after 0.4 mg/kg and by 73% after 0.5 mg/kg. These findings are consistent with the current view that convulsions in poisoning with GABA-lytics will not occur unless at least one half

TABLE 2. Effects of Norbornane and Picrotoxin on ^{3}H-TBOB (5 nM) Binding to Membranes of Whole Brain in Decerebellated Mice *Ex Vivo*

Parameter	Control, fmol/mg protein	Dose, mg/kg					
		norbornane			picrotoxin		
		0.1	0.4	0.5	0.5	1.0	2.0
Ligand binding, % of normal	144±11	72±12*	51±10*	27±13**	96±12	101±8	103±10

Note. *$p<0.01$, **$p<0.001$ in comparison with the control group. The poisons were injected intraperitoneally, and the mice were killed 30 min later. The norbornane doses of 0.4 and 0.5 mg/kg and the picrotoxin dose of 2 mg/kg led to fourth-degree convulsions in some mice.

of the chloride-ion channels have been blocked [5,14]. No specific ^{3}H-TBOB binding was in evidence after picrotoxin injection in equitoxic doses. Our results indicate that NB acylates the target receptors *in vivo*.

To summarize, NB exhibited high affinity for the chloride-ion channel protein in $GABA_A$ receptors of rat brain and, as indicated by radioligand analysis using ^{3}H-TBOB, reduced the density of chloride-ion channels in the striatum and cerebellum of rats and in the whole brain of mice, the reduction observed in mice being dose-dependent. The clinical picture of NB poisoning was characterized by slow progression of convulsions and severe clonic-tonic convulsions were developed by mice when no less than half of the chloride-ion channels had been blocked in their brain. Picrotoxin in equitoxic doses did not alter the density of these channels in mouse brain.

The results of the present study allow NB to be considered as an acylating ligand of chloride-ion channels in $GABA_A$ receptors, which possibly explains the observed slow development of convulsive syndrome in the NB-poisoned rodents.

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