Development of Increased Convulsibility in Mice After a Single Norbornan Injection

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 123, No. 1, pp. 60-62, January, 1997
Original article submitted September 28, 1995

A single intraperitoneal injection of norbornan markedly increases convulsant activities of picrotoxin and 3-mercaptopropionic acid in mice. Norbornan may be useful in the investigation of the mechanisms of convulsions.

Key Words: *norbornan; GABAlytics; receptors; convulsions*

The formation of increased convulsibility is the major pathogenic mechanism of epilepsy [4,13]. In experimental animals, this condition is reproduced by repeated injections of a convulsant [3,5,7]. However, the formation of kindling often requires several weeks [5], which complicates the research and makes it more expensive. The problem can be solved by the use of compounds inducing enhanced convulsibility after a single administration. In the present study we assessed the sensitivity of mice to the GABAlytics picrotoxin and 3-mercaptopropionic acid (3-MPA) after a single administration of the convulsant norbornan in LD_{16}.

MATERIALS AND METHODS

Experiments were carried out on male albino mice (body weight 22-25 g). Norbornan was dissolved in 50% dimethyl sulfoxide. Picrotoxin (Sigma) was suspended in physiological saline with Tween-80 (Sigma). 3-MPA (Sigma). Muscimol (Serva, 1 mg/kg), and baclofen (Sigma, 5 mg/kg) were dissolved in physiological saline. All the compounds were injected intraperitoneally. Convulsive activity was evaluated as previously [2]. The LD_{50} was calculated by probit-analysis.

Radioligand analysis. Washed synaptic membranes isolated from the cerebral cortex of intact male albino rats were incubated with norbornan, tetramethylenedisulfotetramine (TETS), picrotoxin, or tert-butylbicyclophosphate in concentrations of 16, 64, and 100 nM for 30 min at 0°C, and washed five times by centrifugation (20,000*g* for 15 min). The density of the chloride ionophore channels remaining in $GABA_A$ receptors was determined with the use of ^{3}H-tertbutylbicycloorthobenzoate (^{3}H-TBOB; Amersham, 29.5 Ci/mmol, 5 nM) [1]. An equivalent amount of the solvent (dimethyl sulfoxide) was added to each control sample. These samples were then treated in parallel with the test samples. Radioactivity was measured in a Rack-beta 1217-802 counter.

RESULTS

The GABAlytic norbornan increased the convulsibility of mice in response to 3-MPA (Table 1). The effect had an hour-long latency and persisted for at least 48 h.

LD_{50} of 3-MPA and picrotoxin against the background of norbornan was determined in a separate series of experiments. The toxicity of picrotoxin increased to a greater extent than that of 3-MPA: 236% *vs.* 118% 24 h after norbornan injection (Table 2). This difference is probably due to the different mechanisms of their toxic action: 3-MPA inhibits GABA synthesis in the brain [9], and picrotoxin blocks chloride channels of $GABA_A$ receptors [11].

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0007-4888/97/0001-0052$18.00

TABLE 1. Convulsive Activity Induced in Mice by 3-MPA (14 mg/kg) After Administration of Norbornan (LD_{16})

Experimental conditions	No. of mice in group	Convulsive activity, % of mice in group	
		1st-2nd grade	3rd-4th grade
3-MPA 30 min before norbornan	6	0	0
3-MPA together with norbornan	6	0	0
Norbornan+3-MPA after:			
30 min	7	0	0
1 h	13	31	0
2 h	21	29	67
4 h	6	17	83
6 h	6	0	100
8 h	11	0	100
24 h	19	16	84
48 h	15	40	0

Note. Convulsive activity was evaluated as described [2]. Convulsions were observed during the first 10-30 min after 3-MPA injection; intact mice given 14 mg/kg 3-MPA showed a moderate adynamia during the first 10-15 min postinjection and normal behavior thereafter. Norbornan was injected in a dose of 0.095 mg/kg (LD_{16}).

TABLE 2. Toxicity of GABAlytics (% of Control Values) in Mice as a Function of Time After a Single Injection of 0.095 mg/kg Norbornan (LD_{16})

Experimental conditions	Toxicity, %
3-MPA (control)	100
Norbornan+3-MPA after:	
1 h	108
6 h	169
24 h	218
48 h	96
Picrotoxin (control)	100
Norbornan+picrotoxin after:	
1 h	271
6 h	427
24 h	336
48 h	156

Note. LD_{50} for 3-MPA and picrotoxin in the control were 29.4±2.5 and 11.1±1.6 mg/kg, respectively.

The unusual effects of norbornan on the convulsant activity of 3-MPA and picrotoxin may be related to toxicokinetic and toxicodynamic characteristics of norbornan, specifically, irreversible binding to chloride channels. This hypothesis was supported by experiments with rat synaptic membranes. Synaptic membranes isolated from the brain cortex of intact rats were incubated with GABAlytics that inhibit chloride channels (tertbutylbicyclophosphate, tetramethylenedisulfotetramine, picrotoxin, or norbornan), washed 5 times, and then analyzed for the content of chloride channels in $GABA_A$ receptors. As Fig. 1 shows, only norbornan irreversibly inhibited chloride channels.

Most of the known acylating ligands blocking chloride channels of $GABA_A$ receptors are the derivatives of 2,6,7-trioxabicyclo[2,2,2]-octanes [6,8]. The neurochemical aspects of physiological activity of these compounds have been studied in detail [10, 12], whereas their toxicity, ability to modify pharmacologic activity of other substances, and behavioral effects, including changes in the susceptibility to convulsants, have not been assessed.

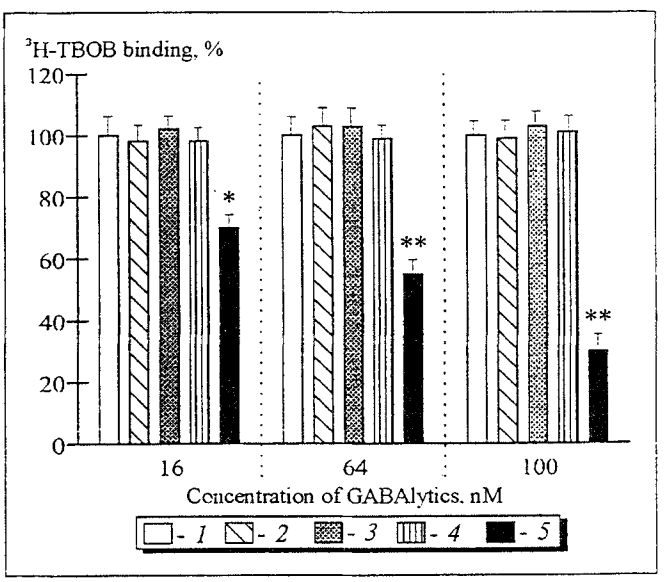

Fig. 1. Effect of preincubation of rat synaptic membranes with GABAlytics and the binding of ^{3}H-tertbutylbicycloorthobenzoate (^{3}H-TBOB, 5 nM) to chloride channels. The values are presented as percentage of the control level. *1*) control; *2*) tetramethylenedisulfotetramine (TETS); *3*) picrotoxin; *4*) tert-butylbicyclophosphate; *5*) norbornan. Ligand binding in the control samples was 163±7 fmol/mg protein. *$p<0.01$, **$p<0.001$ compared with the control.

Thus, mice given a single injection of norbornan in a dose equal to LD_{16} exhibited increased convulsibility in response to picrotoxin and 3-MPA. This phenomenon may be attributed to the irreversible inhibition of chloride channels of $GABA_A$ receptors by norbornan and dysfunction of GABAergic structures. Norbornan is a promising candidate for the use in the investigation of the mechanisms by which convulsions of various origins develop.

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