Delayed Effects of 1,2-Epoxypropyltrimethylammonium Chloride on Behavioral Reactions in Rats

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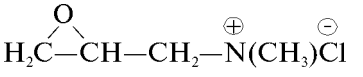
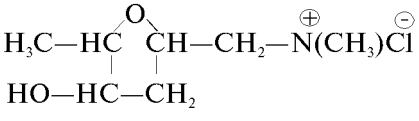
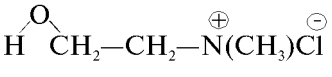
We studied immediate and delayed effects of intraventricular injection of 1,2-epoxypropyltrimethylammonium chloride on behavioral reactions in rats. Apomorphine-induced yawning increased and orientation and exploratory activity was improved 144 h postinjection, which indicates activation of the brain dopaminergic system during this period.

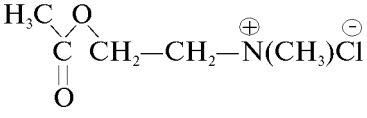
Key Words: *1,2-epoxypropyltrimethylammonium chloride; apomorphine; neurotoxins; animal behavior*

The use of synthetic neurotoxins, *e.g.* 1,2-epoxypropyltrimethylammonium chloride (EPTA), is a promising trend in studies of molecular mechanisms of brain function and a tool for modeling some CNS diseases [3,5,6,8]. Chemical structure of EPTA is similar to that of choline and cholinoreceptor agonists (acetylcholine and muscarine).


EPTA


Muscarine


Choline chloride


Acetylcholin chloride

It was hypothesized that EPTA carrying a highly reactive epoxy group can covalently and selectively modify proteins involved in acetylcholine reception and choline absorption by neurons from the extracellular space.

This hypothesis was confirmed experimentally: intraventricular injection of EPTA led to degenerative changes in some brain structures [7]. It was therefore interesting to find out how the development of degenerative (and at the next stage compensatory) processes induced by EPTA can modify integral animal behavior.

We studied immediate and delayed effects of a single intraventricular injection of EPTA on rat behavior.

MATERIALS AND METHODS

The study was carried out on male Wistar rats (100-160 g). The dose of EPTA was selected by injecting 2, 20, and 200 μg of the agent in 5 μl 0.9% NaCl into left cerebral ventricle III. Controls were injected with 5 μl 0.9% NaCl. Effect of EPTA in a dose of 20 μg on stereotyped behavior was studied on rat model of apomorphine-induced yawning [4]. To this end, experimental and control rats were subcutaneously injected with 25 μg/kg apomorphine simultaneously or after a certain period following intraventricular injection. The rats were put into a glass cylinder (18 cm in diameter), and after 5 min yawns were counted. The test was carried out for 40 min. Motor, orientation, and exploratory activities were evaluated in the open field test [2].

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The results were processed using Fisher precise test.

RESULTS

Slight tremor, general excitation, and intensification of spontaneous motor activity were observed 1.5 min after injection of 2 μg EPTA. One minute after injection of 20 μg EPTA severe tremor and convulsions were observed. Convulsions disappeared after 5-10 min and III degree catalepsy developed. Touch with a glass stick provoked vocalization and convulsive reaction. Five hours postinjection the animals did not apparently differ from controls, but high tactile sensitivity persisted for 6 days. In rats receiving 200 μg EPTA convulsions and tremor developed 1 min postinjection. After 20 min convulsions became weaker, the animals were motioless, touching induced backward or circular movements, convulsions, and tremor. These changes persisted for 5 h postinjection. Blood was detected in the urine and feces. Three of 9 rats died within 3 days.

Hence, injection of EPTA in doses of 2 and 20 μg into the lateral cerebral ventricle led to characteristic acute reactions: locomotor and postural disorders, convulsions of different severity, respiratory disorders, increased tactile sensitivity. All these disorders developed rapidly, were dose-dependent, and depended on the time elapsed after the injection. These pharmacological effects indicate that EPTA affects mainly the cholinergic systems of the brain. EPTA in a dose of 200 μg induced mainly nonspecific toxic effects. Therefore in further experiments, including studies of delayed effect of EPTA, we used this agent in a dose of 20 μg.

The effect of EPTA on other receptor systems of the brain was studied in the apomorphine-induced yawning test [4]. Preliminary experiments showed that unlike cholinomimetics (acetylcholine) and acetylcholinesterase inhibitors (physiostigmine), EPTA caused no changes in apomorphine-induced yawning 5 min, 24, 48, and 72 h after injection. Hence, EPTA produced no direct effects on the dopaminergic system and the effects of apomorphine and EPTA are not synergistic (Fig. 2).

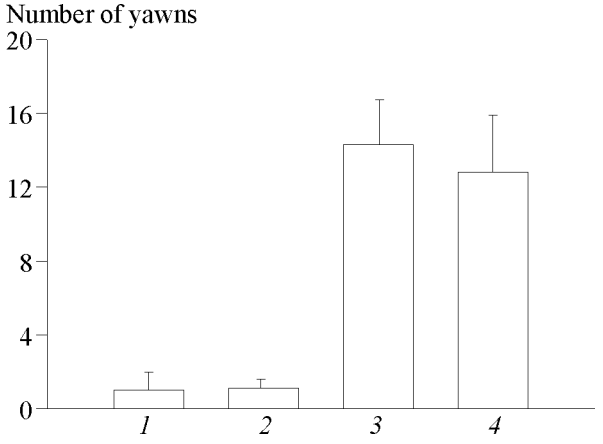

Fig. 1. Effect of EPTA and apomorphine on the yawning reaction in rats. *1*) control; *2*) EPTA (20 μg intraventricularly); *3*) apomorphine (25 μg/kg subcutaneously); *4*) EPTA+apomorphine.

These results and our previous data on high affinity of EPTA to cerebral muscarinic acetylcholine receptors [7] suggest that the acute toxic effects of low doses of EPTA are realized via structures involved in cholinergic transfer of nerve pulses.

Open field testing 72 h after intraventricular injection of 20 μg EPTA revealed no changes in the locomotor and exploratory activities (Table 1), while 144 h postinjection facilitation of the orientation and exploratory activity was observed. Both horizontal and vertical motor activities and the number

TABLE 1. Effect of Intraventricular Injection of EPTA (20 μg) on Open-Field Behavior in Rats

Parameter	Time postinjection, h			
	72		144	
	control (*n*=10)	experiment (*n*=10)	control (*n*=12)	experiment (*n*=12)
Motor activity, arb. units				
horizontal	81	68.4	61.5	98.7*
vertical	9.71	10.7	7.6	10.6*
Number of runs away from the wall	1.25	1.5	0.83	1.75*
Number of visits to the center	0.2	0.1	0.01	0.37*
Number of boluses	4.8	5.4	4.2	4
Number of urinations	0.6	0.8	0.3	0.5

Note. *$p<0.025$ compared to the control.

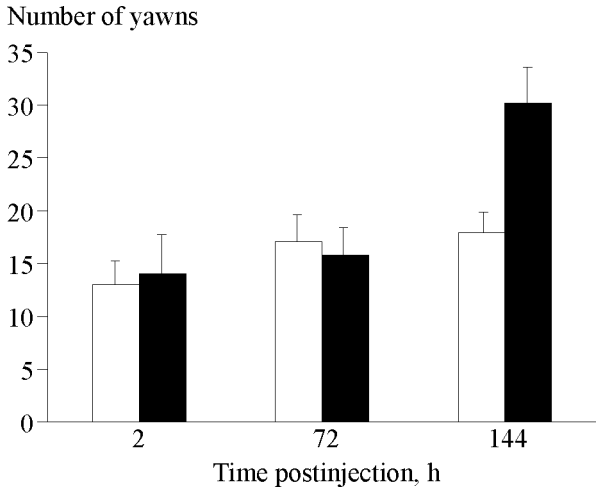

Fig. 2. Effect of intraventricular injection of EPTA (20 μg) on apomorphine-induced yawning in rats. Light bars: control; dark bars: experiment. *p<0.002 compared to the control.

of visits to the central zone markedly increased, which attested to an anxyolytic effect of EPTA. We previously showed that this pattern of open-field behavior can be attained by autostimulation through bipolar electrodes implanted into substantia nigra pars compacta [1] enriched with dopaminergic neurons.

On the other hand, 144 h after injection of EPTA the number of yawns induced by dopamine receptor agonist apomorphine increased 2-fold in comparison with the control (Fig. 3).

Hence, the effects observed 144 h after intraventricular injection of EPTA are realized via activation of the dopaminergic system. It seems that they do not result directly from destructive effect of EPTA on various brain formations, which was detected 48 h postinjection [5]. It is more likely that 6 days postinjection the compensatory processes provoked by destruction become more intensive and lead, among other things, to activation of the dopaminergic system in the brain.

These results substantiate the use of EPTA for modelling of CNS diseases associated with the development of degenerative processes in the system of cholinergic transfer of nerve pulses.

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