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Effects of Picamilon and Isopicamilon on the Formation of Picrotoxin-Induced Convulsive Activity in Rats

O. V. Denisenko,[1,2] O. A. Shandra,[1] L. M. Karpov,[2] and L. I. Siomik[2]

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Seizures were induced in rats using repetitive injections of picrotoxin, PTX (i.p., every 30 min in a dose of 0.9 mg/kg in the first injection and 0.7 mg/kg in subsequent injections). Picamilon (PM) and isopicamilon (IPM) in doses of 20 or 50 mg/kg were i.p. injected into animals 30 min prior to injection of PTX. Epileptiform activity (EFA), recorded from the cerebral structures under conditions of preliminary systemic injections of PM and IPM, could be divided into two types characterized by exclusive development of only spike-wave discharges, SWDs (61.3 %) and regular cortical spike activity with generation of separate short-lasting SWDs (38.7%). In rats with EFA of the first type, the frequency and duration of seizure SWDs decreased significantly after injections of PM and IPM in doses of 50 mg/kg. In rats with EFA of the second type, the intensity of SWDs decreased even after injections of these agents in smaller doses (20 mg/kg). The use of IPM as an agent with a protective anticonvulsive action was more effective.

Keywords: epileptiform activity (EFA), spike-wave discharges (SWDs), regular cortical spike discharges, picamilon (PM), isopicamilon (IPM).

INTRODUCTION

It was demonstrated that GABA-ergic drugs possess clear neuroprotective and therapeutic properties [1-4]. One of such preparations is picamilon, PM N-nicotinoyl-gamma-aminobutyric acid, sodium salt) [5]. Picamilon is widely used as an agent exerting positive cerebrovascular, nootropic, and tranquilizing effects [6, 7]. It is obvious that the mechanisms underlying realization of such effects of PM, its analogs, and derivatives capable of suppressing and discontinuing the spreading of epileptic activity due to modulation of the state of GABA-ergic system should be subjected to detailed investigation.

In experiments on rats, we studied the effects of PM and isopicamilon (IPM) on the formation of convulsive activity after repetitive injections of picrotoxin (PTX) in subconvulsive doses during several hours.

METHODS

Chronic experiments were carried out on 40 male albino rats (body mass 180-250 g). Preliminary operations were performed under complex anesthesia (70 mg/kg sodium thiopental + 7 mg/kg calipsol, i.p.). Monopolar nichrome electrodes in varnish isolation (tip diameter 0.10-0.15 mm) were stereotaxically implanted in the frontal cortex, ventral hippocampus, and mediodorsal thalamus according to the coordinates of the stereotaxic atlas [8]. Recording of electrical activity from these structures under conditions of injections of a convulsant (PTX) and the above-mentioned GABA mimetics and examination of behavioral phenomena were performed on the 7th day after preliminary surgery. Mass electrical activity of the above-listed cerebral structures was recorded under conditions of free behavior of rats within 60 min prior to injection of the convulsant and 5-6 h after such injection. We used a differential amplifier, DL304 (NeuroBioLab, Russia), connected to an ADC (L-154, L-KARD, Russia). Electrical activity was recorded and analyzed using a multichannel oscillograph, PowerGraph (National Instruments, USA).

Epileptiform activity (EFA) was induced by repeated i.p. injections of PTX (Sigma, USA) each 30 min in doses of 0.9 mg/kg in the first injection and 0.7 mg/kg in subsequent injections. The total dose of PTX received by rats in the experiment did not exceed 6.5 mg/kg. Thirty minutes before PTX injections, some of the animals were also i.p. injected with PM or IPM (Consortium–PIK, Russia) in doses of 20 mg/kg

[1] Odessa National Medical University, Ministry of Public Health of Ukraine, Odessa, Ukraine.
[2] Mechnikov Odessa National University, Odessa, Ukraine.
Correspondence should be addressed to O. V. Denisenko (ksenia_den@mail.ru)

(n = 16) or 50 mg/kg (n = 15). Animals of the control group (n = 9), were injected 30 min prior to PTX injection with 0.9% NaCl solution in a similar volume.

Principles of analysis of the frequency-amplitude characteristics of spike-wave discharges (SWDs) and their complexes, separate spike potentials, etc. were described earlier [3]. The intensity of convulsive activity in experimental rats was estimated visually using a 6-point scale [9].

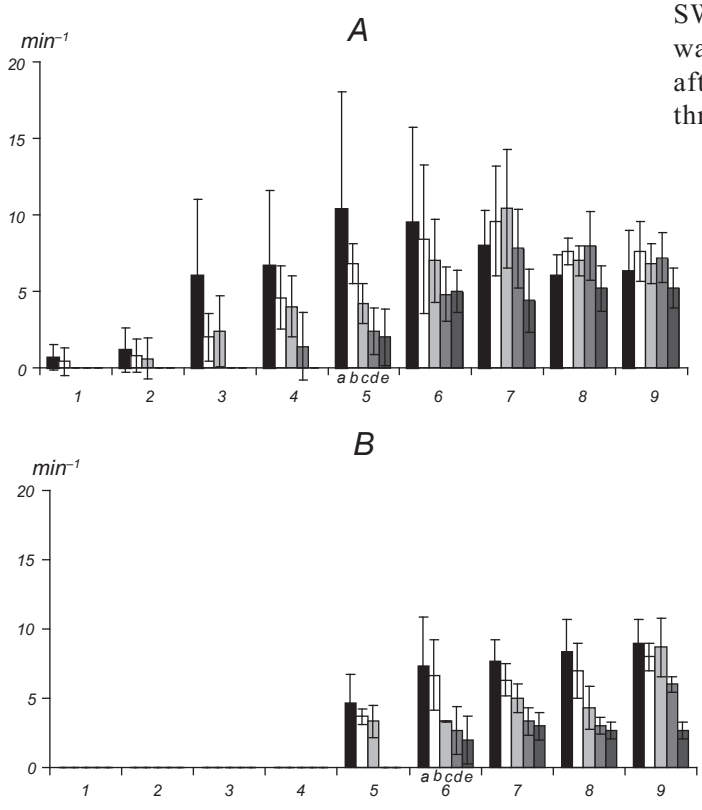
The data obtained were treated statistically using standard approaches. Intergroup differences were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

To induce EFA, the rats were repetitively injected each 30 min with PTX in doses that were initially insufficient to induce behavioral changes in most animals. After such injections of PTX in subconvulsive doses, EFA appeared; according to the peculiarities of this activity, we could divide the latter, using electrographic indices, into two types. The first type corresponded to the development of only SWDs (in 66.7% of animals), while regular cortical spike activity with generation of separate short-lasting SWDs (in 33.3%) was considered the second type. The intensity of seizures after nine injections of the convulsant in rats without generation of regular spikes and with generation of such EEG phenomena was dissimilar and corresponded to 3.0 ± 0.63 and 2.0 ± 0.10 points, respectively.

Under conditions of preliminary injections of derivatives of nicotinic acid and GABA, EFA was also induced with the formation of two types of activity. In 19 of 31 rats (61.3%), we observed the development of SWDs, while additional generation of regular spikes in the frontal cortex was found in 12 of 31 (38.7%) animals. In the case where we injected PM and IPM in doses of 50 mg/kg, the first SWDs appeared only after the fourth injection of PTX accompanying PM injection and after the fifth injection after IPM introduction. In rats without generation of regular electrographic spikes under conditions of preliminary injections of GABA analogs, the maximum increase in the SWD frequency was observed after the seventh injection of the convulsant in the case of introductions of PM and IPM in doses of 20 mg/kg and only after the eighth injection in the case of greater doses of these agents (Fig. 1A).

The dynamics of duration of discharges with convulsive manifestations in experimental animal groups were of the same type, but the level of rise in this index depended on the injected agent and its dose (Fig. 2A). In animals injected with GABA derivatives in doses of 50 mg/kg, the duration of SWDs generated after the fifth injection of PTX was ten times smaller than that in the control, while after the sixth PTX injection these complexes were three to four times shorter. Within the final stages



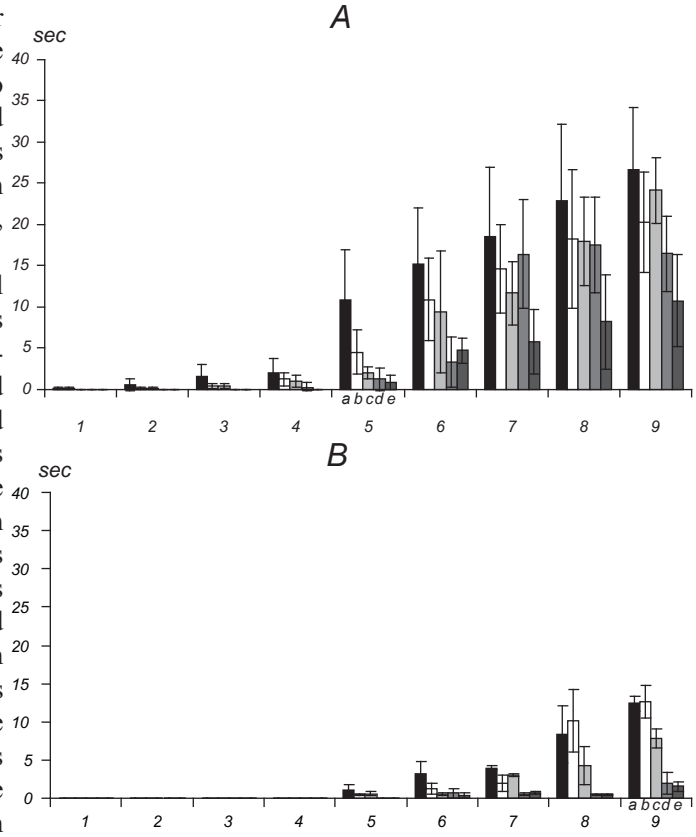
F i g. 1. Effects of preliminary injections of picamilon (PM) and isopicamilon (IPM) in doses of 20 and 50 mg/kg on the frequency of spike-wave discharges in the case of accelerated formation of picrotoxin-induced seizures in rats. A) Discharge frequency in the absence of generation of regular spikes in the frontal cortex; B) discharge frequency when such spikes were generated. *$P < 0.05$ and **$P < 0.01$are cases of significant differences of the studied index in rats of the experimental group, compared with that in control animals. a-e) After injections of picrotoxin, PTX (a), 20 mg/kg IPM + PTX (b), 50 mg/kg IPM + PTX (c), 20 mg/kg PM + PTX (d), and 50 mg/kg PM + PTX (e).

of formation of EFA without generation of regular spikes after injection of 50 mg/kg IPM, the increase in the duration of convulsive discharges was two to four times smaller than that in control animals and in rats injected with 20 mg/kg IPM. Mean intensities of seizures in animals preliminarily injected with PM and IPM were 2.0 ± 0.71 and 2.25 ± 0.50 points, respectively.

In rats with the development of regular cortical spike activity against the background of injections of GABA derivatives, we also observed a dose-dependent pattern of the dynamics of the examined indices (Figs. 1B and 2B). In animals injected with PM and IPM in doses of 20 mg/kg, periods of immobilization were observed only after the fifth PTX injection. In animals injected with 20 mg/kg PM, the seventh to ninth PTX injections were accompanied by the development of discharges that were close in their parameters to those recorded after isolated injection of PTX. Isopicamilon injected in the same dose provided smaller rises in the frequency and duration of SWDs. The corresponding differences were 2.2 and 5.7 times after the sixth PTX injection and 2.0 times after the eighth injection, compared with analogous values in control animals.

Injections of GABA derivatives in doses of 50 mg/kg resulted in a 1.5- to 3.0-fold decrease in the frequency of convulsive discharges; after the final introduction of PTX, this index in animals injected with PM was half as much as that in rats with no such injections. In these rats, the duration of convulsive discharges remained the same; after the final PTX injections, it differed 6-16 times from the analogous index in control rats. Against the background of preliminary injections of GABA derivatives (50 mg/kg) and generation of regular spikes in the cortex, convulsive manifestations in most animals were limited by only myofascial flinching. The mean severity (in points) of convulsions in the case of prior injections of PM and IPM were 1.33 ± 0.58 and 1.0 ± 0.0, respectively.

Therefore, convulsive manifestations in rats subjected to preliminary systemic injections of PM and IPM under conditions of accelerated formation of PTX-induced EFA were expressed to a lesser extent, compared with analogous manifestations in the control group. The development of regular spike activity in the cortex, especially against the background of preliminary injections of PM and IPM, can be considered a phenomenon related to modulation of convulsive activity. Convulsions



F i g. 2. Effects of preliminary injections of picamilon (PM) and isopicamilon (IPM) in doses of 20 and 50 mg/kg on the duration of spike-wave discharges (SWDs) in the case of accelerated formation of picrotoxin-induced seizures in rats. A) Duration of discharges in the absence of generation of regular spikes in the frontal cortex; B) duration when such spikes were present. Vertical scale) Total duration of SWDs during 1-min-long observation interval, sec. Other designations are the same as in Fig. 1.

formed in the course of generation of spike cortical activity were less intense. Under conditions of PM injections, the frequency and duration of epileptiform discharges decreased only in the case where the dose of this agent was 50 mg/kg. After preliminary injections of IPM even in smaller doses (20 mg/kg) into the rats demonstrating generation of regular cortical spikes, the frequency and duration of convulsive SWDs were smaller. The maximum decrease in the intensity of seizures against the background of a significant drop in the frequency and duration of epileptiform convulsive discharges was observed after injections of 50 mg/kg IPM.

Among the mechanisms underlying realization of the effects of PM and its derivatives, two types can be distinguished, neuromediator and metabolic [1, 6]. A neuromediator mechanism is based, first of all, on the effect on the GABA-ergic system. In addition, PM suppresses the activity of MAO and

acetylcholinesterase, activates the processes of aerobic and anaerobic oxidation, increases the energy status of cerebral cells, and activates the antioxidant system. Picamilon is used as a cerebroprotector in some pathological and borderline states [7]. In our experiments, IPM appeared to be somewhat more effective than PM from the aspect of the protective anticonvulsive action.

Experiments were carried out in accordance with the International Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1985), as well as with the regulations of the Ethics Committees of the Odessa National Medical University of the Ministry of Public Health of Ukraine and the Mechnikov Odessa National University.

The authors, O. V. Denisenko, O. A. Shandra, L. M. Karpov, and L. I. Siomik, confirm that they have no conflict of interest.

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