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EFFECT OF NICOTINIC AND MUSCARINIC CHOLINOMIMETICS AND CHOLINOLYTICS ON EPILEPTOGENESIS IN A PENICILLIN FOCUS IN THE DORSAL HIPPOCAMPUS

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Most investigators prefer the penicillin model of epilepsy, for epileptiform changes produced under these conditions correspond most closely to the clinical forms of epilepsy in man [12, 15]. This process is evidently based on inhibition of GABA release from nerve endings [10, 11] and a decrease in the number of GABA receptors [9].

Cholinergic mechanisms are known to play an important role in the formation of epileptiform activity. Mainly muscarinic cholinomimetics and muscarinic cholinolytics are used to study this problem [13, 14]. However, analysis of the experimental data obtained by Gerasimyan [4], who studied many clinically effective anticonvulsants (derivatives of succinimides), reveals that all these preparations have a marked nicotinic cholinolytic action.

In this investigation the role of muscarinic (M) and nicotinic (N) cholinergic mechanisms in the formation of epileptiform activity induced by application of penicillin was studied.

EXPERIMENTAL METHOD

Chronic experiments were conducted on six rabbits weighing 3-3.5 kg, with electrodes implanted into the dorsal part of the hippocampus, the mesencephalic reticular formation, and the sensomotor cortex. An epileptogenic focus was created by introduction of 250 U of a solution of the sodium salt of benzylpenicillin in a volume of 1 µl through a chemical electrode [4] implanted into area CA1 and CA2 of the dorsal hippocampus. Eterofen (a Soviet anticholinergic drug) was used in a dose of 8 mg/kg, gangleron (1,2-dimethyl-3-diethylaminopropyl p-isobutoxybenzoate hydrochloride), in a dose of 3 mg/kg, metamizil (2-diethylamino-1-methylethyl ester of benzylic acid hydrochloride) in a dose of 0.5 mg/kg, and galanthamine in a dose of 1 mg/kg. The drugs were injected intravenously. In each experiment the animals remained under observation for 2.5 h. Experiments in which penicillin alone was injected into the hippocampus served as the control. The number of interictal discharges in the focus and the number of seizures were analyzed. These parameters were counted for 15 consecutive 10-minute periods. The number of spikes was counted during 1 min of recording and the number of seizures during 10 min of observation. Values obtained in the control experiments were taken as 100.

The drugs were injected 20 min before formation of the epileptogenic focus or 30 min after the appearance of epileptiform activity. In the second case mean values of the number of interictal epileptiform discharges and of seizures, counted during 30 min of recording, were

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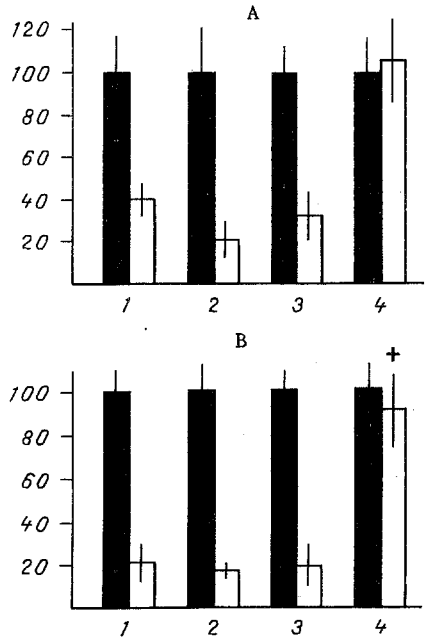

Fig. 1. Changes in number of interictal spikes (A) and frequency of seizures (B) under the influence of galanthamine alone or in combination with cholinolytics injected prophylactically (20 min beforehand). Ordinate, intensity of effect (in %). Black columns denote control values, unshaded columns — experiments in which drugs were used: 1) galanthamine (1 mg/kg), 2) galanthamine (1 mg/kg) + gangleron (3 mg/kg), 3) galanthamine (1 mg/kg) + eterofen (8 mg/kg), 4) galanthamine (1 mg/kg) + metamizil (0.5 mg/kg). +) Increase in duration and intensity of motor seizures.

TABLE 1. Complete Suppression of Epileptogenesis in a Penicillin Focus in the Dorsal Hippocampus of Rabbits under the Influence of Galanthamine, Eterofen, Gangleron, and Their Combinations (%)

Time of injection of preparations	Galanthamine	Eterofen	Gangleron	Galanthamine + gangleron	Galanthamine + eterofen
20 min before formation of focus	70	55	60	90	90
30 min after formation of focus	50	40	45	80	80

Legend. More detailed data on the effects of eterofen and gangleron were published previously [8].

taken as 100. The location of the recording and chemical electrodes was verified histologically. The experimental results were subjected to statistical analysis [1] ($P = 0.05$, $n = 6$).

EXPERIMENTAL RESULTS

In response to injection of 250 U of benzylpenicillin into the dorsal hippocampus epileptiform manifestations, in the form of single and grouped spikes, and subsequently in the form of spike-wave complexes, appeared on the electroencephalogram (EEG) of all the animals, and were recorded initially in the focus but later in other brain structures also. The motor components of the seizure developed after irradiation of epileptiform activity from the focus into different parts of the brain and, in particular, into the cerebral cortex. During the periods between seizures interictal discharges were recorded on the EEG in all structures, and most constantly in the focus itself. Such changes were observed for 3-4 h. It was more

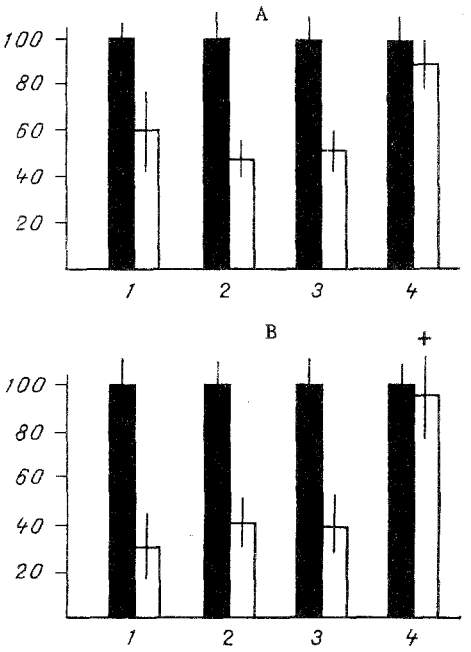

Fig. 2. Changes in number of interictal spikes and seizures under the influence of galanthamine alone or in combination with cholinolytics, injected after the formation of the epileptogenic focus in the dorsal hippocampus. Legend as to Fig. 1.

effective to use the preparations before formation of the epileptogenic focus, for eterofen, gangleron, and galanthamine in 55-70% of experiments, and a combination of galanthamine with eterofen or gangleron in 90% of experiments completely prevented the appearance of epileptiform manifestations on the EEG and in the animals' behavior (Table 1). Galanthamine, injected before formation of the epileptogenic focus, considerably reduced the number of seizures and spikes (Fig. 1A, B). Spike activity was abundant: Its amplitude and frequency were lower than when penicillin alone was injected. The EEG also returned more rapidly to normal (35 ± 4.6 min sooner than in the control). Combined administration of galanthamine with gangleron or eterofen led to more effective depression of epileptogenesis and to the appearance of spindle-like activity on the EEG, against the background of which the interictal spikes were less marked.

Injection of galanthamine in combination with metamizil always potentiated the seizures. Although the number of seizures in these experiments was virtually the same as in the control (compare Figs. 1B and 2B), their course was much more severe and their duration was longer due to an increase in the ionic phase. After injection of galanthamine in combination with metamizil, high-amplitude slow waves developed on the EEG, against the background of which the interictal spikes were easily masked. However, in the reticular formation, where the amplitude of the waves was not increased quite so much, the spikes were distinctly visible and their frequency was higher than in experiments in which metamizil or galanthamine alone was injected.

The results of these experiments show that the anticonvulsant activity of gangleron and eterofen is enhanced if they are used in combination with galanthamine. The increase in severity and duration of the motor seizures in animals receiving combined treatment with metamizil and galanthamine compared with these parameters after injection of metamizil alone also will be noted.

It can thus be considered that both M-cholinergic and N-cholinergic mechanisms take part in the formation of a penicillin epileptogenic focus, and that under these circumstances the direction of their effect is opposite, in agreement with the notions of reciprocity of interaction between M- and N-cholinergic mechanisms, developed by the present writers, within the limits of the single cholinergic system of the body. Potentiation of epileptiform activity by M-cholinolytics and its depression by acetylcholinesterase (AChE) inhibitors can evidently be explained to some degree by their influence on the character of GABA mediation, for it has been shown [3] that the M-cholinomimetics oxotremorine and arecoline, and also the AChE inhibitor physostigmine raise the threshold of convulsions induced by intravenous injection of

picrotoxin into mice, whereas atropine, on the contrary, lowers it. It was found that GABA potentiates the effect of physostigmine and exhibits antagonism toward atropine. Potentiation of activity of the ascending reticular activating system through stimulation of its muscarinic acetylcholine receptors by galanthamine also leads to strengthening of the tonic inhibitory influence of the cortex on deep brain structures, which evidently leads to depression of epileptiform activity in the focus and impairs its irradiation into other brain structures. Simultaneous blockade of nicotinic acetylcholine receptors by gangleron or eterofen reciprocally enhances the M-cholinomimetic properties of galanthamine even more, and makes it more effective. The possibility of the direct effect of N-cholinolytics on brain structures and intracentral relations between them likewise cannot be ruled out. Combined administration of metamizil and galanthamine also gives rise to a N-cholinomimetic effect, promoting a state of preparation of the brain for seizures [2]. Besides other antiepileptic agents, the use of N-cholinolytics as anticonvulsants is thus indicated, and to make them more effective, they should be used in combination with AChE inhibitors.

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