Pharmacology Biochemistry and Behavior, Vol. 2, pp. 1–7. ANKHO International Inc., 1974. Printed in the U.S.A.

Influence of Anticholinergic Drugs on After-discharges and Activity of Epileptogenic Foci in Rabbit Hippocampus

W. A. GUSEL

Department of Pharmacology, Leningrad Pediatric Medical Institute
Leningrad, Litovskaya ul. 2, 194100, USSR

(Received 1 November 1973)

GUSEL, W. A. *Influence of anticholinergic drugs on after-discharges and activity of epileptogenic foci in rabbit hippocampus.* PHARMAC. BIOCHEM. BEHAV. **2**(1) 1–7, 1974. – The influence of M-anticholinergic drug Metamyzil and N-anticholinergic drug Gangleron on the thresholds of after-discharges (AD) and the activity of a penicillin-produced epileptogenic focus in the hippocampus was studied in rabbits with chronic bipolar electrodes implanted in the cortex and subcortical structures. Both anticholinergics increased AD thresholds under direct administration into hippocampus at the point of stimulation (1–160 mkg). Metamyzil induced a more marked effect than Gangleron. When injected into the area of epileptogenic focus in the hippocampus, both anticholinergics (15–20 mkg) did not influence the intensity of interictal epileptiform discharges but decreased the number of fits. Gangleron equally affected the activity of the epileptogenic focus under both conditions of intravenous (1–3 mg/kg) and direct administration into the hippocampus. Contrary to Gangleron, when Metamyzil was intravenously administered (0.5–2.5 mg/kg) it markedly depressed the ascending activating system of the reticular formation and intensified interictal epileptiform activity and fits. It was concluded that the blocking of cholinergic systems of the hippocampus on one hand prevents the circulation of excitement along neuron circuits and, on the other hand, it does not influence the interictal epileptiform discharges in the EEG, which suggests that the fluctuation of the membrane potential of epileptogenic neurons were not affected.

Anticholinergic drugs Hippocampal seizures After-discharges Penicillin epileptogenic focus Intrahippocampal injections

THE hippocampus, which is one of the main structures of the limbic system, takes part in the regulation of normal behavior in animals (e.g. emotional reactions and wake–sleep behavior) and in the origin of seizure disorders [11, 12, 21]. In accomplishing various functions of the limbic system, the reverberation of excitement in short and long circuits of neurons is of great importance [1,26]. Numerous fields of hippocampal units take part in the formation of these circuits [25]. The suggested presence of cholinergic systems in the hippocampus [7, 19, 20] makes probable their participation in spreading of excitement in neuron circuits. Thus, the aim of the present work was to study the role of cholinergic systems in the formation and spreading of hypersynchronous discharges in the hippocampus caused by its electric stimulation or by the formation of a discrete penicillin epileptogenic focus in this structure. To accomplish this purpose a drug which blocks muscarinic–cholinergic (M-cholinergic) structures – Metamyzil (β-diethylaminopropylbenzilic acid ester hydrochloride) and a drug that blocks nicotinic-cholinergic (N-cholinergic) structures – Gangleron α-diethyl-amino-1,2-dimethylpropyl-p-isobutoxibenzoic acid ester hydrochloride) were used. They were injected locally into hippocampus in the site of stimulation (or in epileptogenic foci) or intravenously.

METHOD

Animals

Experiments were carried out on 47 rabbits (2.7–3.4 kg) of both sexes.

Recording Techniques

Bioelectrical activity of the left and right dorsal hippocampus, the ventral amygdaloid region (nucl. amygdala basalis), the reticular formation of the mesencephalon (nucl. reticularis tegmenti) and the sensori-motor region of brain cortex was recorded by stereotaxically implanted chronic bipolar electrodes. All animals were anesthetized with morphine (10 mg/kg) and chlorpromazine (4 mg/kg), both injected intravenously. To record the potentials of hippocampus and its stimulation and to administer the solutions of anticholinergic drugs and penicillin into this structure (to form an epileptogenic focus) a chemotrode was used [4]. It consists of a cannula to administer microquantities of solutions and a bipolar electrode for recording and stimulating. Recording of potentials of other brain structures was carried out by means of bipolar electrodes made of nichrome wire insulated by glass except for the tip of 0.2–0.3 mm in length; the distance between electrodes

being 0.5–0.8 mm. We began to record potentials 3–5 days after the operation using an MT-014 Orion electroencephalograph (parameters of filters: 0.2–12 Hz).

Studying the Hippocampal afterdischarges

The threshold of hippocampal afterdischarges (AD) was determined by stimulation of the structure through the implanted chemotrode before and after the administration of each gradually increasing dose of anticholinergic drugs into the structure. The parameters of stimulation (generator of rectangular impulses ESL-I) were as follows: 50 imp/sec; 1.5 msec; 5 sec. The stimulation of the hippocampus was made repeatedly raising the amplitude of impulses by 0.5 v every time until reaching the threshold. Under this voltage AD were recorded in the symmetrical point of the contralateral hippocampus. After AD were observed, the next stimulation was made not earlier than 20 minutes and during this period (5–10 min before the next stimulation) a solution of anticholinergic drug (0.001 ml) was injected under the stimulating electrode. The following increasing concentrations of Metamyzil: 0.1%, 0.2%, 1% and 2% containing 1, 2, 10 and 20 mkg of drug in 0.001 ml respectively (pH 6.4–4.6 depending on concentration) and of Gangleron: 0.1%, 0.2% and 1.5% containing respectively 1,2 and 15 mkg of drug in 0.001 ml (pH 6.8–6.3) were used. The solutions were prepared with bidistilled water. The administration of the latter (pH 6.9) into hippocampus in our experiments like in those of Borodkin *et al.* [4] did not change the AD thresholds.

Injections of solutions of increasing concentrations were made with the interval of 40–80 min until the changes of AD threshold were obtained. Administration of solutions of still higher concentrations was continued after the primary change of the excitability of hippocampus. In order to inject solutions of increasing concentrations the cannula (one of two parts of the chemotrode) was not discarded. Before the injection of 0.001 ml of new solution was made, the cannula was filled with 0.0002 ml of this solution for replacing the former solution. In this way, injection dose consisted of the dose in 0.001 ml of latter solution and of the 1/5 dose of the former solution. In performing experiments with injection of cholinolytics into the hippocampus the suggestion was checked whether the administration of solutions into the point of stimulation can lower the resistance between two stimulating electrodes. If it were so the fall of voltage on the object of stimulation and of the amplitude of the excitatory stimulus could be decreased and that could be considered as the rise of the AD threshold. To check this supposition, an oscilloscope in parallel to the stimulating bipolar chemotrode was switched on to the generator of rectangular impulses (resistance on the output – R_i = 10 ohm). The known resistance (R) was switched in consecutive order with a chemotrode (Fig. 1). Having measured the threshold amplitude of AD on the screen of the oscillograph without switching on of resistance R and after its switching on, the resistance of the load, that is the resistance between the stimulating electrodes – (R of the rabbit: R rab) was determined.

$$R_{rab} = \frac{R(A-B)}{B}$$

Where:

R = value of the known, switched in consecutive order with the load, resistance;

A = amplitude (in mm) of the threshold value of rectangular impulses on the screen of oscillograph before the switching on of resistance R;

After the administration into the point of stimulation of Metamyzil or Gangleron which increased the AD threshold, the above mentioned measurements were made again. It appeared that the resistance between stimulating electrodes did not change. On the basis of these model experiments, the conclusion was drawn that the rise of AD thresholds in hippocampus after the administration of anticholinergic drugs into the site of stimulation was not an artifact but a result of their specific influence on the excitability of the structure.

Studying the Activity of Epileptogenic Foci

Epileptogenic focus in dorsal hippocampus (field CAI) of control and experimental rabbits was made by administration of 0.001 ml of penicillin-potassium (50 units) in bidistilled water through the chemotrode. Experiments on the same rabbit were made every other day and penicillin was administered into right and left hippocampus in turn. Five injections were made into each hippocampus. During 100 min after administering of penicillin into hippocampus the number of fits during every 10 min and the amount per minute of interictal epileptiform discharges on EEG in the hippocampus were recorded. The results of the first administration of penicillin into the given hippocampus of control and experimental rabbits (the mean number of fits and epileptiform discharges during the time of observation) were assumed as 100%. In next administrations of penicillin into the same hippocampus, changes of the mentioned parameters were determined under injection of 0.001 ml of bidistilled water to control rabbits (5–10 min before the injection of penicillin) and of either 0.001 ml of 2% solution of Metamyzil (20 mkg; 10 experiments) or 0.001 ml of 1.5% solution of Gangleron (15 mkg; 8 experiments) to experimental animals. The temperature of the solutions injected into the brain was 37° C.

In the other series of experiments the number of interictal epileptiform discharges (per 1 min of EEG record) was counted several times during the period from the 20th to the 40th minute after the administration of penicillin into hippocampus, when the pathologic activity of foci became stabilized. The mean number of spikes per 1 min was assumed as the control intensity of interictal discharges. From the 40th to 50th minute after formation of the focus, Metamyzil (0.05 mg/kg – 7 experiments; 0.5–2.5 mg/kg – 6 experiments) or Gangleron (0.5–3 mg/kg – 13 experiments) was administered intravenously to experimental animals.

Data of the experiments were treated statistically using Student's *t* test. The localization of subcortical electrodes was determined histologically.

RESULTS

The Influence of Anticholinergic Drugs on the AD Thresholds

Both anticholinergic drugs when administered into hippocampus under the stimulating electrode caused the rise of AD thresholds (Fig. 2, A,B). The results of the experiments are given in Table 1. As one can see from Table 1 Meta-

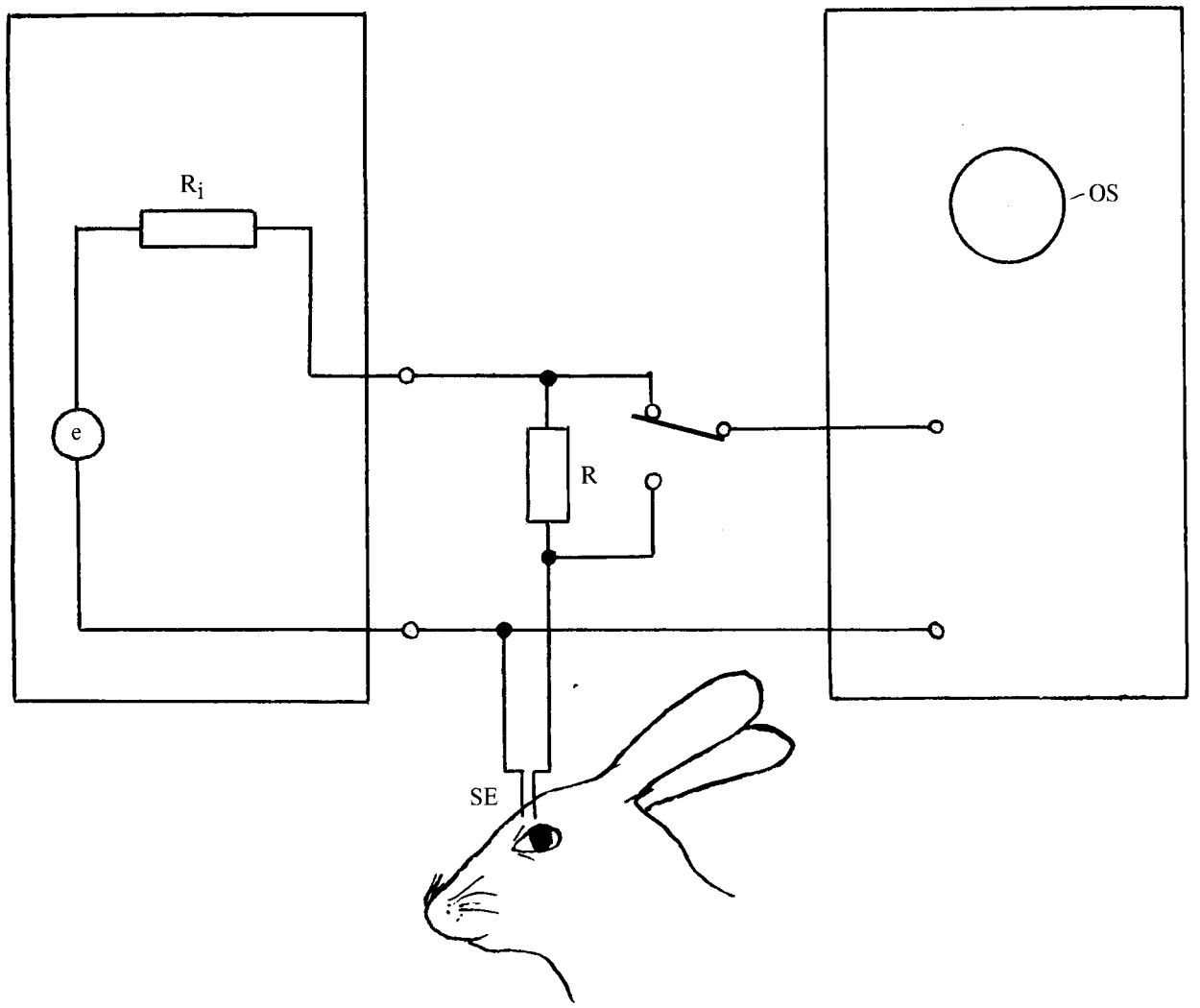

FIG. 1. Scheme of electric circuit to measure resistance between stimulation electrodes before and after administration of solutions of anticholinergics into the point of stimulation. R_i = resistance on the output of generator of rectangular impulses; R = the known resistance switched on consecutive order to stimulating electrodes; e = generator of rectangular impulses; OS = oscilloscope; SE = stimulating electrodes.

TABLE 1

THE INFLUENCE OF METAMYZIL AND GANGLERON ON AD THRESHOLDS

Substances	The first dose of the agent administered into the hippocampus (mkg)	NUMBER OF EXPERIMENTS Total	With a rise of AD threshold	With a rise of AD threshold by the first dose	With a step-like rise of AD threshold	The mean value of the AD threshold after Metamyzil and Gangleron
Metamyzil	1 or 2	7	7	5	5	197.8%*
	10 or 20	8	8	4	5 from 6	
Gangleron	2	7	5	3	2	146.5%*
	10 or 15	8	4	3	1	

*$p<0.05$

myzil depressed the AD-threshold in a larger number of experiments and more intensively than Gangleron.

Influence of Anticholinergic Drugs on the Activity of an Epileptogenic Focus in Hippocampus

Administration of cholinolytics directly into the area of epileptogenic foci. A typical picture of manifestations of pathologic activity of an epileptogenic focus in the hippocampus on EEG is given in Fig. 3. It is seen that interictal epileptiform discharges (in the form of biphasic spikes) are transformed into bioelectrical activity which is characteristic of causing a fit in a rabbit. The formation of hippocampal epileptiform focus, its activity and spreading of seizure discharges from hippocampus has been elucidated in many studies [3, 6, 9, 10, 12, 15, 27].

Preliminary experiments have showed that the administration of solutions of substances or bidistilled water into the focus, which is already developed in the hippocampus, resulted in the more frequent and more severe fits. That made us in further experiments administer drugs into the hippocampus of experimental rabbits (and bidistilled water into the hippocampus of control animals) before (and not after) the production of a penicillin-induced epileptogenic focus.

The results of these experiments are given in Fig. 4A and B. Pretreatment with either Metamyzil or Gangleron did not influence the intensity of interictal discharges. At the same time both anticholinergics statistically significantly decreased the number of fits namely: Metamyzil to 62% and Gangleron to 39% (of the number of fits after the first control injection of penicillin into the hippocampus).

Placement of electrodes into the amygdala and reticular formation was necessary for observing the effects of drugs on the spikes conducted from hippocampus or secondary ones in these structures. The intensity of these spikes was smaller than in hippocampus. There was no differences in the influence of drugs on the pathological activity in amygdala and reticular formation as well as in hippocampus. Therefore we measured interictal spikes in the lead of hippocampus.

Intravenous administrations of Metamyzil and Gangleron. Gangleron in doses of 1–3 mg/kg i.v. like after direct administration into the area of epileptogenic focus in hippocampus did not influence essentially the intensity of interictal epileptiform discharges (Fig. 4 B). However, gangleron in these doses eliminated fits for 30–70 min from the time of administration. In a dose of 0.5 mg/kg Gangleron did not influence the frequency of fits. Intravenous administration of Metamyzil in a dose of 0.05 mg/kg did not lead to the marked change of the intensity of epileptiform discharges. This treatment did not inhibit arousal reaction to sensory stimuli (light, sound). Metamyzil in doses of 0.5–2.5 mg/kg which blocked the arousal reaction, caused a statistically significant increase of intensity of interictal epileptiform discharges (up to 123%). These data agree with the results obtained by us earlier on the other model of epilepsy [16]. After intravenous administration of Metamyzil, especially in doses of 0.5–2.5 mg/kg, behavioral and electroencephalographic fits sharply increased.

DISCUSSION

Data presented in this paper show that an M-anticholinergic drug Metamyzil and an N-anticholinergic drug Gangleron when administered directly into the rabbit hippocampus are able to depress after-discharges in this

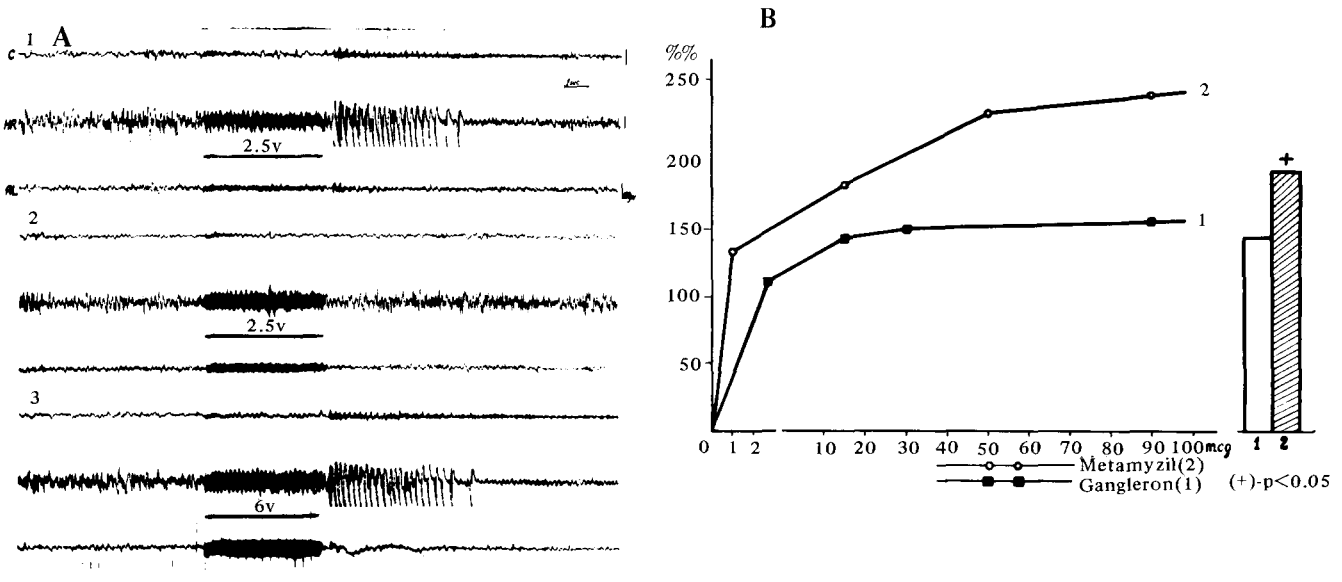

FIG. 2. Influence of direct administation into hippocampus of Metamyzil and Gangleron on the threshold of AD. A. Depression of AD after administration under the stimulating electrode of 10 mkg of Metamyzil. 1. AD threshold in the left hippocampus = 2.5 v. 2. AD absence in stimulation of the left hippocampus by the threshold amplitude of current (2.5 v) after administration of Metamyzil. 3, AD threshold increased from 2.5 v to 6 v after administration of Metamyzil. C = sensorimotor cortex; HR = right dorsal hippocampus; AL = left amygdala nuclei. B. Comparative characteristic of depression of AD thresholds by Metamyzil and Gangleron with statistical data of mean values of AD threshold changes by different doses of anticholinergic drugs. On abscissa - doses of the substance in mkg. On ordinate - values of AD threshold changes in %% of the initial.

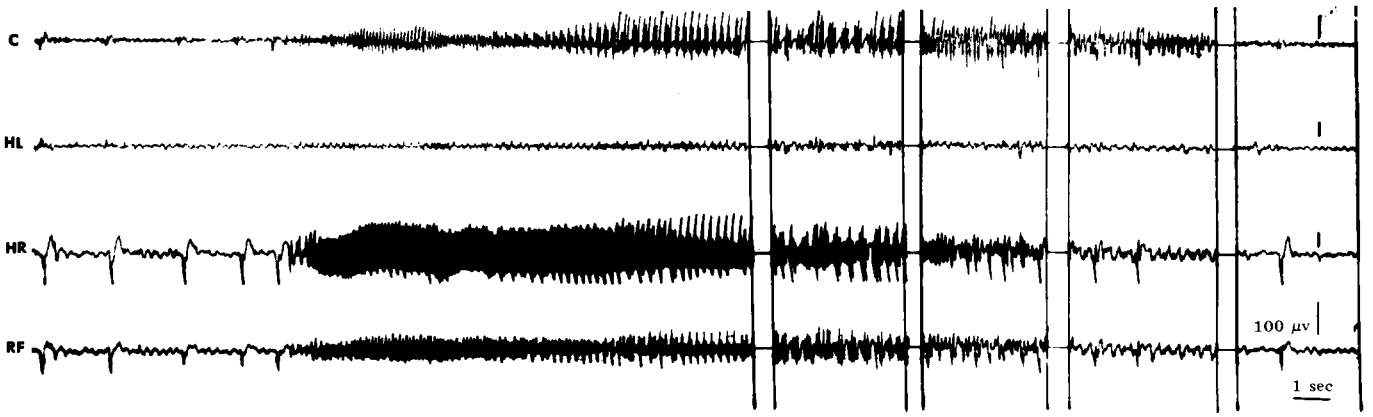

FIG. 3. EEG – Interictal epileptiform discharges which are transformed in a fit. Epileptogenic focus is located in the right dorsal hippocampus. C = sensomotor cortex; HL and HR = left and right dorsal hippocampus; RF = mesencephallic reticular formation.

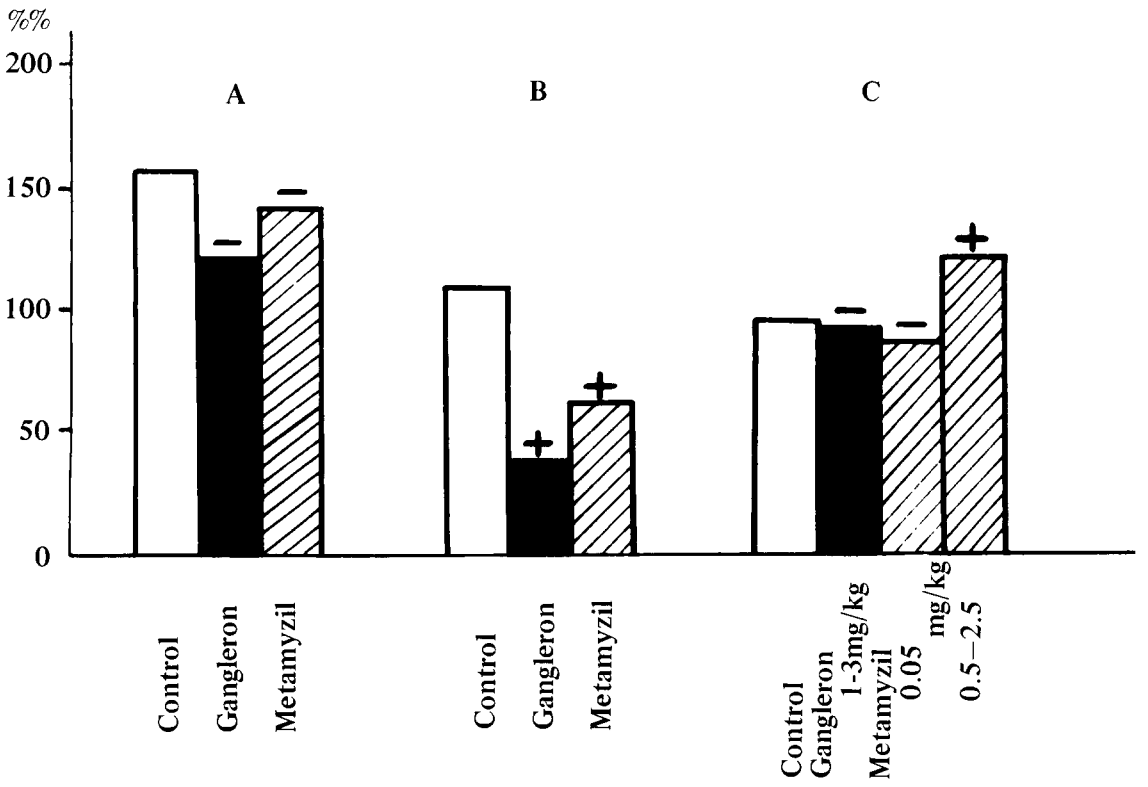

FIG. 4. Influence of anticholinergic drugs on the activity of epileptogenic focus in rabbit hippocampus. A and B. Intensity of interictal epileptiform discharges (A) and the number of fits (B) in rabbits in control (under repeated administration of penicillin only into hippocampus) and after preliminary administration of Metamyzil and Gangleron into the area of epileptogenic focus in %% to the results of the first administration of penicillin into the given hippocampus of control and experimental rabbits. C. Intensity of interictal epileptiform discharges on EEG after intravenous administration of Metamyzil and Gangleron in %% to the initial intensity of pathologic activity. /+/ over the columns = $P<0.01$; /–/ over the columns = data that are not statistically significant.

structure. Metamyzil caused a more marked depression in the hippocampus than Gangleron. First, it increased the AD threshold in all experiments, whereas Gangleron increased it in only 9 of 15. Second, additional administration of Metamyzil in 10 of 13 experiments, after getting the first changes of AD thresholds, caused a more pronounced depression of hippocampus; whereas, the additional administration of Gangleron led to a further depression only in 3 of 15 experiments. From the above mentioned data it follows that in the area CA_1-CA_2 of hippocampal pyramidal cells, where the chemotrodes were implanted both in basal and apical dendritic layers, M-cholinergic systems prevail over N-cholinergic ones. This suggestion agrees with results of experiments with iontophoretic administration of cholinergic substances into the hippocampus [7].

The depression of AD by anticholinergics suggests that blocking of cholinergic systems prevents circulation of excitability in neuron circuits in the formation of which some cell fields of hippocampus take part. This supposition is proved by data on the influence of anticholinergic drugs on the activity of the epileptogenic focus in the hippocampus. Blocking of M- or N-cholinergic systems by Metamyzil or Gangleron injected into the area of epileptogenic focus caused the depression of fits in animals. It is known that for the occurrence of both fits and AD the spreading of excitability in neuron circuits is necessary [18]. The same results were obtained in experiments of Baker and Benedict [2]. They have observed that M-anticholinergic drug scopolamine injected into the area of hippocampal epileptogenic focus caused by administration of picrotoxin or strychnine into this structure, blocked afterdischarges accompanying spike and wave complexes. The suggestion about the cholinergic nature of the temporo-ammonic tract, which connects the entorhinal cortex with the gyrus dentatus and hippocampal pyramidal cells [19], speaks in favour of the possible localization of action of anticholinergics. Preservation of these links is necessary for appearance of seizure discharges in the hippocampus [13,14] because the entorhinal cortex as well as Schaffer's collaterals is a necessary link for connecting the areas of hippocampus CA_1 and CA_3-CA_4 into one single circuit available for circulation of excitement.

Intravenously injected M-anticholinergic drug Metamyzil through depressing the ascending reticular activating system causes nondirect activation of hippocampus, which has reciprocal relations with it [4,5]. Let us assume that this nondirect activation of the hippocampus caused the intensification of interictal epileptiform activity and fits in animals after intravenous administration of Metamyzil because its direct injection into epileptogenic focus did not change the intensity of pathologic discharges. Gangleron (unlike Metamyzil) does not change the relations of hippocampus and reticular formation [5]. That is why, probably, under both its intravenous administration and direct injection into the epileptogenic focus, the depression of fits in animals is observed and the intensity of interictal epileptiform discharges does not change.

The presence of a benzoic nucleus in the structure of Metamyzil and Gangleron might suggest the existence of a local anesthetic properties in these drugs. In this way depression of AD and seizure fits by Metamyzil and Gangleron due to their intrahippocampal injections could be a result of a local anesthetic effect of the drugs, and not a result of an anticholinergic effect. But the identity of effects of Gangleron under its intrastructural and intravenous injections on epileptogenic foci makes this suggestion hardly believable. Single, sporadically recorded EEG interictal epileptiform discharges clearly correlate with dramatic oscillations of membrane potential of epileptogenic neurons, so called "paroxismal depolarization shifts" (PDS) [8, 22, 23]. The absence of influence of anticholinergics in their administration into the area of epileptogenic focus on single epileptiform discharges in our experiments as well as in the works of Baker and Benedict [2] suggests that the blocking of cholinergic systems in the hippocampus probably does not prevent the appearance of PDS of the membrane potential of the neurons located in the epileptogenic focus.

REFERENCES

1. Ajrapetianz, E. Sh. and T. S. Sotnichenko. *Limbica.* Nauka. Leningrad, 1967 (in Rus.).
2. Baker, E. W. and F. Benedict. Influence of increased local cholinergic activity on discharge patterns of hippocampal foci. *Electroenceph. Clin. Neurophysiol.*, **28:** 499–509, 1970.
3. Blum, B. A. Comparative study on hippocampal seizure discharges induced by direct and by indirect hippocampal stimulation in the cat and in the monkey. *Confina Neurol.*, **31:** 316–326, 1969.
4. Borodkin, Ju. S. EEG analysis of influence of some neurotropic substances on intracentral regulation systems and relations between them. Doc. thesis, Leningrad, 1967 (in Rus.).
5. Borodkin, Ju. S., N. A. Losev, and V. A. Kraus. Dependence of matamyzil and glipin effects on hippocampus and mesencephalic reticular formation from permeability of the blood-tissue barrier of these structures. *Farmakologia i Toxikologia (Moscow)*, **33:** 259–263, 1970 (in Rus.).
6. Cadilhac, J. *Hippocampe et epilepsie.* Montpellier, Impr. Paul Dehav, 1955.
7. Curtis, D. R. and J. M. Crawford. Central synaptic transmission-microelectrophoretic studies. *Ann. rev. Pharmacol.*, **9:** 209–240, 1969.
8. Dichter, M. and W. A. Spencer. Penicillin-induced interictal discharges from the cat hippocampus. I. Characteristics and topographical features. *J. Neurophysiol.*, **32:** 649–662, 1969.
9. Elul, R. Regional differences in the hippocampus of the cat. I. Specific discharge patterns of the dorsal and ventral hippocampus and their role in generalized seizures. *Electroenceph. Clin. Neurophysiol.*, **32:** 649–662, 1969.
10. Goodfellow, E. F. and W. T. Neimer. The spread of afterdischarges from stimulation of the rhinencephalon in the cat. *Electroenceph. Clin. Neurophysiol.*, **13:** 710–721, 1961.
11. Green, J. D. The hippocampus. In: *Handbook of Physiology*, Sec. 1., v.II. Ed. J. Field. Washington, 1960, 1373.
12. Green, J. D. The hippocampus. *Physiol. Rev.*, **44:** 561–608, 1964.
13. Green, J. D. and T. Shimamoto. Hippocampal seizures and their propagation. *Archs Neurol. Pscyhiat.*, **70:** 687–703, 1953.
14. Green, J. D. and W. R. Adey. Electrophysiological studies of hippocampal connections and excitability. *Electroenceph. Clin. Neurophysiol.*, **8:** 245–262, 1956.
15. Guerrero-Figueroa, R., A. Barros, R. G. Heath and G. Gonzalez. Experimental subcortical epileptiform focus. *Epilepsia*, **5:** 112–139, 1964.
16. Gusel, W. A. Influence of drugs interacting with cholinergic and adrenergic systems on experimental epileptogenic foci in rabbit's hippocampus. *Farmakologia i Toxikologia (Moscow)*, **33:** 406–411, 1970 (in Rus.).

17. Iljuchenok, R. Ju. *Neurohumoral mechanisms of brain stem reticular formation.* Nauka. Moscow, 1965 (in Rus.).
18. Kreindler, A. *Experimental epilepsy.* Amsterdam, Elsevier Publish. Co. 1965.
19. Kultas, K. N., N. B. Potapina and O. S. Vinogradova. Distribution of acetylcholinesterase in structures of hippocampal formation of rabbits. *Arch. anat. histol. embriol. (Moscow),* **37:** 33–39, 1969 (in Rus.).
20. Lewis, P. R. and O. S. Shute. The cholinergic limbic system: projections to hippocampal formation, medial cortex, nuclei of ascending cholinergic reticular system, and the subfornical organ and supra-optic creast. *Brain,* **9:** 521–540, 1967.
21. MacLean, P. D. The limbic system and its hippocampal formation. *J. Neurosurg.,* **11:** 29–44, 1954.
22. Matsumoto, H., G. F. Ayala and R. J. Gumnit. Neuronal behavior and triggering mechanism in cortical epileptic focus. *J. Neurophysiol.,* **32:** 688–703, 1969.
23. Okudzava, V. M. Neuron interrelations in the brain cortex under seizure activity. In: *Materials of XI meeting of USSR physiol. society,* Leningrad, 1970, v. I, p. 35 (in Rus.).
24. Penfield, W. and G. Jasper. *Epilepsy and functional anatomy of human brain,* Foreign Literature, Moscow, 1958, p. 482, (in Rus.).
25. Raisman, G., W. M. Cowan and T. P. S. Powell. The extrinsic afferent, commissural and association fibres of the hippocampus. *Brain,* **89:** 83–108, 1966.
26. Serkov, F. N. In: *General and particular physiology of nervous system,* Ed's Chernigovsky V. N. *et al.,* Nauka, Leningrad, 1969, chapt. 17, p. 423, (in Rus.).
27. Walker, A. E. and G. B. Udvarhelyi. Dissemination of acute focal seizures in the monkey. II. From subcortical foci. *Archs Neurol.,* **12:** 357–380, 1965.