ANTIEPILEPTIC EFFECTS OF A COMBINATION OF SODIUM VALPROATE AND THE CALCIUM ANTAGONIST RYODIPINE ON A MODEL OF PENICILLIN-INDUCED FOCAL EPILEPTIC ACTIVITY

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Combination pathogenetic therapy (CPT) [3], consisting of the combined administration of preparations targeted in accordance with their mechanism of action on different pathogenetically interconnected components of a pathological system [3], as experimental [4, 5] and clinical investigations [2, 7] have shown, is capable of achieving a more complete therapeutic effect, reducing the doses of preparations used, and thus at the same time reducing the likelihood of development of side effects. When CPT is planned, attention must be paid both to the specific character of action of the preparations and the basic pathogenetic mechanisms of onset of the particular form of epileptic activity (EpA) [3, 6].

An important role in the mechanisms of epileptogenesis is played by disturbance of GABA-ergic inhibition [13] and increased inflow of Ca^{2+} into the neuron [1, 9, 10, 15]. The present investigation was accordingly undertaken in order to study the efficacy of a combination of preparations acting on the above-mentioned stages of epileptogenesis: valproate, which potentiates GABA-ergic processes [11], and a new calcium antagonist, ryodipine, belonging to the 1,4-dihydropyridine class, on a model of penicillin-induced focal EpA. The writers showed previously that this combination of preparations leads to increased antiepileptic efficacy on a model of generalized metrazol-induced EpA [6].

EXPERIMENTAL METHOD

Experiments were carried out on 52 male Wistar rats weighing 210-240 g. The animals were kept under ordinary animal house conditions on a standard diet. To create a model of focal EpA, 24 h before the experiment and under hexobarbital anesthesia (150 mg/kg, intraperitoneally) and local procaine anesthesia, a burr-hole measuring 2 × 4 mm was drilled in the animal's skull above the sensomotor cortex of the left cerebral hemisphere, leaving the dura intact, and a monopolar silver cortical electrode was applied in order to record electrical activity from this region of the cortex (ECoG). The reference electrode was implanted into the nasal bones of the skull. The external leads of the electrodes were fixed to the surface of the skull with stomatologic paste, and a capsule was formed around the burr-hole. To prevent drying of the exposed area of the brain the capsule was filled with physiological saline and covered above with waterproof film, which was fixed around the edges with stomatologic paste. Next day, to create a focus of EpA, the film was removed from the capsule and a piece of filter paper, soaked in a solution of the sodium salt of benzylpenicillin in a concentration of 32,000 IU/ml was applied to the exposed area of the cortex. The ECoG was recorded on a EEG 8S electroencephalograph (Hungary) in the unanesthetized, unrestrained animals. Considering the possible effects of different factors, effects of valproate and ryodipine, alone or in combination,

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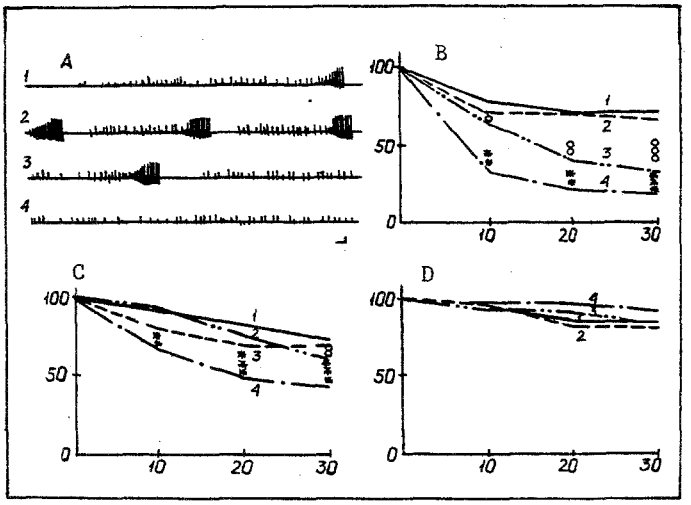

Fig. 1. Characteristic pattern of epileptic activity (EpA) in penicillin-induced focus in rat sensomotor cortex and change in EpA parameters under the influence of valproate, ryodipine, and a combination of both. A) EpA after penicillin application: 1) after 2 min (IID, appearance of the 1st ID), 2) after 25 min (stage of stable generation of IID and ID), 3) after 70 min, and 4) after 80 min (gradual weakening of EpA). Calibration: 10 sec, 500 μV. Change in parameters of EpA under influence of preparations: B) frequency of ID generation, C) frequency of IID generation, D) amplitude of IID. Abscissa, time after injection of preparations (in min); ordinate, parameters studied (in %, taking initial values before injection of preparations as 100%). 1) Control, 2) ryodipine, 3) valproate, 4) valproate + ryodipine; $^{**}p < 0.02$, $^{***}p < 0.001$ compared with corresponding values in control animals; $^{\circ}p < 0.05$, $^{\circ\circ}p < 0.02$, $^{\circ\circ\circ}p < 0.01$ compared with value before injection.

were studied simultaneously and parallel with effects of penicillin alone. The preparations were injected intraperitoneally against the background of consistent generation of ictal discharges in the focus, 20-30 min after application of penicillin: sodium valproate 150 mg/kg (14 rats), ryodipine in a 30% solution of dimethyl sulfoxide (DMSO) 0.8 mg/kg (nine rats), and both preparations together in the same doses (12 rats). Control animals received the same volume (0.2 ml) of the solvent – physiological saline (seven rats) or DMSO (seven rats).

The experimental results were analyzed on an Olivetti computer system (Italy). Amplitude–frequency characteristics and duration of the foci of EpA were determined.

EXPERIMENTAL RESULTS

Application of penicillin to the sensomotor cortex led to the appearance of EpA: separate interictal spike discharges (IID) appeared, their amplitude gradually increased, and after 6-15 min ictal seizure discharges (SD) appeared; after 20-30 min a stage of marked seizure activity supervened, and was characterized by the regular appearance of ID and continued for 30-40 min, after which the ID became less frequent and the generation frequency and amplitude of the IID also decreased (Fig. 1A). The mean duration of the foci of EpA from the time of application of penicillin to complete disappearance of EpA averaged 99.21 ± 5.88 min. In animals of the control groups, injection of physiological saline and DMSO 20-30 min after application of penicillin, when generation of ID had

stabilized, had no effect on the character of EpA in the focus. Later these animals were pooled into a single control group.

Valproate, injected at the stage of stable EpA, had an antiepileptic effect in the form of reduction of the generation frequency of ID, which was observed immediately after injection and was most marked after 30 min (Fig. 1B). Meanwhile, comparison of the time course of changes in the frequency of ID after injection of valproate into these animals and of physiological saline into the control rats revealed no significant differences between these two groups of animals. Moreover, 30 min after injection, valproate caused a significant decrease in the duration frequency of IID (Fig. 1C). Valproate had no effect on the amplitude of IID (Fig. 1D) and did not shorten the duration of the foci of EpA (75.50 ± 5.66 min).

In the dose used, ryodipine had no significant effect on the amplitude and frequency characteristics of the EpA focus. The mean duration of the foci was 88.12 ± 8.56 min.

In response to a combination of valproate and ryodipine, compared with administration of valproate alone there was a greater and more significant degree of depression of EpA, in the form of a decrease in the generation frequency of ID and IID. Potentiation of the anticonvulsant effect was not observed with respect either to the amplitude of IID or the duration of the foci of EpA (85.17 ± 6.69 min).

The antiepileptic effect of valproate is associated with its potentiation of inhibitory GABA-ergic processes [8, 11, 12, 14], whereas that of ryodipine is associated with blockade of the CA-inward current, when strengthened during hyperactivation of neurons and the appearance of EpA [1, 9, 10, 15]. The action of each of the preparations used is thus aimed at different initial pathogenetic components of epileptogenesis, and that is why their combined use leads to potentiation of the final antiepileptic effect. This investigation, like a previous one [6], is evidence of the value of CPT in the form of combined administration of valproate and ryodipine.

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