EFFECT OF ORGANIC CALCIUM ANTAGONISTS AND MAGNESIUM ON THE DEVELOPMENT OF METRAZOL KINDLING

G. N. Kryzhanovskii, M. N. Karpova, and O. Yu. Pantov

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Calcium antagonists and N-methyl-D-aspartate (NMDA) receptors inhibit local and generalized epileptic activity induced by various convulsants [1, 6, 11]. The effect of these substances on the long process of formation of predisposition to seizures, which is usually observed in epilepsy, merits special study. Kindling, a chronic effect produced by electrical stimulation of brain structures [8] or by injection of epileptogens in subconvulsive doses [3, 4, 7] can serve as an experimental model of this process. We have studied the effect of organic calcium antagonists [Finoptin (verapamil) and Riodipine* (of the 1,4-dihydropyridine class)], and also of Mg^{2+} (magnesium sulfate), injected systemically, on the formation of predisposition to seizures in rats induced by daily injection of metrazol in a subconvulsive dose.

EXPERIMENTAL METHOD

Experiments were carried out on 85 male Wistar rats. The animals were kept under ordinary animal house conditions on a standard diet. In Series I randomized animals were used without any preliminary selection as regards sensitivity to metrazol, but none of them responded with seizures to metrazol in a dose of 30-40 mg/kg, i.e., this dose of metrazol was subconvulsive for them. These animals were injected daily with metrazol in a dose of 30 mg/kg intraperitoneally. The experimental animals of this series were given Finoptin ("Orion," Finland) in a dose of 10 mg/kg (10 rays), Riodipine in dimethylsulfoxide (DMSO) in a dose of 2 mg/kg (10 rats), and magnesium sulfate in a dose of 0.15 ml of 5% solution/100 g body weight (10 rays). In the experiments of Series II animals giving a convulsive reaction of 1 to 3 points to injection of metrazol in a dose of 40 mg/kg were chosen, and they could be taken as relatively more sensitive to the epileptogenic action of metrazol. Metrazol was injected into these rats in the same subconvulsive dose of 30 mg/kg. The drugs were given to the experimental animals of this series in a rather higher dose: Finoptin up to 15 mg/kg (7 rats), magnesium sulfate 0.3 ml of a 5% solution/100 g body weight (7 rats), and the two preparations were given together in the same doses (8 rats). The control rats of both series were given injections of the same volume (0.1 ml) of the solvent, namely physiological saline (Series I, 10 rats; Series II 14 rays) and DMSO (Series I, 10 rats). The substances were injected intraperitoneally 15 min before each injection of metrazol. The severity of the seizure response to injection of metrazol was estimated daily and expressed in points: 1) shaking or tilting of the head, 2) infrequent separate chronic convulsions of the whole body; 3) a series of chronic convulsions of the whole body or clonus of the forelimbs; 4) tonico-clonic convulsions with standing up on the hind limbs (the "kangaroo" posture); 5) single clonico-tonic convulsions with the animal falling on its side and a phase of tonic extension; 6) repeated clonico-tonic convulsions and (or) death of the animal. The convulsive response of the animals of each group was assessed by the averaged number of points, counting only those animals in which this response appeared. In the experiments of Series III the effect of Finoptin (15 and 30 mg/kg) on the seizure response was determined in rats receiving metrazol (30 mg/kg) and physiological saline (15 min before metrazol) for 45 days. The significance of differences was estimated by Student's test.

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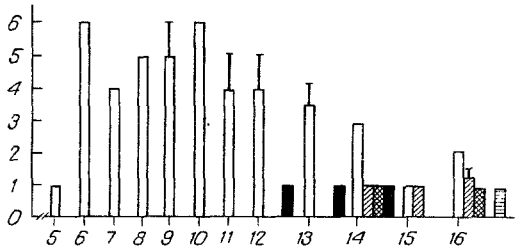

Fig. 1. Severity of seizure response in randomized rats after daily injections of metrazol in subconvulsive dose, preceded by injection of physiological saline (unshaded columns), DMSO (black columns), Finoptin (obliquely shaded columns), Riodipine (horizontally shaded columns), and magnesium (cross-hatched columns). Here and in Figs. 2 and 3: abscissa, time of experiment (in days); ordinate, average severity of seizures (in points).

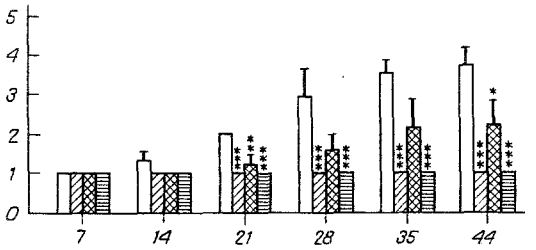

Fig. 2. Severity of seizure response in animals relatively sensitive to metrazol after injection of the latter in a subconvulsive dose, preceded by injection of physiological saline, Finoptin, magnesium, or both preparations together. Here and in Fig. 3: unshaded columns – physiological saline, obliquely shaded – Finoptin, cross hatched – magnesium, horizontal shading – Finoptin + magnesium.

EXPERIMENTAL RESULTS

Seizures with a severity of 1 point were recorded in the control animals of Series I, receiving metrazol after physiological saline, on the 5th day after daily injections of metrazol (Fig. 1). Maximal manifestation of the seizure reaction (up to 6 points) was observed from the 6th through the 10th day. Later a gradual diminution of the average severity of the seizures was observed, amounting to 2 points on the 16th day.

Since the seizure response was assessed on the basis of the averaged number of points and the animals of this series differed in their sensitivity to metrazol, the marked increase in the seizure response during the first 5-10 days in the control animals can be linked with the enhanced response of the most sensitive of the animals to the epileptogenic action of metrazol (one of the rats died). The reduction in severity of the seizures may also be linked with potentiation of the inhibitory mechanisms, which may take place during the development of kindling [3, 12].

considerable delay in the development of the seizure response was observed in experimental animals receiving Finoptin, magnesium, and Riodipine. It did not appear until after the 14th-16th injections of metrazol, and its severity was rated at 1 point. In animals receiving DMSO, seizures rated at 1 point were recorded after the 12th injection of metrazol (Fig. 1).

Thus preliminary injection of these calcium antagonists, including Mg^{2+}, significantly delayed the development of predisposition to convulsions. The great delay of development of predisposition to seizures under the influence of Riodipine may probably be the sum of two effects: the action of the drug itself and the action of its solvent DMSO.

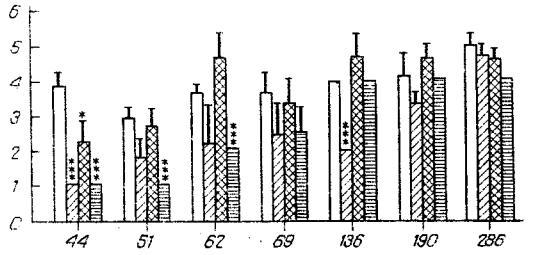

Fig. 3. Severity of seizure response in rats at different times after discontinuing administration of metrazol, Finoptin, magnesium, or both preparations together, in response to testing dose of metrazol.

In animals of Series II, which can be taken to be more sensitive to metrazol, injection of the epileptogen in the same subconvulsive dose (30 mg/kg) induced a seizure response with an intensity of 1 point as early as on the 2nd day. No differences were observed between the response of the control rats, receiving physiological saline, and animals receiving Finoptin or magnesium or a combination of both, during the first 2 days (Fig. 2). Differences began to appear on the 14th day and were significant in the 3rd week. They persisted also during further administration of metrazol up to the 6th-7th week; in the control rats, moreover, there was a further increase in the severity of the convulsions (4 points), whereas in the experimental animals the seizure response was maintained at the level of 1 point. A tendency could be detected for the appearance of a greater kindling-inhibitory effect following administration of Finoptin, and also of a combination of Finoptin and magnesium.

In the animals of Series III (7 rats), which responded after the end of a 45-day period of kindling to the testing dose of metrazol (30 mg/kg) by a seizure response of 3-5 points, injection of Finoptin (15 min before metrazol) had no significant effect on the severity of the seizures: their intensity was the same as after injection of metrazol alone, without Finoptin.

To study the duration of preservation of this state of enhanced predisposition to seizures (EPS) after the end of administration of metrazol (from the 45th day) animals of all groups periodically received the testing dose of metrazol (30 mg/kg). The experiments showed that the EPS persisted in rats throughout the period of observation, namely for 8 months (Fig. 3). It is an interesting fact that after discontinuation of the Ca-blockers (after the 45th day of the experiments) a tendency appeared for the severity of the seizures to increase in response to the testing subconvulsive dose of metrazol. This is evidence of some enhancement of predisposition to seizures. In those animals which received a combination of Finoptin and magnesium, this tendency appeared much later.

In the control animals not receiving the drugs, EPS persisted at the same level throughout the period of observation. These data can be compared with clinical observations showing an increase in severity of epileptic fits after discontinuation of anticonvulsive maintenance therapy.

The results of these experiments, like those obtained previously [3, 4, 7], are evidence that chronic administration of metrazol in subconvulsive doses causes a gradual increase in predisposition toward convulsions, manifested as the onset of seizures or an increase in their severity in response to subsequent injections of the epileptogen. This EPS syndrome may continue for a long time even after administration of the convulsant has ceased, as was noted in our previous investigations [4] and also by other investigators [15]. We also know that EPS persists for a long time after kindling of brain structures induced by electrical stimulation [8, 14]. The use of calcium antagonists and also of magnesium delays the development of EPS significantly. It is a very important fact that this effect is observed with the use of calcium antagonists of different types, including magnesium, the spectrum of whose action includes voltage-dependent blockade of NMDA-channels, which possess high permeability for Ca^{2+} [5], and blockade of voltage-dependent Ca-channels. The results of these experiments agree with data on the inhibitory effect of MK-801 (a noncompetitive antagonist of NMDA receptors) on the development of electrical kindling of the amygdala and hippocampus [10, 13].

The general conclusion can be drawn from all the data that calcium antagonists and magnesium can inhibit the development of EPS during kindling and that EPS in connected with involvement of the Ca-current of neuron membranes. It is remarkable that in animals which are more sensitive to metrazol (Series II), and which received Finoptin or magnesium with metrazol, no differences could be found in the severity of the seizures compared with the control animals initially (during the first 2 weeks), and marked differences did not appear until later. It can be tentatively suggested that this effect is connected with the fact that stronger activation of Ca-channels takes place in animals sensitive to metrazol, so that the preparations used become effective only if they act for a comparatively long time. Meanwhile, in animals receiving Finoptin and magnesium with metrazol, a latent tendency toward activation of Ca-channels, compensated under these conditions, evidently takes place under the influence of the chronic action of metrazol. This tendency is manifested when drups controlling activity of Ca-channels are discontinued.

Organic calcium antagonists and magnesium, at least under the conditions specified above, do not prevent completely the development of the EPS syndrome. The change in state of the Ca-channels during kindling is not the only mechanism of plastic changes in neurons responsible for the development of the EPS syndrome.

The effect of DMSO thus observed is probably connected with its effect, not on the development of the EPS syndrome, but on the realization of the seizure response itself. This may be connected with the antioxidative activity of DMSO [9], for inhibitors of lipid peroxidation weaken the seizure response [2].

The results of the experiments with Finoptin, like data obtained by other workers who used MK-801 [10], are evidence that calcium antagonists and antagonists of NMDA-receptors, by inhibiting the development of EPS, at the same time have no significant influence on realization of the seizure response itself (see Series III). This suggests that the mechanisms of chronic epileptogenesis (in this case in the form of kindling-dependent metrazol-induced EPS) and the seizure response are different. It follows from this conclusion that not all anticonvulsants can be true antiepileptic remedies, and conversely, that substances abolishing epileptogenesis are not necessarily anticonvulsants. This conclusion will determine the strategy for the search for substances of both classes.

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