ANTIEPILEPTIC EFFECTS OF IOS-1.1212, A NEW CALCIUM CHANNEL BLOCKER

M. N. Karpova, O. Yu. Pankov, R. N. Glebov, G. J. Duburs, S. K. Germane, and V. E. Kluša

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Increased admission of Ca^{2+} into the neuron is one cause of hyperactivation of the neuron and the genesis of epileptic activity (EpA) [1, 12]. Recently, in order to terminate EpA, various calcium channel blockers have been used [1-5, 8, 9, 13]. The list of these substances includes the 1,4-dihydropyridines [3-5, 8, 9, 13].

The aim of the present investigation was to study the effect on EpA of a new preparation belonging to the 1,4-dihydropyridine class, namely IOS-1.1212 [2,6-dimethyl-3,5-bis-(2′-propoxyethoxycarbonyl)-4-(2-difluoromethoxyphenyl)-1,4-dihydropyridine] which differs from classical dihydropyridines (nifedipine, riodipine) in causing selective dilatation of cerebral vessels [10], it exhibits greater affinity for the brain dihydropyridine receptors, and also has a marked effect on function of the CNS [11].

EXPERIMENTAL METHOD

Experiments were carried out on 187 male Wistar rats weighing 170-200 g and on 100 male JCR:JCL mice weighing 18-22 g, on models of focal and generalized EpA, electroshock, and pharmacological kindling. As a model of focal EpA in rats, on the day before the experiment a hole measuring 2 × 4 mm was drilled by the method described previously [2] in the animal's skull above the region of the left sensomotor cortex, the dura mater was removed (only in experiments with penicillin application), and monopolar silver cortical electrodes were inserted to record electrical activity from the above-mentioned region of the cortex (ECoG). Foci were created by applying filter paper soaked in a solution of the sodium salt of benzylpenicillin (20,000 IU/ml) or a 0.3% solution of strychnine. The ECcG was recorded on an EEG8S electroencephalograph (Hungary), in unanesthetized, unrestrained animals The preparation was dissolved in dimethylsulfoxide (DMSO) and injected intraperitoneally in doses of 2 and 10 mg/kg (100% DMSO) against the background of stable EpA in the penicillin-induced focus or of developing EpA in the strychnine-induced focus, and also 30 min before creation of the focus. Control animals were given injections of the same volume of solvent (0.1 ml).

Acute generalized EpA was induced in rats by intraperitoneal injection of metrazol in a dose of 75 mg/kg. The latent periods of the first seizure manifestations, the time of the most marked seizures (with the animal falling on its side), and the number of animals which died were recorded, IOS-1.1212 also was injected intraperitoneally 30 min before injection of metrazol in doses of 2 and 10 mg/kg. Seizures were induced in mice by intravenous injection of 1% metrazol solution and 0,01% strychnine solution at the rate of 0.01 ml/sec (until lethal tonicoclonic convulsions developed) 1 and 3 h after intraperitoneal injection of the preparation in doses of 1.5 and 5 mg/kg, in the form of an aqueous suspension, made up with the aid of Tween-80. Maximal electroshock was induced in the mice by applying a current of 50 mA with a frequency of 50 Hz and for a duration of 0.2 sec. IOS-1.1212 (1.5 and 5 mg/kg, intraperitoneally) was injected 1 and 3 h before electroshock.

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TABLE 1. Effect of IOS-1.1212 on Focal EpA Induced by Application of Penicillin to Rat Cerebral Cortex (M ± m)

Group of animals	Number of animals	Before injection			20-30 min after injection			Duration of existence of focus, min
		number of IID in 1 min	amplitude of IID, μV	number of ID in 1 min	number of IID in 1 min	amplitude of IID, μV	number of ID in 1 min	
1) Control, physiological saline	6	12,76±1,12	1265±114	0,43±0,05	14,13±1,24	1378±108	0,41±0,08	159,12±18,68
2) Control, DMSO	11	12,54±1,37	1402±272	0,44±0,09	12,25±4,59	1058±230	0,37±0,09	164,20±20,50
3) Experiment, 2 mg/kg	6/7	13,00±2,22	1330±151	0,34±0,06	13,12±1,65	1248±178	0,08±0,03**	141,44±16.05
4) Experiment, 10 mg/kg	12/15	12,21±1,24	876±77	0,42±0,07	7,41±0,98*	579±70*	0,17±0,04**	86,80±7,40*

Legend. Numerator gives number of animals in which IOS-1.1212 caused the appearance of EpA, denominator gives total number of animals in group. *$p < 0.02$, **$p < 0.01$ Compared with corresponding values in column before injection.

Pharmacological kindling was carried out by daily intraperitoneal injection of metrazol in a subconvulsive dose of 30 mg/kg. In series I, randomized animals without any previous selection for sensitivity to metrazol were used. IOS-1.1212 was injected intraperitoneally in a dose of 10 mg/kg into the experimental animals (10 rats) 15 min before each injection of metrazol. Animals of the control group (10 rats) received an injection of DMSO. To reduce the scatter of the animals relative to sensitivity to metrazol, which correlates with scatter in the effects of the anticonvulsant, a method devised by ourselves was used [4], with preliminary testing in the form of a seizure reaction to a near-threshold (or minimally acting) dose; this was 40 mg/kg. In sensitive animals this dose induced a seizure response rated at 1-3 points. These animals were used in the experiments of series II; kindling was induced by injecting metrazol in the same subconvulsive dose of 30 mg/kg daily for 30 days. The experimental animals of this series (10 rats) also received IOS-1.1212 intraperitoneally in a dose of 2 mg/kg 30 min before each injection of metrazol The control animals (10 rats) under similar experimental conditions were given the same volume (0.1 ml) of DMSO The severity of the seizure reaction was estimated on a 6-point scale [4]. The significance of differences was estimated by Student's test.

EXPERIMENTAL RESULTS

Penicillin-Induced Focal EpA. The first interictal spike discharges (IID) were recorded 3-5 min, and seizure ictal discharges (ID) 7-15 min after application of penicillin. The stage of marked seizure activity began after 25-35 min and was characterized by the regular appearance of IR and continued for 30-40 min, after which there was a gradual decrease in the frequency of ID generation and also in the frequency and amplitude of IID. The average duration of existence of the foci of EpA from the time of application of penicillin until complete disappearance of EpA was 164 ± 20 min.

In animals of the control groups (Table 1; groups 1 and 2) injection of physiological saline and DMSO at a time of stable ID generation (25-30 min after penicillin application) did not change the character of EpA. Injection of IOS-1.1212 in a dose of 2 mg/kg during this period caused a decrease in the frequency of ID generation 20 min after injection; the frequency of generation and amplitude of IID and also the duration of existence of the foci of EpA were unchanged. If the dose was increased to 10 mg/kg, not only was the frequency of ID generation reduced, but so also were the frequency and amplitude of IID (Table 1); the duration of existence of the EpA foci was less than in animals of the control groups.

Strychnine-Induced Focal EpA. Application of strychnine to the dura mater of the cerebral cortex of animals (six rats) led after 30-60 sec to the appearance of single discharges, whose amplitude and frequency gradually increased. The duration of existence of the EpA focus from the moment of application of strychnine until complete disappearance of the discharges, was 40-60 min.

IOS-1.1212 in a dose of 2 mg/kg (eight rats) injected 5-12 min after strychine application, had no effect on the subsequent character of EpA formation. The duration of existence of the focus under the influence of the compound was not significantly reduced, but averaged 34.3 ± 2.9 min; in animals (eight rats) receiving the solvent it was 41.3 ± 3.2 min. Increasing the dose of the compound to 10 mg/kg (eight rats) likewise had no effect on the character of EpA and did not

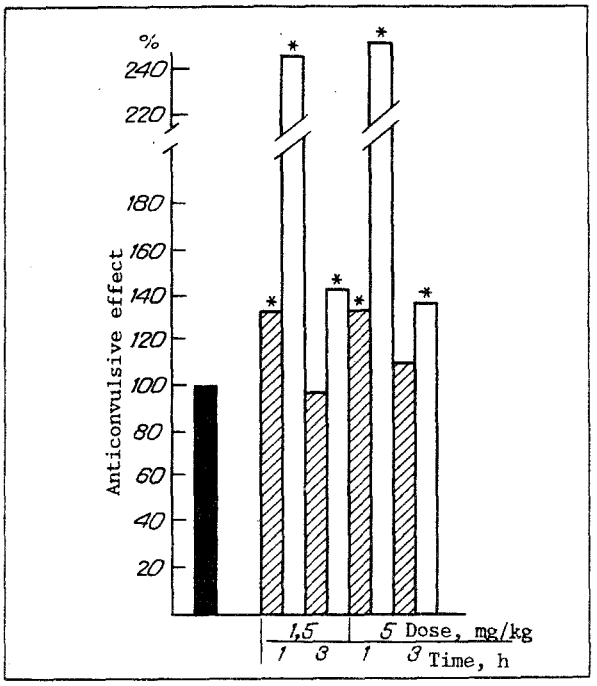

Fig. 1. Effect of IOS-1.1212 on seizures induced in mice by metrazol (intravenous titration). Black column — control, obliquely shaded column — clonic phase of seizures, unshaded — tonic phase of seizures; *p < 0.05.

shorten the duration of existence of the focus: it was 39.5 ± 1.7 min in the experimental animals and 35.3 ± 5.5 min in the controls.

Preliminary injection of IOS-1.1212 in doses of 2 and 10 mg/kg (eight rats in each group) 30 min before creation of the strychnine-induced focus of EpA had no antiepileptic effect.

Acute Generalized EpA. Preliminary injection of IOS-1.1212 in a dose of 2 mg/kg (14 rats) increased the latent period of the first seizures from 23.2 ± 2.6 sec in the control (14 rats) to 32.6 ± 2.6 sec in the experiment ($p < 0.05$); it delayed the onset of the clonic phase from 37.4 ± 1.8 sec in the control to 50.1 ± 2.9 sec in the experiment ($p < 0.001$) and also the development of generalized seizures (the animal falling on to its side) from 83.2 ± 3.7 sec in the control to 160.0 ± 14.6 sec in the experiment ($p < 0.001$). The mortality rate in the group of control animals was 71% and in the group of experimental animals 50%; the time of death of the control animals was 545.8 ± 110.6 sec and in the experimental animals 1420.0 ± 201.7 sec ($p < 0.01$). Increasing the dose of the drug to 10 mg/kg (14 rats) had no significant effect on the increase in its antiepileptic activity compared with that observed when a dose of 2 mg/kg was used.

Preliminary injection of the compound also had a protective action on mice with metrazol seizures (Fig. 1); this effect was particularly marked against the tonic phase, and when injected 1 h before metrazol. Meanwhile the compound was ineffective against strychnine seizures and electroshock. In a dose of 1.5 and 5 mg/kg the compound significantly (by 43%) increased the antiepileptic activity of phenobarbital in electroshock (ED_{50} for phenobarbital was 35.5 mg/kg, and for phenobarbital combined with the action of the compound it was 20.5 mg/kg).

Metrazol Kindling. In the control animals of series I, into which metrazol was injected after physiological saline, seizures rated at 1 point were recorded on the 5th day after daily injection of metrazol (Fig. 2). In the experimental animals receiving IOS-1.1212 the development of the seizure reaction was delayed: it began only after the 14th injection of metrazol and its severity was rated at 1 point. In animals of the control group receiving DMSO, seizures of 1 point were recorded after the 12th injection of metrazol (Fig. 2).

In the animals of series II, which could be regarded as more sensitive to metrazol, injection of the drug evoked a seizure reaction rated at 1 point as early as on the 2nd day. In animals receiving the drug convulsions with a severity of 1 point were recorded on the 3rd day after injection of metrazol. Later, for 2 weeks no difference was observed between the response of the control rats (receiving physiological saline and DMSO) and the experimental rats receiving the drug (Fig.

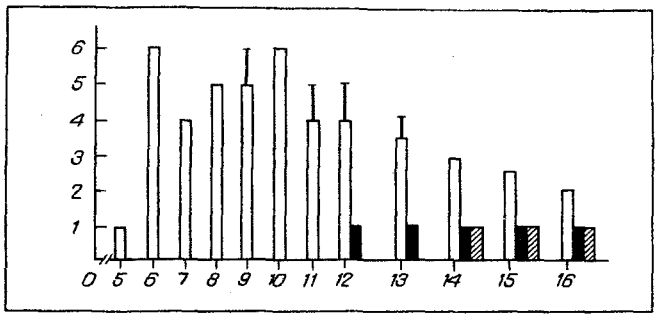

Fig. 2. Severity of seizure reaction in randomized rats receiving daily injections of metrazol in a subconvulsive dose after preliminary injection of physiological saline, DMSO, and IOS-1.1212 in a dose of 10 mg/kg. Here and in Fig. 3: abscissa, duration of experiment (in days); ordinate, mean severity of seizures (in points); unshaded column – physiological saline, black column – DMSO, obliquely shaded – IOS-1.1212.

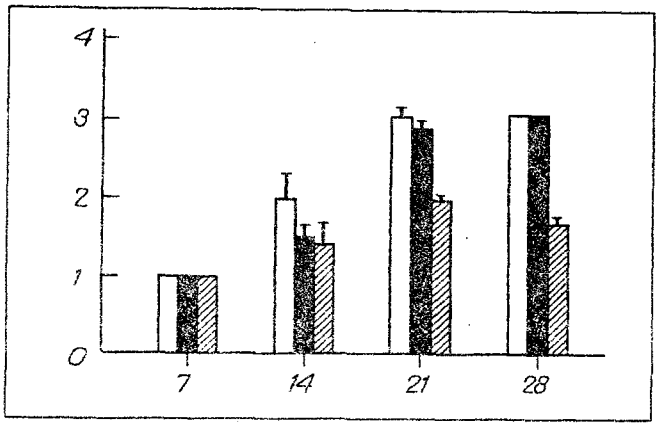

Fig. 3. Severity of seizure reaction in animals relatively sensitive to metrazol, in response to injection of the latter in a subconvulsive dose after preliminary injection of physiological saline, DMSO, and IOS-1.1212 in a dose of 2 mg/kg.

3). Differences began to be noted and were significant in the 3rd week (after the 17th day); they also persisted during subsequent injections of metrazol (4 weeks).

Thus IOS-1.1212 has an antiepileptic action against acute generalized tonicoclonic convulsions induced by metrazol, in cases of chronic exposure to an epileptogen in the form of metrazol kindling, and in focal penicillin-induced EpA. Meanwhile the compound had no effect on strychnine-induced focal and generalized EpA. The differences found with regard to the efficacy of the compound in metrazol-, penicillin-, and strychnine-induced EpA were evidently due to differences in the trigger mechanisms of the seizure action of these convulsants: the effects of penicillin and metrazol are linked with suppression of GABA-ergic inhibition [7, 14], whereas those of strychnine are linked with glycinergic inhibition [6, 15]. The spectrum of the anticonvulsant action of IOS-1.1212 coincides with the analogous anticonvulsant activity of dihydropyridine calcium antagonists such as nimodipine and nitrendipine, which likewise had no effect on strychnine-induced seizure activity [9].

The writers showed previously that riodipine, a new preparation belonging to the 1,4-dihydropyridine class, also has a suppressive action of EpA [3]. The IOS-1.1212 used in the present investigation not only has an inhibitory effect on EpA, but also leads to normalization of the cerebral circulation [10]. A further search for preparations of this and other classes which not only possess antiepileptic activity, but also have a positive effect on the hemodynamics and metabolism of the brain, which are altered in convulsive processes, would seem to be promising.

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ACTION OF MORPHINE ON NEURONAL INPUT SYSTEMS OF THE SPINAL CORD INVOLVED IN NOCICEPTIVE PRESSOR REFLEX FORMATION

I. A. Belyantseva and E. V. Lukoshkova

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The complex defensive reaction evoked by nociceptive afferent stimuli of somatic nerves includes, besides various motor reflexes, autonomic reflexes, of which one is a rapid rise of arterial blood pressure (BP) [12]. Morphine, which acts in the region of entry of afferent stimuli into the brain, namely through opiate receptors of posterior horn neurons of the spinal cord (intrathecal injection), can suppress both the sensation of pain and the motor components of this reaction [13]. Meanwhile it has been reported that the circulatory component of the defensive reaction, consisting of powerful pressor reflexes developing in response to electrical stimulation of somatic nerves, is not reduced after intrathecal injection of morphine [1, 7]. This dissociation in the action of morphine on transmission of nociceptive stimuli in the spinal cord appears enigmatic, more especially if it is recalled that: 1) after intravenous injection morphine reduces pressor reflexes developing in response to electrical stimulation of (A + C)-afferents of somatic nerves [2]; 2) if injected either intravenously [2, 8] or intrathecally, namely into the region of entry of the stimulated nerves into the brain [10], morphine suppresses reflex responses of sympathetic neurons evoked by volleys of somatic C-afferents; 3) a reflex rise of BP under general anesthesia is induced by stimuli from somatic afferents of this same type [4, 5, 9, 11]. It can accordingly be postulated that opiate receptors are present in the membrane of some of the input neurons of the spinal cord involved in the formation of nociceptive pressor reflexes, and their activation should lead to weakening of these reflexes.

Laboratory of Biomechanics and Regulation of the Circulation, Institute of Experimental Cardiology, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR V. N. Smirnov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 112, No. 10, pp. 365-368, October, 1991. Original article submitted January 16, 1991.

