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


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Increased admission of Ca²⁺ 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'-propoxyethoxy carbonyl)-4-(2-difluormethoxyphenyl)-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 ECoG 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.
TABLE 1. Effect of IOS-1.1212 on Focal EpA Induced by Application of Penicillin to Rat Cerebral Cortex (M ± m)

<table>
<thead>
<tr>
<th>Group of animals</th>
<th>Number of animals</th>
<th>Before injection</th>
<th>20-30 min after injection</th>
<th>Duration of existence of focus, min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>number of IID in 1 min</td>
<td>amplitude of IID, μV</td>
<td>number of IID in 1 min</td>
</tr>
<tr>
<td>1) Control, physiological saline</td>
<td>6</td>
<td>12.76±1.12</td>
<td>1266±114</td>
<td>0.43±0.05</td>
</tr>
<tr>
<td>2) Control, DMSO</td>
<td>11</td>
<td>12.54±1.37</td>
<td>1402±272</td>
<td>0.44±0.09</td>
</tr>
<tr>
<td>3) Experiment, 2 mg/kg</td>
<td>6/7</td>
<td>13.00±2.22</td>
<td>1330±151</td>
<td>0.34±0.06</td>
</tr>
<tr>
<td>4) Experiment, 10 mg/kg</td>
<td>12/15</td>
<td>12.21±1.24</td>
<td>876±77</td>
<td>0.42±0.07</td>
</tr>
</tbody>
</table>

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 strychnine 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