The Effect of Isradipine on Maximal Electroshock Seizures in Mice

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ABSTRACT. 1. The aim of this study was to investigate the possible anticonvulsant effect of a dihydropyridine calcium antagonist, isradipine, which easily crosses the blood–brain barrier displaying high affinity and specificity for the brain L-type voltage-sensitive calcium channel, on maximal electroshock seizures in mice.

2. Isradipine at IP doses of 2.5 mg/kg and 5.0 mg/kg was found to cause a statistically significant increase in the convulsion threshold of maximal electroshock seizures in a dose-dependent manner (P=0.047 and P=0.022, respectively).

3. It was concluded that the mode of action of the anticonvulsant effect of isradipine is related to blockade of the intraneuronal calcium currents, which play an important role in epileptic activity.

KEY WORDS. Isradipine, maximal electroshock seizure, mice

INTRODUCTION

There is evidence that central nervous system excitability and various ions play important roles in the induction of epileptic activity. Regulation of the brain excitability is mediated by the inhibitory systems acting through γ-aminobutyric acid A (GABA A) and glycine receptors coupled to the calcium channels (Grenningloh et al., 1987; Scheffield et al., 1987); by postsynaptic GABA B (Andrade et al., 1986), (α-2-adrenergic and μ-opioid (Aghajanian and Wang, 1986) receptors coupled to the potassium channels by G proteins; and by the excitatory systems acting through the N-methyl-D-aspartate, quisqualate and kainate receptors (Davies et al., 1987; Jones et al., 1984; McLennan, 1983; Watkins and Evans, 1981) stimulated by amino acids such as glutamate and aspartate. Because most of these receptors are coupled to Na+, K+, Cl− and especially Ca2+ channels, agents modifying conductance of these ions play an important role in producing or preventing epileptic activity.

There are many studies about the agents or procedures that increase the intraneuronal influx of calcium ions, which is an important factor for the induction of epileptic activity, causing epileptiform activity (Heinemann and Louvel, 1983; Heinemann and Pumain, 1981; Hotson and Prince, 1981; Pumain et al., 1983; Shelton et al., 1987), and, conversely, that block calcium channels and are effective against experimental epileptiform models such as electroconvulsions (Desmott et al., 1975), sound-induced seizures (De Sarro et al., 1988), amygdalar and hippocampal kindling (Ashton and Wauquier, 1979; Vezani et al., 1988), pentylentetrazole convulsions (Dolin et al., 1988; Meyer et al., 1986) and high-pressure-induced seizures (Dolin et al., 1988). In addition, calcium channel inhibitors have also been found to antagonize BAY-K 8644-induced seizures potently (Shelton et al., 1987).

Dihydropyridine calcium antagonists display high affinity and specificity for L-type calcium channels (Gaggi et al., 1995a, 1995b; Van-Luijtelea et al., 1995) and, among the voltage-sensitive calcium channel subtypes, for L, N and T (Schwartz, 1992; Tsien et al., 1988).

The aim of this study is to examine the effect of the dihydropyridine-derivative calcium-channel antagonist isradipine, which easily crosses the blood–brain barrier, displaying high affinity and specificity for the brain’s L-type voltage-sensitive calcium channels (Gaggi et al., 1995a, 1995b), on maximal electroshock seizures in mice.

MATERIALS AND METHODS

In this study, male and female adult Swiss albino mice weighing 20–30 g were used (n=140). These mice were not reused in subsequent experiments.

The electroshocks were evoked through a current transmitter producing 60-Hz square waves (Ugo Basile, ECT unit). Flow duration of the current and the duration of each square wave were fixed at 0.2 sec and at 0.4 msec, respectively (Swinyard et al., 1952, 1963). During the shock, electrodes were attached to each animal’s ears, and the animals lay on their backs, their tails being fixed. Thus, observation of the tonic and clonic convulsions that appeared during the seizure was assured (Ewart, 1972). In mice, maximal electroshock seizures consist of a latency period lasting 1.6 sec followed by a short initial flexion period, then 13.2 sec of tonic hindlimb extension and 7.6 sec of terminal clonus. Total duration of the seizure is 22.3 sec (Swinyard et al., 1963). The shock was applied in the afternoon between 2:00 and 5:00 p.m.

At the beginning of our study, convulsive current 50 (CC 50) value, 46 mA, was determined from our previous studies, and its confidence interval was calculated by the Litchfield and Wilcoxon method (Akkan et al., 1988; Eşkan et al., 1990; Litchfield and Wilcoxon, 1949; Özyazgan et al., 1992).

The subjects of our study were divided into four groups, and they were given intraperitoneal saline (n=50), 1.25 mg/kg (n=30), 2.5
TABLE 1. Effect of intraperitoneally applied isradipine on maximal electroshock seizures in mice

<table>
<thead>
<tr>
<th>Dose (mg/kg) IP</th>
<th>n</th>
<th>Number</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (saline)</td>
<td>50</td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td>Isradipine (1.25)</td>
<td>30</td>
<td>15</td>
<td>50</td>
</tr>
<tr>
<td>Isradipine (2.5)</td>
<td>30</td>
<td>8</td>
<td>27*</td>
</tr>
<tr>
<td>Isradipine (5.0)</td>
<td>30</td>
<td>7</td>
<td>23***</td>
</tr>
</tbody>
</table>

P = 0.047; **P = 0.022.

mg/kg (n=30) or 5.0 mg/kg (n=30) isradipine, respectively. Electroshock applications were performed 1.5 hr (tmax) after the injections.

The chi-square method (with the Yates correction) was used in the statistical analyses.

RESULTS

As expected, half of the mice in the control group seizure after applying the CC50. Percentages of the seized mice in the groups given IP isradipine at doses of 1.25 mg/kg, 2.5 mg/kg and 5.0 mg/kg were 50%, 27% (P=0.047) and 23% (P=0.002), respectively. In the groups treated with 2.5 mg/kg and 5.0 mg/kg IP isradipine, the number of seized mice significantly decreased in a dose-dependent manner. In other words, the maximal electroshock seizure threshold significantly increased in these groups.

All findings are shown in Table 1 and Figure 1.

DISCUSSION

Excitatory and inhibitory systems regulating the central nervous system excitability and agents or procedures affecting the ion conductance, such as Na+, K+, Cl- and Ca2+, play an important role in the induction of epileptic activity (Andrade et al., 1986; Davies et al., 1982; Genninholoh et al., 1987; Watkins and Evans, 1981). Activities of all these systems are regulated largely by ion channels such as Na+ and Ca2+ channels. Therefore, agents affecting these ion channels may cause an increase or blockade in the epileptic activity (De Sarro et al., 1988; Dolin et al., 1988; Heinemann and Pumain, 1981; Pumain et al., 1983; Vezzani et al., 1988).

Clinically used antiepileptic drugs are known to affect calcium channels, as well as sodium channels (MacDonald and McLean, 1986). Calcium entry into the neurons has been shown to be through three different voltage-dependent calcium channels called L, N and T (Gaggi et al., 1995a, 1995b; Tsien et al., 1988). These calcium channels differ in their voltage dependency for activation and inactivation, rates of inactivation and individual channel conductances. In addition, they have different agonist and antagonist pharmacology. L-channel conductance is large, and L current is long lasting and slowly inactivated (MacDonald and Meldrum, 1989). Phenytoin and barbiturates block both L and N currents without affecting the T current (Gross and MacDonald, 1987; Gross et al., 1989; MacLean and MacDonald, 1983); trimethadione and ethosuximide affect the T current (Gross et al., 1989). The dihydropyridine-derivative calcium-channel blocker used in this study, isradipine, easily crosses the blood–brain barrier and displays high affinity for cerebral L-type voltage-sensitive calcium channels (Carboni and Wójcik, 1988; Gaggi et al., 1995a, 1995b). Studies show that dihydropyridine-derivative calcium-channel blockers do not have such effects (Karpova et al., 1993; Kryzhánovski et al., 1993a, 1993b; Palmer et al., 1993). In another study, convulsions caused by the dihydropyridine-derivative L-type voltage-dependent calcium channel agonist BAY K-8644 were significantly lowered in these groups. Our results are consistent with the findings in the literature. In many studies, dihydropyridines (nifedipine, rilapride, etc.) were demonstrated to prevent maximal electroshock seizures in mice and to potentiate the antiepileptic effects of barbital, phenytoin and valproate, but the differently structured calcium channel blockers, such as verapamil and diltiazem, do not have such effects (Karpova et al., 1993; Kryzhánovski et al., 1993a, 1993b; Palmer et al., 1993). In another study, convulsions caused by the dihydropyridine-derivative L-type voltage-dependent calcium channel agonist BAY K-8644 were claimed to be antagonized by calcium channel blockers (Shelton et al., 1987). In addition, anticonvulsant effects of the calcium channel blockers were established in other experimental epilepsy models (Ashton and Wauquier, 1979; De Sarro et al., 1988; Desmedt et al., 1975; Dolin et al., 1988; Vezzani, 1988). In another study, however, it was claimed that nimodipine, a dihydropyridine-derivative calcium-channel blocker, did not prevent electroshock seizures in mice, in contrast with previous studies (Sills et al., 1994).

Considering the data in the literature (Larkin et al., 1988; Overweg et al., 1984, 1986) and results of this study, we concluded that calcium channel blockers—especially dihydropyridine derivatives—might be useful in certain epilepsy types in humans and might be combined with barbital, phenytoin or barbiturates, especially in the therapy-resistant cases. However, further studies are needed for the use of the calcium channel blockers in some types of human epilepsy.

References


