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The effect of tizanidine on maximal electroshock seizures (MES) in mice

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Abstract

Tizanidine, an α-2 adrenergic agonist, is a centrally active muscle relaxant and a spasmolytic drug. The aim of our study was to investigate the activity of tizanidine on maximal electroshock seizures (MES) in mice. In the first part of the study, convulsive current 50 (CC 50) value to produce seizures was found. Then, tizanidine was given intraperitoneally (IP) at the doses of 0.5, 1, and 2 mg/kg, and orally (PO) at the doses of 5, 10, 20, 40 mg/kg. We found that tizanidine at the doses of 1 and 2 mg/kg IP and 40 mg/kg PO caused a significant protection against MES. In the second part of the study, after pretreatment with yohimbine, an α-2 adrenergic receptor blocker, at the dose of 2 mg/kg, anticonvulsant effect of tizanidine is diminished. We concluded that the mode of action of the anticonvulsant effect of tizanidine may be mediated by the central α-2 adrenergic receptors.

Keywords: Tizanidine; Maximal electroshock seizures; Mice; Yohimbine; α-2 adenergic; Receptor

Tizanidine is a centrally active muscle relaxant which is an α-2 adrenergic agonist. It differs from other muscle relaxant drugs due to its different chemical structure and its mechanism of action. Its chemical structure is 5-chloro-4-(2-imidazolin-2yl-amino)2,1,3-benzothiazole (Sayers et al., 1980). Tizanidine reduces increased muscle tone by depressing the polysynaptic pathways that activate the motor units (Sayers et al., 1980). Its site of action is shown to be both spinal and supraspinal structures (Davies et al., 1982). The increased muscle tone is caused by the hyperactivity of the α and γ motor systems depressing the polysynaptic neurons (Newman et al., 1982). Furthermore, recent studies show that its muscle relaxant actions may occur through its effect on the basal ganglia, particularly substantia nigra reticulata and entopeduncular nucleus (Fromm et al., 1993; Türski et al., 1986). The muscle relaxant effect of tizanidine may be due to the presynaptic modulation of excitatory amino acid release from the spinal interneurons via interaction with central noradrenergic α-2 receptors (Lang and Riley, 1992). In addition it is observed that during the clinical treatment with tizanidine the level of MHPG (3-methoxy-4-hydroxyphenyglycol) decreases significantly CNS. This compound is the metabolite of noradrenaline produced in the CNS. This result can be explained on the basis of tizanidine being a centrally active α-2 agonist reducing the release of noradrenaline in the CNS. It shows that tizanidine decreases the turnover of the central noradrenaline (Davies et al., 1983). It was also suggested that tizanidine causes an increase in the content 5-hydroxytriptamine (5-HT) (Sayers et al., 1980).

Tizanidine was reported to have anticonvulsant properties in audiogenic seizures in DBA/2 mice, photically-induced epilepsy of Senegalese baboons, and seizures induced by strychnine in mice (Sayers et al., 1980). The aim of our study was to show anticonvulsant activity of tizanidine in electrically-induced seizures in mice, and to ascertain whether there was an interaction of tizanidine with α-2 adrenoceptors.

1. Materials and methods

In this study, Swiss albino mice of either sex weighing between 20–30 g were used. Each experiment and control group consisted of 40 mice. We used a stimulator that produces 60 Hz square wave (ECT unit, Ugo Basile) to produce electroshock seizures. During the shock,

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the duration of the current was 0.2 sec and the duration of each square wave was 0.4 msec (Swinyard et al., 1952). All seizures were produced at definite daytime, between 1:00–3:00 P.M.

In mice, maximal electroshock seizures (MES) include a latency period lasting 1.6 sec followed by a short flexion period, the tonic extension of hindlimbs lasting about 13.2 sec, and the terminal clonus lasting for about 7.6 sec. Total duration of the seizure is 22.3 sec (Swinyard et al., 1963).

In the MES experiments, first convulsive current 50 (CC 50) values were found. CC 50 value shows the current necessary to produce generalized seizures in 50% of mice. In order to calculate this value, different groups of mice were stimulated by currents of 30, 40, 50, 60, and 46 mA. Using Litchfield and Wilcoxon method, the percentage value of the frequency of positive signs were plotted on a semilogarithmic scale. It was found out that 46 mA was the current at which 50% of mice had seizures as it was shown before (Akkan et al., 1988). All of the experiment were carried on by stimulating the mice by 46 mA.

Chi-square test was used in the statistical analysis to compare the percentage of positive signs in the experiment groups with the control. In the first part of the experiments, tizanidine was given IP at doses of 0.5, 1, and 2 mg/kg and orally at the doses of 5, 10, 20, and 40 mg/kg. In the second part, we administered 2 mg/kg yohimbine to find out the interaction between tizanidine and α-2 adrenoceptors.

2. Results

2.1. Intraperitoneal treatment with tizanidine

We stimulated the mice 30 min after IP administration. 0.5, 1, and 2 mg/kg tizanidine were given IP and the frequencies of convulsions were 35%, 22.5%, and 10%, respectively. We compared this group with a control group treated with 0.5 ml sterile saline solution IP. Chi-square values were 1.278, 5.409, and 13.393, respectively. Treatment with 1 and 2 mg/kg IP tizanidine produced significant protection against MES ($p < 0.01$ and $p < 0.001$, respectively) (Fig. 1).

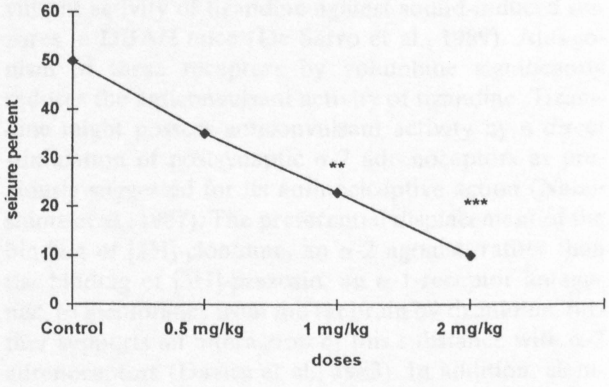

Fig. 1. The dose response curve of the tizanidine given IP. Treatment with 1 and 2 mg/kg tizanidine produced significant protection against MES. Horizontal axis shows the doses given and vertical axis shows the percentage of seizures observed ($n = 40$ in each group). *$p < 0.1$, **$p < 0.01$, ***$p < 0.001$, relative to control.

2.2. Oral treatment with tizanidine

We stimulated mice 2 h after oral tizanidine administration at the doses of 5, 10, 20, and 40 mg/kg. The frequencies of convulsions were 52.5%, 47.5%, 32.5%, and 15%, respectively. The control group was treated with 0.5 ml sterile saline solution PO. Chi-square values were 0, 0, 1.856, and 9.629, respectively. Tizanidine given 40 mg/kg PO protects against MES ($p < 0.001$), after 2 h (Fig. 2).

In another group we produced MES 3 h after oral tizanidine administration at the doses of 10, 20, and 40 mg/kg to find out long-term pharmacological effects. The frequencies of convulsions were 52.5%, 32.5% and 10%, respectively. Chi-square values were 0, 1.856, and 9.649, respectively. We again observed that 40 mg/kg tizanidine was effective in protection ($p < 0.001$) (Fig. 2).

2.3. Oral treatment with yohimbine

We produced MES 2 h after 2 mg/kg yohimbine administration PO. This drug was chosen as the α-2 adrenoceptor antagonist. This dose was used on the account that LD 50 of the drug was 40 mg/kg when PO. We observed that 85% of the mice had seizures ($\chi^2 = 9.629$, $p < 0.01$). The control group was treated with 0.5 ml sterile saline solution PO (Fig. 3).

2.4. Pretreatment with yohimbine

We treated mice first with 2 mg/kg yohimbine PO and then with 2 mg/kg tizanidine IP. The frequency of

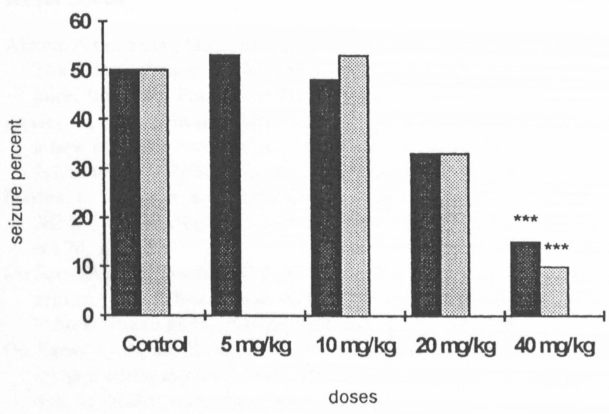

Fig. 2. The comparison of the effect of tizanidine given orally on MES after 2 h (darker columns) and 3 h (lighter columns). It is shown that there is a significant between the control group and tizanidine groups at the dose 40 mg/kg after 2 and 3 h. Horizontal axis shows the doses given and vertical axis shows the percentage of seizures observed ($n = 40$ in each group). *$p < 0.1$, **$p < 0.01$, ***$p < 0.001$, relative to control.

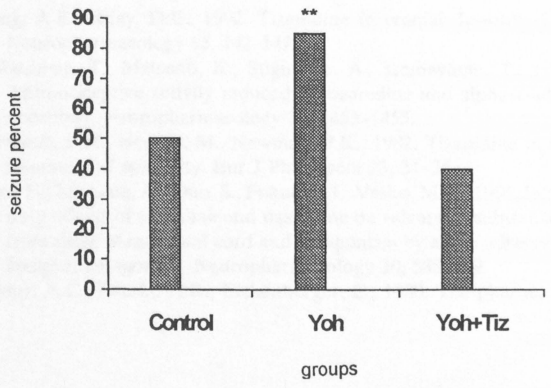

Fig. 3. The comparison of the effect of yohimbine (Yoh) and yohimbine and tizanidine (Yoh + Tz) on the control of MES. Treatment with yohimbine significantly increased seizures whereas tizanidine reversed this effect back to the control level. Horizontal axis shows the doses given and vertical axis shows the percentage of seizures observed (n = 40 in each group). **$p < 0.01$, relative to control.

the seizures was 40% which was not significantly different from the control group treated only with 0.5 ml sterile saline solution ($\chi^2 = 0.455$, $p > 0.05$). Thus it was shown that pretreatment with yohimbine reduced the anticonvulsant effect of tizanidine (Fig. 3).

3. Discussion

The present results show that α-2 adrenergic receptors may be important in mediating the anticonvulsant activity of tizanidine against MES in Swiss albino mice. This effect may be observed when the drug is PO or IP. Although there is an observation that when tizanidine is PO its maximum plasma concentration is achieved after 3 h in human beings, we observed no difference between its effect after 2 h and 3 h in mice. As Hutchinson showed, we assume that it has a long plateau concentration in the plasma after it is PO (Hutchinson, 1989).

α-2 adrenergic receptors may mediate the anticonvulsant activity of tizandine against sound-induced seizures in DBA/2 mice (De Sarro et al., 1989). Antagonism of these receptors by yohimbine significantly reduces the anticonvulsant activity of tizandine. Tizanidine might possess anticonvulsant activity by a direct stimulation of postsynaptic α-2 adrenoceptors as previously suggested for its antinociceptive action (Nabeshima et al., 1987). The preferential displacement of the binding of [3H]-clonidine, an α-2 agonist, rather than the binding of [3H]-prazosin, an α-1-receptor antagonist, to membranes from the rat brain by tizanidine, further supports an interaction of this substance with α-2 adrenoceptors (Davies et al., 1983). In addition, clonidine which has structural features in common with those of tizanidine and in common with those of tizanidine and interacts with α-2 adrenoceptors, is well known for producing an anticonvulsant action.

Several other mechanisms may contribute to the anticonvulsant activity of tizanidine. It was suggested that anticonvulsant action of tizanidine against strychnine-induced seizures was mediated by increasing glycine neurotransmission (Sayers et al., 1980). Tizanidine has no direct effect on the GABA system since it has been shown to have no effect on biciculine-induced seizures in mice (De Sarro, 1989). Although it is observed that tizanidine reduces aspartate release rat spinal cord slices, there is no evidence that tizanidine interacts with excitatory amino acids in the CNS (Davies et al., 1983; Davies et al., 1982).

Finally, clonidine blocks the depressant responses to purines in the cerebral cortex of the rat (Ono et al., 1991). This effect occurs at much smaller doses of clonidine than those which affect responses to noradrenaline or serotonin.

Tizanidine possesses chemical structural similarities with clonidine and both might act on purinergic neurotransmission (De Sarro et al., 1984). Aminophyline antagonized the anticonvulsant, sedative, and hypothermic effects of tizanidine and did not show a direct proconvulsant action when 25 mg/kg 15 min after tizanidine administration in DBA/2 mice (De Sarro et al., 1989). The role of purinergic system on the anticonvulsant activity of tizanidine on MES needs to be established. Clonidine and other α-adrenoceptor agonists may act at a presynaptic level at dosages used as anticonvulsant compounds (Fogelholm and Murros, 1992). However, yohimbine used in this study has been suggested to block postsynaptic adrenoceptors. Thus it seems possible that at the doses used, tizanidine may be acting postsynaptically and its reversal by yohimbine may be due to postsynaptic antagonism.

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