MODULATION OF CONVULSIVE THRESHOLD OF PENTYLENE TETRAZOLE BY ZINC

N. KHANNA, A. GARG, K.K. SHARMA AND R. KHOSLA

Department of Pharmacology, University College of Medical Sciences & G.T.B. Hospital, Delhi-110 095

ABSTRACT

In the present study which was aimed to see the effect of zinc on pentylenetetrazole induced convulsive threshold in rats we found that zinc sulfate 100 µg intracerebroventricularly and pentylenetetrazole (50 mg/kg intraperitoneally) produced dose related seizure activity ; however, pretreatment with zinc decreased the threshold, increased the severity, incidence of multiple seizures and total duration of pentylenetetrazole induced seizures. Diazepam (5 mg/kg intraperitoneally) pretreatment increased the threshold and lowered the incidence of convulsions in zinc induced seizures which further confirms that the convulsive effect of zinc is through inhibition of gamma amino butyric acid.

KEY WORDS : Zinc, Pentylentetrazole, Diazepam, GABA.

INTRODUCTION

Various electrical and chemical techniques have been devised to induce convulsions in laboratory animals as models of epilepsy. Electroshock methods include supramaximal electroshock, psychomotor electroshock, minimal electroshock threshold, focal stimulation of cortex, isolated cortical stabs etc. In chemical methods various chemicals like pentylenetetrazole (PTZ, metrazol, leptazol, cadiozol, corazol), strychnine, isoniazid, nicotine, caffeine etc. are injected. These methods are useful only as acute experimental models.

Chronic experimental models of epilepsy in animals have been reported. They are produced by application of cobalt, alumina oxide and penicillin to different areas of the brains of animals such as monkeys, cats and rats (1).

Only preliminary data is available—that too electrophysiological only which implicate zinc in epileptic

electrical activity in brain. There are other preliminary evidences also which point out the interference of gamma amino butyric acid (GABA) transmission by zinc. Chemoconvulsions induced by pentylenetetrazole is a well established model of clinical petitmal epilepsy. But it is not known what is the effect of changing concentration of zinc in the brain on the convulsive threshold of pentylenetetrazole induced convulsions. Therefore, it was decided to study the effect of zinc on pentylenetetrazole induced convulsive threshold in rats.

MATERIALS AND METHODS

In the present study healthy adult male wistar rats weighing 180-220 g were used. The animals were housed in temperature and light controlled conditions and had free access to food and water. Food (but not water) was withheld 12 hours prior to the experiment.

The various drugs used for this study were zinc sulfate, pentylenetetrazole diazepam. They were dissolved in saline. Zinc sulfate was administered intracerebroventricularly (i.c.v.) while the other drugs were given intraperitoneally (i.p.). Zinc sulfate was given in a dose of 10-1000 µg. For i.c.v adminis-

Address for correspondence

Dr. N. Khanna, Reader, Deptt. of Pharmacology, University College of Medical Sciences & G.T.B. Hospital, Delhi-110 095

tration of zinc sulfate a polythene cannula was chronically implanted in the left lateral ventricle as described by Noble, Wurthman and Axelrod (2). Rats were allowed two days to recover postoperatively. The drug was injected into the ventricle slowly with the aid of a Hamilton micro syringe in constant volume of 10 μ l. Pentylenetetrazole was given in a dose of 50-125 mg/kg. i.p. and idazepam was given 5 mg/kg i.p. one hour before zinc sulfate was given.

The control group represented treatment with pentylenetetrazole only while the experimental group was pretreated with zinc sulfate 100 μ g, 30 minutes prior to pentylenetetrazole treatment.

Continuous observations were made with reference to general behavior, time of onset, type, duration and disappearance of seizures.

RESULTS

In order to study the effect of zinc per se on convulsions, zinc sulfate was administered in escalating doses of 10-1000 μ g. Figure 1 is showing graded dose response of zinc sulfate in rats. Zinc sulfate in a low dose of 10 μ g shows no seizure activity and mortality but in dose of 100 μ g, there was seizure activity in 60% and death in 16% of the animals, whereas higher doses of 500 and 1000 μ g 100% seizure activity and mortality.

The i.c.v. administration of zinc sulfate also elicited behavioural changes in rats which were dose dependent as shown in Table 1. At low dose of 10 μ g rats showed stereotyped behaviour while at the

dose of 100 μ g there was jumping, vocalization and clonic convulsions in the animals. At the subsequent higher dose of 500 μ g there was jumping, running, vocalization, circling, extension of limbs followed by clonic convulsions and the animals died within one hour. At the highest dose tested i.e. 1000 μ g there were severe tonic clonic convulsions and the animal died within 15 minutes.

Pentylenetetrazole was given i.p. in gradually increasing doses and incidence of seizures and death was recorded as shown in Figure 2. Low doses of pentylenetetrazole i.e. 50 and 75 mg/kg produced seizures in 60 and 100 per cent of rats respectively and there was no mortality. In higher doses of 100 and 125 mg/kg, convulsions were seen in 100% and death in 30% and 100% of the animals respectively.

When low doses of zinc sulfate (100 μ g) and pentylenetetrazole (50 mg/kg) were given together there was approximately 100% seizure activity and moreover the duration of seizure activity was increased to 153 minutes from 50 minutes as shown in Figure 3. The behavioural changes consisted of jerks, circling movement, clonic convulsions, body bended towards contralateral side and straub tail phenomena was also observed.

Pretreatment of rats with diazepam 5 mg/kg i.p. one hour prior to zinc sulfate 100 mg i.c.v. reduced the incidence of seizures from 84 to 50% (Figure 4). Moreover the onset of seizures was increased from less than 10 minutes for zinc sulfate treated group to more than 90 minutes.

Table 1 : Effect of intraventricular injection of zinc on rat behaviour.

Dose (μ g)	Behavioural effects
10	Stereotyped behaviour
100	Jumping, running, vocalization, clonic convulsions
500	Jumping, running, vocalization, circling, extended limbs, clonic convulsions, died in 1 h
1000	Severe tonic clonic convulsions, died at 15 minutes

DISCUSSION

The possible involvement of trace metals in convulsive disorders has been given more attention in recent years (3). Studies suggest that post-traumatic epilepsy may be related to iron released from haemoglobin. Intracortical injection of purified bovine hemoglobin produced chronic foetal spike activity in rats (4). Sub-pial or cortical infection of ferrous or ferric chloride into cat, rat and monkey can produce epileptiform discharges (5, 6). Intracerebral injection of ferrous sulfate into rabbits or cortical iron injection into rats can produce recurrent seizures, used as model of post-traumatic epilepsy (7, 8). These materials showed that iron may be causally related to the development of trauma-induced epileptiform focus in humans.

Zinc is another important trace metal in the human body. The participation of zinc in seizure initiation and propagation has been demonstrated by several experimental studies in animals. Mice maintained on a zinc deficient diet display significant protection against electrically induced seizure, whereas mice treated with zinc experience an exacerbation of seizure activity (9). The serum zinc level was found to be higher in baboons prone to seizures than in nonsusceptible animals; D-penicillamine afforded protection against such seizures (10). Intracortical injection of zinc in rats or intracerebral injection of zinc in rabbits can produce convulsions or generalized clonic seizures (11, 12). All of these investigations suggest that zinc metabolism may be altered in patients and in animals with convulsive disorders.

Pentylenetetrazole is a potent convulsant commonly used to produce epileptiform discharges in animal nervous systems. It is able to induce two different types of seizures in rodents: (1) minor metrazole seizures (clonic) considered to be a model of human absence type seizures by some authors (13) and (2) major, i.e. generalized tonic-clonic seizures that could be elicited by higher doses of pentylenetetrazole.

The mechanism by which pentylenetetrazole provokes convulsions in animals has been investigated by measuring its influence in vitro on the activities of several enzymes of glutamate metabolism in rat homogenates (14). Pentylenetetrazole does not affect the specific activities of glutamine

synthetase, glutaminase, or glutamate decarboxylase; it inhibits those of glutamate dehydrogenase and aspartate aminotransferase, and stimulates γ -amino butyric acid (GABA) aminotransferase (14). The overall consequence of the action of pentylenetetrazole on the activities of these enzymes should be an increase in the concentration of glutamate and a decrease in that of GABA (14). This modulation of glutamate and GABA metabolism by pentylenetetrazole could contribute to the triggering of convulsions (14).

In our study, we observed that both zinc sulfate and pentylenetetrazole are convulsants in Wistar rats and produce dose related seizure activity. Low doses of zinc sulfate (100 μ g i.c.v.) and pentylenetetrazole (50 Mg/kg i.p.) which were producing convulsions in approximately 60% of the rats were selected to check whether there was any potentiation of seizure activity. When zinc sulfate was given 30 minutes prior to pentylenetetrazole; the combination produced approximately 100% seizure activity and moreover the duration of seizure activity was increased to 153 minutes from 50 minutes. The pretreatment with zinc decreased the threshold, increased the severity, incidence of multiple seizures and total duration of pentylenetetrazole induced seizures.

Since pentylenetetrazole causes decrease in the concentration of GABA (14) and preliminary evidence is available which points out the interference in GABA transmission by zinc and since diazepam, a standard anticonvulsant has an indirect GABA mimetic action by increasing its release or by facilitating its actions; we injected diazepam in a dose of 5 mg/kg one hour before zinc sulfate and observed that incidence of seizures which was 84% in zinc treated rats came down to 50%. Moreover the onset of seizures was increased from less than 10 minutes for zinc treated group to more than 90 minutes.

On the basis of our study which was aimed to see the effect of zinc on pentylenetetrazole induced convulsive threshold it appears that zinc sulfate has a proconvulsant effect on pentylenetetrazole induced seizures in rats and the convulsive effect of zinc is decreased by diazepam which further confirms that the convulsive effect of zinc is probably mediated through interference of GABA transmission.

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