The Effect of Zinc on the Central Nervous System : Increase in Brain Excitability and Initiation of Seizures Following Systemic and Intracerebral Administration of Zinc

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INTRODUCTION

The significance of zinc, a ubiquitous and essential element, in the biological systems has commanded increasing attention in recent years.

In 1951, TOKUOKA[25] reported that the caudal resection of the pancreas was effective in alleviating the epileptic seizures in genuine epilepsy, and assumed that the disturbance of zinc metabolism caused by this procedure might have some connection with the alleviating effect. Since then, in our laboratory, the relationship of zinc to brain excitability has been studied experimentally. NAGASUE (1957)[15] observed that zinc deficiency elevated the Metrazol-seizure threshold, while zinc intoxication lowered it, in the sucking mice. However, the mechanism of action of zinc on brain excitability was poorly explained.

Since zinc does not readily cross the blood-brain barrier,[20] systemic administration of zinc is insufficient to investigate its direct effect on the brain. Haley and McCormick (1957)[9] used intracerebral injection technique to study the central effects produced by several drugs in conscious mice. This method seems to answer the purpose.

The present study is undertaken in an attempt to elucidate the effects of systemically and intracerebrally injected zinc on the central nervous system in the neuropharmacological aspects.

MATERIAL AND METHODS

All experiments were performed on adult male mice of dd strain, 18 to 25 g in weight, at room temperature (17-21°C). The animals were maintained on

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Oriental Chow and were allowed free access to food and water throughout the experiments. Anaesthesia was not used in all procedures.

Systemic administration of zinc. Zinc chloride was dissolved in 0.9 % NaCl solution, adjusted to pH 2.0 by adding HCl. In one series of experiments, zinc was given subcutaneously in a single dose of 0.5, 2 and 12 mg kg body weight. In another series, the animals received subcutaneous injection of zinc in a daily dose of 2 mg/kg for 2 weeks and was tested 18 to 20 hours after the last injection. The injection volume was 0.05 ml. In each series control mice received the same volume of 0.9 % NaCl solution (pH 2.0).

Adrenalectomy. Adrenalectomy was performed under ether anesthesia on the day before the experiment, through two separate lumbar incisions.

The operated mice were maintained on 0.9 % NaCl solution in drinking water. On successive administration of zinc, the animals received a daily dose of 2 μg of dexamethasone phosphate (Decadron).

Determination of brain excitability. The electroshock seizure threshold (EST) was measured using Woodbury and Davenport's apparatus (1952).[34] As the electrical stimulus, 60 cycle/sec. alternating-current was applied by means of corneal electrodes for 0.2 sec. As the amount of the current is increased, the response to electroshock changes as follows[34] : (1) no response ; (2) furor, characterized by violent running or hopping movements and shrill squealing ; (3) subthreshold stunning ; (4) minimal or threshold seizure, characterized by facial clonus and rhythmic movements of the jaws and ears ; (5) submaximal seizures, pattern of which is clonic type ; (6) maximal seizures, consisting of the tonic flexor phase of the hindlimbs, the tonic extensor phase of the hindlimbs and the clonic phase of the entire body. According to Takahashi et al. (1961),[24] EST was defined as the current to produce clonic movements involving the head and forelimbs only.

For determination of the EST, the current required to evoke the EST seizure in 50 % of animals (EST-50), with the 95 % confidence limits, was calculated by the method of Litchfield and Wilcoxon (1949).[14] To represent changes in EST, EST in control mice was measured and the average was used as the basis for comparison of the experimental values.

Intracerebral injection technique. Intracerebral injection was performed by our modification of the method described by Haley and McCormick (1957).[9] The animals were grasped firmly and the scalp was incised to expose the skull. The site of injection was located 1.5 mm on either side of the midline and 2 mm anterior to the lambda. A hypodermic needle attached to a microsyringe was inserted perpendiculary through the skull into the brain at a depth of 3 mm from the surface of the skull, and 0.01 ml of solution was slowly injected. Studies using Evans' blue showed that most of the injected dye was distributed

in the ventricular spaces. An animal which exhibited any neurological deficit was discarded. All chemicals used were of reagent grade quality. Zinc chloride (Katayama's guaranteed reagent) was dissolved in warm Ringer's solution prepared with ion-free water in various concentrations. Other metals were also used as the chloride. The injected solutions were freshly prepared for each experiment.

Administration of anticonvulsants. The anticonvulsant agents chosen for this study were: diphenylhydantoin sodium (Aleviatin); phenobarbital (Phenobal); and acetazolamide sodium (Diamox). Their doses and routes of administration were as follows: diphenylhydantoin sodium, 100 mg/kg, intraperitoneal; phenobarbital, 50 mg/kg, subcutaneous; and acetazolamide sodium, 100 mg/kg, intraperitoneal. These drugs were administered by their respective routes 30 min., 30 min. and 3 hours prior to the intracerebral injection of zinc. The injection volume never exceeded 0.1 ml. On the other hand, anticonvulsant effects of calcium and magnesium were studied. Calcium and magnesium, as the chloride, were dissolved in Ringer's solution containing zinc to be injected intracerebrally. The effect of calcium was also studied 30 min. after the intraperitoneal injection.

RESULTS

The effect of subcutaneously injected zinc on brain excitability

The effect of subcutaneously injected zinc on EST is shown in Fig. 1.

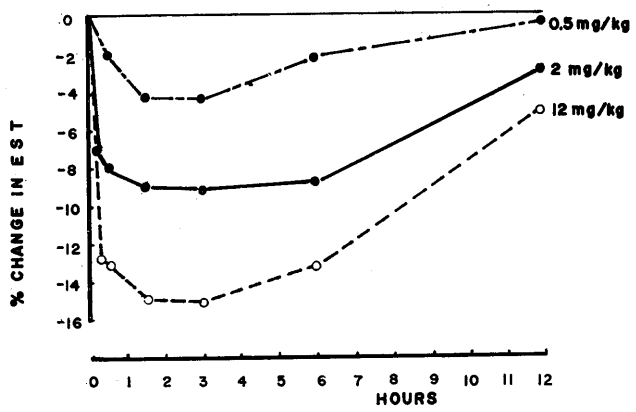

Fig. 1. Effect of single doses of zinc on EST of intact mice. Change in EST is expressed as % change from control value: 6.6 mA (6.8–6.4 mA). Zinc, as the chloride, was dissolved in 0.9 % NaCl solution, adjusted to pH 2.0 by adding HCl, and 0.05 ml of the solution was injected subcutaneously. Twelve to 18 mice, weighing 21 to 25 g, were used for each EST-50 determination.

As is evident, zinc produced a marked lowering in EST. Irrespective of the injected dose, the EST curve was practically the same; the EST was conspicuously

decreased as early as 10 minutes after the injection and showed a peak change in 3 hours. Thereafter, the EST-lowering effect was gradually decreased and largely disappeared in 12 hours. The rapid onset of the effect suggests the implication of the adrenal gland, for reasons mentioned in the DISCUSSION. Thus, the effect of zinc on EST was studied in adrenalectomized mice. As shown in Fig. 2, changes in EST were minimal, being in striking contrast to the marked decrease in EST in intact mice. These data indicate that the decrease in EST produced by zinc is mediated, in some manner, by the adrenal gland.

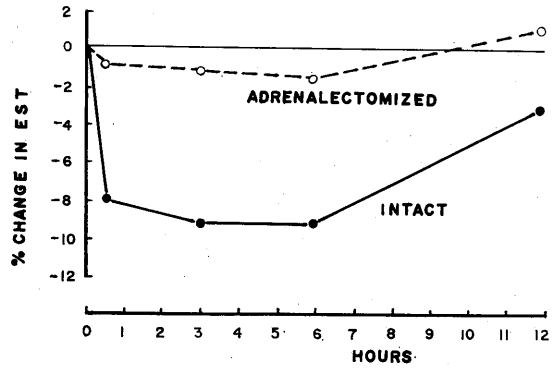

Fig. 2. Effect of a single dose of zinc (2 mg/kg, subcutaneously) on EST of adrenalectomized mice. The EST change in intact mice is shown for comparison. Change in EST produced by zinc is expressed as % change from the corresponding control values. Adrenalectomized mice were maintained on 0.9 % NaCl solution in drinking water. Twelve to 18 mice, weighing 21 to 25 g, were used for each EST-50 determination.

Similar results were obtained on the successive administration of zinc. As shown in Fig. 3, zinc produced a decrease in EST in intact mice, while this effect disappeared in adrenalectomized mice; the EST appeared to have an increasing tendency. Since there was no significant change in body weight between zinc-intoxicated and control mice, the decrease in EST produced by zinc undoubtedly represents an increase in brain excitability.[34]

The effect of intracerebrally injected zinc on brain excitability

In order to investigate the direct effect of zinc on the brain, various concentrations of zinc were intracerebrally injected and changes in EST were studied. The EST test was applied 50 sec. after the injection, since intracerebral injection of a higher concentration of zinc produced behavioral responses with a latency of less than 1 minute, as will be mentioned later. Changes in EST are shown in Table 1. So far as we studied, the EST was decreased as zinc concentration was increased. Its decrease amounted to 0.9 mA, being equivalent to 15 % of control value, at a concentration of 3 mM.

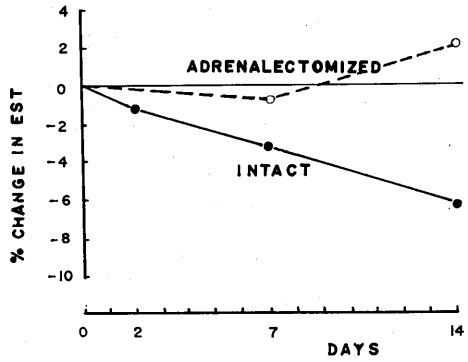

Fig. 3. Effect of successive doses of zinc (2 mg/kg, subcutaneously) on EST of intact and adrenalectomized mice. Change in EST produced by zinc is expressed as % change from the corresponding control values. EST test was applied 18 to 20 hours after the last injection. Adrenalectomized mice were maintained on 0.9% NaCl solution in drinking water and received a daily dose of 2μg of dexamethasone phosphate. Twelve to 18 mice were used for each EST-50 determination.

Table 1. Effect of intracerebrally injected zinc on EST of intact mice

Zinc Concentration mM	No. of Mice	EST-50 mA	95 % Confidence Limits mA	% Change from Control
Control	24	6.1	6.5 – 5.7	
1	18	5.8	6.1 – 5.5	– 5
2	12	5.4	5.8 – 5.0	–11
3	12*	5.2	5.5 – 4.9	–15

EST test was applied 50 sec. after intracerebral injection of zinc. Zinc, as the chloride, was dissolved in Ringer's solution and injection volume was 0.01 ml. Animals weighed 20 to 23 g.
*A few animals had produced running movements when the test was applied.

In this case, it might also be possible that intracerebrally injected zinc has some effect on the endocrine system and the autonomic nervous system by its action on the hypothalamus.[2)13)17)33)] In such a chain of reactions, the adrenal gland plays the leading part. Therefore, the effect of zinc on EST was studied in adrenalectomized mice. The result is shown in Table 2. The EST-lowering effect of zinc was not affected by adrenalectomy. This excludes the possibility that the adrenal gland is participated in the lowering in EST produced by intracerebral injection of zinc, and supports the conclusion that this effect is produced by the direct action of zinc on the brain.

As the control experiments, the effect of 3 mM of cobalt (II) and iron (II) on EST was studied. Cobalt decreased EST by 6 % while iron had little effect

on EST. The pH of Ringer's solution containing the metals varied from 5.2 to 5.4 and was much the same as that of Ringer's solution alone.

Table 2. Effect of intracerebrally injected zinc on EST of adrenalectomized mice

Group	No. of Mice	EST-50 mA	95 % Confidence Limits mA	% Change from Control
Control	18	6.3	6.5 – 6.1	
3mM Zinc	18*	5.3	5.6 – 4.9	−16

EST test was applied 50 sec. after intracerebral injection of zinc. Injection volume was 0.01 ml. Adrenalectomy was performed on the day before experiments. Adrenalectomized mice, weighing 20 to 24 g, were maintained on 0.9 % NaCl solution in drinking water.

* A few animals had produced running movements when the test was applied.

Initiation of seizures following intracerebral injection of zinc

Behavioral responses produced by intracerebral injection of zinc in conscious mice varied with the concentration of zinc administered. Although zinc had little effect on behavior at 2 mM (1.3 μg per 0.01 ml), a concentration of 3 mM of zinc (2.0 μg per 0.01 ml) produced violent running or hopping movements, within 80 sec. after the injection. This response lasted 2 to 4 minutes, after which the animals apparently resumed their normal activity. With still higher concentrations (2.6 and 3.3 μg Zn per 0.01 ml), the movements were suddenly replaced by tonic seizures with a latency of about 50 sec. The tonic seizures, essentially similar to the maximal seizures produced by electroshock, consisted of a tonic flexion lasting about 2 sec. and tonic extension, followed by death. On rare occasions, when the animals were survival, the tonic extension was followed by a whole-body clonus. Clonic seizures were seen in only a few animals. The clonic seizures, if occurred, lasted about 15 sec., after which the animals showed a generalized depression of 30 sec. duration, followed by secondary running movements.

The relationship between zinc concentration and the incidence of the separate responses produced by zinc is shown in Table 3. The running or hopping movements were easily produced, while the tonic extensor seizures and death required higher concentrations. The latency of the responses appeared to exhibit a reducing tendency, as zinc concentration was increased. The mortality rate coincided closely with the incidence of the tonic extensor seizures, since death occurred only in the mice which exhibited such responses.

Control experiments. The effect of 5 mM of bivalent heavy metals on behavior was studied as the control experiments. The pH of each solution was between 5.0 to 5.4 except copper solution which was slightly more acidic.

Table 3. Incidence of responses produced by intracerebral injection of zinc

Zinc Concentration		No. of Mice	Type of Response					
			Running or Hopping Movements		Seizures			Mortality
mM	μg per 0.01 ml		%	Latency* sec	Clonus	Tonic Extension %	Latency* sec	%
2	1.3	12	0		0			0
3	2.0	12	33	54 (33–80)	0			0
4	2.6	14	86	39 (25–75)	14	50 36	52 (41–87)	36
5	3.3	14	100	39 (23–70)	0	86 86	46 (30–80)	78

Zinc, as the chloride, was dissolved in Ringer's solution and 0.01 ml of the solution was injected. Animals weighed 18 to 22 g.

* Figures in parentheses indicate range of values obtained.

Immediately after intracerebral injection of Ringer's solution, the animals were depressed for approximately 10 sec. and then resumed their normal activity. Such response was scarcely changed by addition of manganese and lead, while cobalt and iron produced nose-scratching movements and facial twitchings. Intracerebral injection of copper caused hopping or rotating movements with a latency of about 15 sec., followed shortly by a violent fall with floundering. One minute later, the animals usually appeared to have a generalized tonus. Thereafter, the animals continued to crouch except for the intermittent paroxysm of running movements or clonic movements involving the limbs. A few animals had tonic extensor seizures about 15 minutes after the injection.

Protection experiments. The protective effect of anticonvulsant agents on the seizures produced by zinc is shown in Fig. 4. Diphenylhydantoin as well as phenobarbital abolished the tonic extensor seizures and death produced by zinc but failed to eliminate the running or hopping movements. The most striking feature produced by acetazolamide was that the major pattern of seizures was converted from tonic extension to clonus. Intracerebral injection of 5 mM of calcium protected the responses to zinc so completely that the animals appeared to be normal. Such protection, although not complete, was also produced by intraperitoneal injection of calcium. This contrasted sharply with the finding that the behavioral responses produced by copper were scarcely affected by calcium. Intracerebral injection of 5 or 10 mM of magnesium protected the seizures but was insufficient to eliminate the running or hopping movements. Even when the movements were not manifest, the animals were excitable and would run if touched.

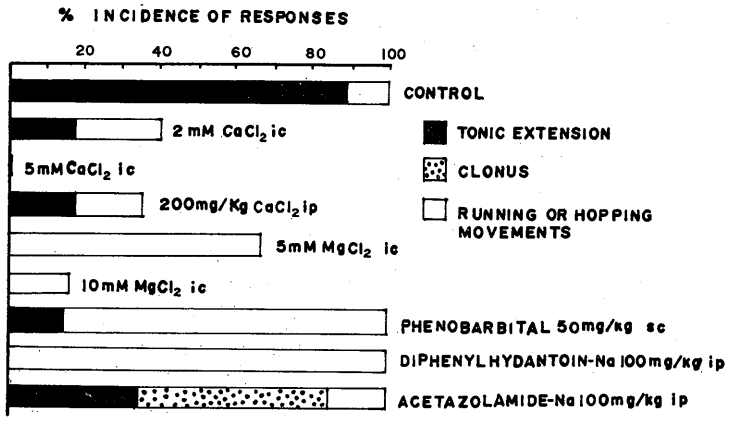

Fig. 4. Protective effect of anticonvulsant agents on the seizures produced by intracerebral injection of 5 mM (3.3μg per 0.01 ml) of zinc. The agents were injected intracerebrally (ic), intraperitoneally (ip) or subcutaneously (sc). Each observation was made on 6 to 14 conscious mice, weighing 18 to 22 g.

DISCUSSION

The decrease in EST produced by systemic injection of zinc occurred with some rapidity. Since zinc does not readily cross the blood-brain barrier,[20] it is unlikely that this effect is caused by the direct action of zinc on the brain.

Lack of the EST-lowering effect of zinc in adrenalectomized mice indicates that this phenomenon is mediated, in some manner, by the adrenal gland. Although intimate relationship of the adrenal gland to brain excitability is well known,[32,33] the mode of action of zinc on this organ is not established. Woodbury (1954)[32] has shown in rats that the effect of adrenocortical hormones on EST reaches its peak 6 hours after administration and largely disappears by the 24 th hour. Except for the rapid onset, the change in EST produced by zinc is nearly consistent with that produced by adrenocortical hormones. This suggests the participation of the adrenal cortex in the EST-lowering effect of zinc. On the other hand, Swinyard (1962)[23] has shown in mice that the increase in brain excitability produced by epinephrine occurs almost instantaneously. Because of the rapid decrease in EST produced by zinc, it seems unreasonable to exclude the implication of the adrenal medulla. This problem is currently under investigation in our laboratory.

The intracerebral injection procedure in conscious mice, described by Haley and McCormick (1957),[9] has been adopted by several investigators[8,11,18,21,27] as a procedure giving a better estimation of the central actions of drugs. The present study using this procedure showed that a lower concentration of zinc decreased EST, while a higher one induced a tonic type of seizure responses.

In this case, the effect of pH was not implicated, since the pH of Ringer's solution was not changed by addition of zinc chloride. Furthermore, the participation of osmotic pressure and anion were ruled out by using the same concentration of respective chloride in the control experiments. Therefore, the observed effects can be ascribed to the action of zinc on the brain.

It is well known that intracerebral injection of various agents induces seizure responses.[11)31)] With respect to heavy metals, Davies et al. (1961)[4)] have reported that subarachnoidal injection of copper (II) produces a seizure response in pigeons. This response, at least in mice, was quite different from that induced by zinc. Although cobalt powder implanted intracerebrally has been shown to produce a chronic convulsive state in mice,[12)] the present study showed that the acute administration of cobalt had little effect on behavior except for a lowering in EST. Therefore, the observed effects produced by zinc seem to be not common for heavy metals but rather specific for this metal. It may also be suggested that the seizure responses produced by zinc may be elicited by different mechanisms of action than those produced by other heavy metals.

The EST test showed that intracerebral injection of zinc decreased the threshold for evoking minimal clonic seizures, the manifestation of which is thought to be related to activity in the centrencephalic system including involvement of the cortex.[29)] On the other hand, the seizure responses produced by zinc in mice were characterized by tonic extension of all limbs. Since tonic extensor seizures are primarily concerned in the spread of seizure discharge through the brain,[3)6)35)] it seems that zinc may have some analeptic action on seizure-transmission. This is supported by the present finding that the tonic extensor seizures produced by zinc were protected by diphenylhydantoin, phenobarbital and acetazolamide, which have been shown to prevent, more or less, the neural spread of seizure discharge.[3)6)7)10)28)] The tonic extensor seizures produced by zinc had an initial flexor component and were protected by the anticonvulsants. These characteristics are similar to those of Metrazol or electroshock seizures, in contrast with those of strychnine seizures.[3)6)26)] Strychnine increases the level of neuronal excitability by selectively blocking inhibition, while Metrazol is thought to stimulate excitatory and inhibitory neurons uniformly.[6)] It is therefore reasonable to presume that zinc directly excites central neurons.

The running or hopping movements, which may be considered to be a motor aura, could not be protected by the anticonvulsants. This corresponds with the fact that forced circling movement in DFP (di-isopropyl fluorophosphate) seizures and aura in clinical seizures are not prevented by diphenylhydantoin and other drugs.[6)26)]

In this respect, it is noteworthy that calcium produced a complete protection against the seizures including the running or hopping movements. Kita et al.

(1965)[11] have observed that intracerebral injection of calcium-binding agents (EDTA-2Na, trisodium citrate etc.) can induce seizures, while seizures induced by intracerebral injection of various agents are commonly, but not always completely, protected by calcium. On the basis of these observations, they have supposed that the depletion of calcium from the cell membrane may be one of the trigger mechanisms of seizures. It has been shown that calcium ion is important in regulating the membrane permeability to sodium and potassium in the excitable tissues,[1)19)] while heavy metals exert general pharmacological effects on the cell membrane permeability and ion transport.[16)] In addition, some investigators have shown that a reciprocal antagonistic action may exist between zinc and calcium.[5)22)] It is therefore reasonable to assume that the seizures produced by intracerebral injection of zinc may be caused by pathological changes, such as the depletion of calcium, in the neuronal membrane. If neurochemical mechanisms governing brain excitability can be ascribed to the extracellular or intracellular distribution of brain electrolytes,[28)32)] such interpretation may also be applied to the changes in brain excitability produced by intracerebral injection of zinc. Further studies into this problem are necessary.

SUMMARY

1. The effect of zinc on the central nervous system was investigated in conscious mice using electroshock seizure threshold tests and whole animal behavioral studies.

2. Single or successive subcutaneous injection of zinc markedly decreased EST. This EST-lowering effect was abolished in adrenalectomized mice, indicating that the decrease in EST following systemic injection of zinc is produced by way of the adrenal gland.

3. Intracerebral injection of lower concentrations of zinc produced a marked decrease in EST in intact and adrenalectomized mice.

4. Intracerebral injection of higher concentrations of zinc produced the following behavioral changes.

a) A concentration of 4 mM or more of zinc (2.6 μg or more Zn per 0.01 ml) produced running or hopping movements, which were followed by convulsive seizures with a latency of about 50 sec. The seizure pattern was almost always tonic flexor-tonic extensor type.

b) Such seizure responses seemed to be rather specific for zinc, since copper produced a different type of seizure responses and several heavy metals had little effect on behavior.

c) Diphenylhydantoin, phenobarbital and acetazolamide protected the tonic extensor seizures but failed to eliminate the running or hopping movements preceding the seizures. Calcium completely protected the behavioral responses to

zinc, while the protective effect of magnesium was insufficient.

5. Possible mechanisms concerning the direct effect of zinc on the brain are discussed.

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