DUAL ACTION OF ZINC ON ILEAL SMOOTH MUSCLE - ZINC-CALCIUM INTERACTION

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Summary : Effect of zinc sulphate was studied on histamine-induced contractions on guinea-pig ileum. In doses of 1.72×10^{-7}M or less no effects were observed. Zinc sulphate in doses of 3.44×10^{-7}M, 6.88×10^{-7}M and 1.72×10^{-6}M produced dual effect. Short exposure of tissue for 10 min to zinc sulphate resulted in significant dose dependent potentiation of histamine effect whereas after washing the tissue of zinc sulphate, histamine response was inhibited significantly and in a dose dependent manner. Higher concentrations of zinc sulphate of 3.44×10^{-6}M and above produced irreversible inhibition of histamine response. The above observations have been explained in terms of zinc-calcium interaction. It is hypothesized that interaction of zinc with calcium may take place extracellularly at membrane level and intracellularly.

Key words : guinea-pig ileum histamine zinc-calcium interaction

INTRODUCTION

Several studies are reported about trace elements recently. This field which was looked upon from the point of view of deficiency diseases has gained importance today because of the clinical background. Though trace elements are present in minute amounts in our body, their presence is essential because of the participation of these trace elements in cellular metabolic processes. Trace elements are contained in metalloenzymes which are involved in physiological and biochemical reactions. Aetiopathology of various diseases is attributed to changes in levels of these trace elements e.g. zinc levels are deficient in patients having myocardial infarction (9,16,24), atherosclerosis (23) and hypertension (21). In case of diabetes reports about zinc levels are controversial (8). Low magnesium concentrations are found in patients having myocardial infarction (1). Excess cadmium levels in body may result in hypertension (21).

Zinc has attracted our attention because of the fact that gastrointestinal disorders like steatorrhoea lead to zinc deficiency (15, 22) and zinc therapy was found to produce beneficial effects in those subjects not responding to usual treatment (15).

Zinc has been shown to have membrane stabilizing property (4). It is likely that zinc supplementation in gastrointestinal disorders associated with steatorrhoea, besides restoring the levels to normal, may itself control motility of gut because of stabilization of biomembrane.

The effects of zinc sulphate on isolated guinea-pig ileum were observed to study the effect of zinc on the ileal smooth muscle. An attempt was also made to study the mode of its action.

MATERIAL AND METHODS

Guinea-pigs of either sex weighing between 350-450 *g* were fasted for 24 hr before sacrifice. Animals were killed by a blow on the head and repidly exsanguinated by cutting the carotid arteries. A piece of ileum was removed, washed and suspended under a resting tension of 0.5 *g* in a 10 *ml* bath containing oxygenated Tyrode solution at 37°±0.5°C. The contractions were recorded on a smoked paper using a frontal writing lever.

Contractions of guinea-pig ileum of sufficient height were obtained with histamine in doses of 2.70×10^{-8}M — 3.60×10^{-6}M which served as control. The preparation was then exposed to zinc sulphate solution added to the bath in doses of 3.44×10^{-7}M, 6.88×10^{-7}M and 1.72×10^{-6}M and was allowed to remain in the contact for 10 min. At this time histamine response for 30 sec was obtained in the presence of zinc sulphate. After this, the preparation was washed and histamine responses were repeated at 10 min intervals to observe the recovery of the tissue. Height of contraction of the control was compared with the histamine response obtained in the presence of zinc sulphate and at 10 min interval after washing the tissue. % potentiation and % inhibition was calculated in terms of the control. Results were statistically analysed by Student's 't' test.

In another set, identical experiments were performed using Tyrode solution devoid of calcium chloride and Tyrode solution containing varying amounts of calcium chloride i.e. containing 1/6th calcium chloride, 1/3rd calcium chloride and normal calcium chloride to see if changes in extracellular calcium levels influence zinc sulphate effect.

RESULTS

Zinc sulphate in doses of 3.44×10^{-7}M, 6.88×10^{-7}M and 1.72×10^{-6}M produced a dual response. Exposure to zinc sulphate for 10 min in the bath produced a significant

dose dependent potentiation of histamine response as compared to control (Fig. 1, Table I). However, histamine response obtained 10 min after washing zinc sulphate from the bath was inhibited significantly in a does dependent manner (Fig. 1, Table II). Recovery

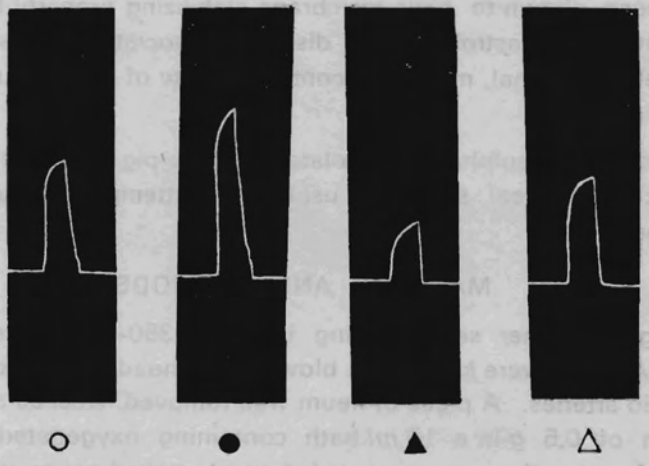

Fig. 1 : Effect of zinc sulphate on histamine-induced contractions of guinea-pig ileum.

- ○ Histamine 1.80 x 10^{-6}M
- ● Zinc sulphate 1.72 × 10^{-6}M for 10 min followed by histamine 1.80 × 10^{-6}M
- ▲ Histamine 1.80 × 10^{-6}M 10 min after washing of zinc sulphate
- △ Histamine 1.80 × 10^{-6}M 20 min after washing of zinc sulphate

TABLE I : Potentiating effect of zinc sulphate on histamine-induced contractions of guinea-pig ileum in normal Tyrode.

Zinc sulphate (dose in M)	*% potentiation Mean ± SE of 6 obs.*	*P value*
3.44×10^{-7}	17.50 ± 4.09	< 0.01
6.88×10^{-7}	40.84 ± 0.43	< 0.01*
1.72×10^{-6}	52.88 ± 2.36	< 0.01*

*P value as compared to previous dose.

from inhibition was seen after 20 min at the doses used in our experiments. No effect was observed in doses of 1.72×10^{-7}M or less while higher doses in the rarge of 3.44×10^{-6}M and above produced irreversible inhibition.

In the second set of experiments Tyrode solution containing varying amounts of calcium chloride was used. Histamine did not produce contraction of ileum in calcium

free solution. While the potentiating effect of zinc sulphate did not significantly differ in varying amounts of calcium (Table III), the inhibitory effect was significantly influenced. Table II shows that the inhibitory effect of zinc sulphate is signiflcantly higher when calcium levels are normal than in 1/3rd calcium. There was no inhibitory effect in presence of 1/6th calcium (Fig. 2).

TABLE II : Comparison of the inhibitory effect of zinc sulphate on histamine-induced contractions of guinea-pig ileum in normal Tyrode and Tyrode containing 1/3 rd calcium chloride.

Zinc sulphate (dose in M)	*% inhibition Mean ± SE of 6 obs.* Calcium chloride		*P value*
	Normal	*1/3 rd*	
3.44×10^{-7}	21.43 ± 1.51	11.92 ± 2.61	< 0,05
6.88×10^{-7}	32.53 ± 2.01*	19.75 ± 1.04*	< 0.01
1.72×10^{-6}	54.11 ± 2.95*	43.50 ± 0.88*	< 0.05

*P value as compared to previous dose P < 0.01

TABLE III : Potentiating effect of 1.72×10^{-6}M zinc sulphate on histamine-induced contractions of guinea-pig ileum in Tyrode containing varying amounts of calcium chloride.

Calcium chloride	*% potentiation Mean ± SE of 6 obs.*	*P value*
Normal	52.88 ± 2.36	
1/3rd	30,62 ± 19.30	N.S.*
1/6th	26.08 ± 11.16	N.S.*

* N.S. Not signiticant. P value as compared to normal.

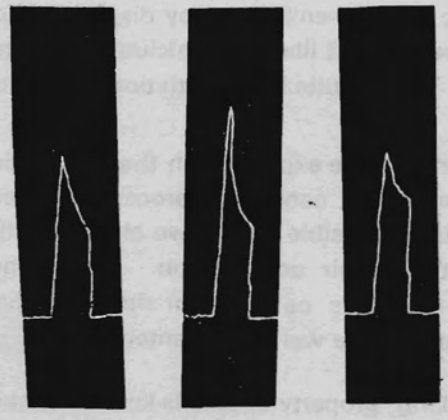

Fig. 2 : Effect of zinc sulphate in presence of Tyrode containing 1/6th calcium.
○ Histamine 1.80×10^{-6}M
● Zinc sulphate 1.72×10^{-6}M for 10 min followed by histamine 1.80×10^{-6}M
▲ Histamine 1.80×10^{-6}M 10 min after washing of zinc sulphate

DISCUSSION

It can be seen from the results that zinc sulphate in doses of 3.44×10^{-7}M, 6.88×10^{-7}M and 1.72×10^{-6}M produces a dual effect on guinea-pig ileal contractions induced by histamine. Initially there was significant dose dependent potentiation of histamine response ($P<0.01$) in the presence of zinc sulphate; later, on washing of the tissue, histamine response was inhibited. The inhibition was dose dependent, irreversible at higher doses and statistically significant ($P<0.01$).

Several possibilities can be thought of as regards effect of zinc on ileal, smooth muscle taking into consideration the fact that zinc is bound extracellularly at membrane level as well as intracellularly (20).

Calcium plays a major role in regulation of contraction of smooth muscle. It is vital in the function of cell microskeleton represented by microtubules and microfilaments (5, 7, 17). It is well-known that dependence on extracellular calcium transport for contraction varies greatly with different tissues. Hudgins and Weiss (12) have reported using vascular smooth muscle that, histamine which has been used as spasmogen in our experiments acts by mobilizing calcium from extracellular fluid. In the presence of varying amounts of calcium in Tyrode solution potentiating effect of zinc though not significant, continued since only a certain threshold of calcium is required to be reached in the smooth muscle cell to trigger the contractile process as is true with vascular smooth muscle (14). Potentiating effect of zinc can be explained on the basis that extracellular binding of zinc to the membrane may displace calcium bound to the membrane, which then enters the cell and liberates calcium from intracellular structures such as sarcoplasmic reticulum. This results in potentiation of histamine response.

Inhibitory effect of zinc can be explained on the assumption that zinc gets bound to intracelluar structures involved in contractile process and competes with calcium for the same binding sites. This is possible if the two elements show affinity for a common binding site due to similarity in their constitution. Zinc is known to compete with cadmium, lead, copper and iron besides calcium for similar binding sites (11). Interactions between dietary copper and zinc are well documented (13).

Calcium uptake blocking property of zinc is known in case of isoproterenol injured myocardium (6). Significance of this phenomenon in other tissues has to be established.

Zinc when used as zinc chelate or zinc sulphate counteracts and prevents cadmium-induced hypertension (10,18,19). This observation is explained on the basis of zinc and cadmium both having affinity for a specific protein metallothionein in kidney (2). This is

relevant to our study because such binding of zinc to metalloenzymes involved in contractile processes in intestine probably occurs to bring about irreversible inhibition of ileal contractions at higher doses of zinc sulphate. Balaraman *et al.* (3) attributed calcium involvement in cadmium-induced hypertension. Calcium deposition over blood vessels occurs in pathological processes like atherosclerosis and hypertesion (25) affecting ageing blood vessels where zinc-calcium antagonism can be rightly thought of.

Zinc sulphate failed to show inhibition in the presence of Tyrode containing 1/6th calcium. However, the typical inhibitory effect of zinc was obvious in presence of Tyrode containing 1/3rd and normal calcium. This suggests that extracellular calcium has to be in sufficient concentration so as to make the inhibitory effect of zinc sulphate obvious.

Based on our findings it can be inferred that zinc has dual action on ileal smooth muscle. It potentiates histamine action initially and inhibits the response later on. Its spasmolytic effect may be beneficial in controlling steatorrhoea. We have tried to explain the results in terms of zinc-calcium interaction. It is hypothesized that the interaction of zinc with calcium may take place extracellularly at membrane level and intracellularly. Further work has to be carried out to confirm this hypothesis.

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