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Effects of Theophylline and Imidazole on Calcium Accumulation in Renal Cortex of Rats treated with Stannous Chloride

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Effect of theophylline and imidazole on the renal cortex calcium concentration was examined in rats intraperitoneally administered with stannous chloride. The increased calcium concentration in renal cortex caused by the tin administration (Sn 3.0 mg/100 g) was markedly decreased by the treatment of imidazole (20 mg/100 g), while was not altered significantly by the treatment of theophylline (2.0 mg/100 g). Theophylline alone significantly increased the calcium concentration in renal cortex compared with that of control. The present study suggests that the action of tin on renal cortex calcium may be dependent on cyclic AMP.

Keywords——tin poisoning; kidney calcium; rats; theophylline; imidazole

Benoy, *et al.*[2] reported that tin was found in a large amount in kidney of rats ingesting orange juice; tin content, 540 ppm. It is possible that tin induces disturbance of the kidneys in tin poisoning. Then, we had initially examined the influence of tin on the calcium metabolism in order to investigate the mechanism of toxic action of tin, and found that the increased calcium after the administration of tin predominantly accumulated in the cortex of kidney.[3] The mechanism by which tin increases kidney calcium remains to be elucidated. More recently, we suggested that the calcium accmulation in the renal cortex caused by the administration of tin is closely connected with an increase in the calcium binding activity.[4]

In the present studies, we attempted to evaluate the role of cyclic AMP on the accumulation of calcium in renal cortex by using drugs which affect the enzyme, cyclic 3′, 5′-nucleotide phosphodiesterase. This report describes the effects of theophylline and imidazole on the calcium concentration in renal cortex of rats treated with stannous chloride.

Methods

Male Wistar strain rats, each weighing approximately 120 g, were used in the present experiments. The animals were kept at a room temperature of 25°±1° and fed commercial lab. chow and tap water freely.

$SnCl_2$, freshly dissolved in hydrochloric acid solution (pH 1.8), was diluted to a final concentration of 3 mg Sn/ml distilled water. This solution was given as a single intraperitoneal injection (1.0 ml/100 g body weight) to the rats and controls were injected HCl solution (pH 1.8).

Imidazole was dissolved in demineralized water. Theophylline was dissolved in demineralized water to which 1.0 N KOH was added until a clear solution was obtained. The final pH of this solution was approximately 10. The animals treated with stannous chloride were given imidazole (20 mg/100 g)[5] or theophylline (2.0 mg/100 g)[6] immediately and 12 hr later by intraperitoneal injection. Controls were injected demineralized water, or KOH solution (pH 10).

The animals were bled by cardiac puncture under light ether anesthesia 24 hr after tin administration. Kidneys were removed immediately after bleed and rinsed with cold 0.25 M sucrose solution. The kidney tissue was separated from the fat capsule and divided macroscopically into cortex and medulla. The concentration of calcium in the renal cortex were determined by atomic absorption spectrophotometry (Perkin-

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3) T. Yamamoto, M. Yamaguchi, and H. Sato, *J. Toxicol. Environ. Health.*, 1, 749 (1976).
4) M. Yamaguchi, H. Sato, and T. Yamamoto, *J. Toxicol. Environ. Health.*, 3, 411 (1977).
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6) H. Wells and W. Lloyd, *Endocrinology*, 81, 139 (1967).

Elmer, Model 303) with a reversed air-acetylene flame after chloric acid digestion.[7] Blood samples obtained by cardiac puncture were centrifuged immediately after the collection. The serum was separated and analyzed immediately. Determination of calcium was made on 0.1 ml aliquots of serum by atomic absorption spectrophotometry after precipitation with 10% trichloroacetic acid.[8]

Results

The effects of theophylline on the calcium concentration in renal cortex of rats after a single intraperitoneal administration of stannous chloride are shown in Table I. The renal cortex calcium concentration in rats treated with tin increased about 2 times ($p<0.01$) when compared to values obtained from control rats. The injection of theophylline to rats treated with tin did not elevate significantly on the calcium concentration of renal cortex after tin administration, while the serum calcium concentration reduced significantly. Theophylline alone increased significantly the calcium concentration of renal cortex compared with that of control.

Table I. Effect of Theophylline on Calcium Concentration in Renal Cortex of Rats treated with Stannous Chloride

Treatment	Calcium concentration	
	Kidney (μg/g wet)	Serum (mg/100 ml)
Control; vehicle	27.1±0.66[a]	9.6±0.10
Tin; vehicle	65.1±3.14[a]	9.5±0.40
Tin; theophylline	57.6±4.42	8.0±0.21[b]
Control; theophylline	40.7±1.31[b]	7.4±0.07[b]

a) Mean±SEM for 5 animals.
b) Significance from the control, $p<0.01$ (Student's t-test).

On the other hand, imidazole markedly reduced the increased calcium concentration in renal cortex caused by tin administration (Table II). The treatment of imidazole to control rats did not increase the calcium concentration of renal cortex, while the calcium concentration in serum lowered significantly.

Table II. Effect of Imidazole on Calcium Concentration in Renal Cortex of Rats treated with Stannous Chloride

Treatment	Calcium concentration	
	Kidney (μg/g wet)	Serum (mg/100 ml)
Control; vehicle	35.1±0.73[a]	9.6±0.23
Tin; vehicle	72.0±5.31[b]	9.3±0.16
Tin; imidazole	39.7±3.03[c]	8.0±0.56[b]
Control; imidazole	37.8±0.40	7.4±0.09[b]

a) Mean±SEM for 5 animals.
b) Significance from the control, $p<0.01$ (Student's t-test).
c) Significance from the tin, $p<0.01$.

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8) J.B. Willis, *Nature* (London), **16**, 249 (1960).

Discussion

The increased renal calcium after tin administration accumulated markedly in the cortex of rats.[3] The calcium binding activity in the soluble fraction from a rat renal cortex was predominantly increased by the tin administration.[4] Presumably, it is possible that the increased calcium binding activity caused by the tin administration stimulates the absorption of calcium through the renal tubulla. In fact, the administration of tin inhibited the excretion of calcium into urine of rats.[9] From these studies, it was proposed that the calcium accumulation in the renal cortex caused by the administration of tin is closely connected with an increase in the calcium binding activity.[4]

In the present studies, we attempted to evaluate the role of cyclic AMP on the accumulation of calcium in renal cortex by using drugs which affect the enzyme, cyclic 3′,5′-nucleotide phosphodiesterase. Inhibition of this enzyme results in an accumulation of cyclic AMP in tissues. Presumably, theophylline inhibits and imidazole activities this enzyme. The treatment of imidazole prevented dramatically the accumulation of renal cortex calcium after tin administration, while theophylline had no effect the accumulation of calcium. Theophylline alone significantly increased the calcium concentration in renal cortex. These data suggest that the action of tin on renal cortex calcium may be dependent on cyclic AMP.

Since an increase in cyclic AMP in kidney cells stimulates the permeability of calcium through the cell membrane,[10] the calcium concentration in kidney cells would be elevate significantly. Accordingly, the data obtained from the present experiments seem to be consistent the point of view that the accumulation of renal cortex calcium induced by the tin administration is caused by the augmentation of calcium influx in the cells.

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