Effect of Cadmium, *in vivo* and *in vitro*, on Intestinal Brush Border ALPase and ATPase

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It is well known that the small intestine of the mammals can transport ^{45}Ca actively, against concentration gradients, from fluid bathing the mucosal surface to fluid bathing the serosal surface. Wasserman and Taylor (1) reported that the calcium-binding protein played a role as carrier in the calcium active transport. It was proven by the technique of fluorescent antibody that this calcium-binding protein was localized in the brush border and Goblet cell of the intestinal mucosa (2). Ingersoll et al. (3) reported that cadmium strongly inhibited the binding activity of the chick intestinal mucosa calcium-binding protein for ^{45}Ca, in vitro. Sugawara (4) reported that the ^{45}Ca binding activity of this protein obtained from the rats administered cadmium was lower than that of the rats unadministered cadmium. These above reports support the implication that the calcium absorption from the small intestine was prevented by the administration of cadmium.

In an attempt to approach the effect of cadmium on the intestinal brush border, we studied the effect of cadmium on the activity of ALPase (orthophosphoric monoester phosphohydrolase) and ATPase (ATP phosphohydrolase ; Mg^{++}-stimulated ATPase and $Mg^{++}Ca^{++}$stimulated ATPase) in the brush borders. It might seem that cadmium action on these enzymes in vivo was different from that in vitro.

METHODS

Male rats of the Wistar strain were divided into two groups ; control group (5 rats) and experimental group (8 rats). The control group was given a cadmium free drinking water (distilled water). The experimental group was given a water containing 100 ppm cadmium ($CdCl_2$) during 30 days. The basal diet was used MNF obtained from Oriental Kobo Chemical Co,. The diet and drinking water were provided ad libitum. On the starting, 9th, 20th and final day, body weight was recorded and the blood was sampled to determin the concentration of calcium and phosphorus in the serum.

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On the 27th or 30th day, the control and experimental rats were decapitated to obtain the duodenum mucosa. The proximal duodenum was removed at the length of about 10 cm and the mucosa layer separated from the underlying muscle layers by the use of a glass slide. The procedures of preparing the brush borders were according to the method of Norman et al (5). The rate of appearance of the product p-nitrophenolate anion was determined at 410 nm. The specific activity was ultimately expressed in standard unit of μmoles of p-nitrophenol released per minute per mg of protein. The ATPase activity was measured by using the assay system of Norman et al (5), and the measurement of P_i released was made by the method of Allen (6). The measurement of protein was according to the method of Lowry et al (7). The rats used in vitro examination was similar age and sex as examination in vivo.

RESULTS AND DISCUSSION

TABLE 1 shows the activity of ALPase and ATPase in a variety of concentration of cadmium. As shown in TABLE 1, the activity of ALPase was slightly decreased by the presence of 10^{-6} M cadmium, and in the 10^{-5} M concentration this activity was about 44 % of the control. The activity of Mg^{++}-stimulated ATPase was not affected by the cadmium of 10^{-7}, 10^{-6} and 10^{-5} M, but in the presence of 10^{-4} M cadmium this activity was about 50 % of the control.

TABLE 1 Effect of Cadmium, in vitro, on ALPase and ATPase

Cd(M)	ALPase	ATPase	
		$Mg^{++}Ca^{++}$	Mg^{++}
Control	3.57	15.52	3.22
10^{-7}		14.01(90)	3.54(110)
10^{-6}	3.36(94)	9.05(65)	3.59(111)
10^{-5}	1.59(44)	3.47(22)	3.82(118)
10^{-4}	0.69(19)	1.90(12)	1.61(50)
10^{-3}	0.68(19)	1.20(7)	1.13(36)

Unit of ALPase activity and ATPase activity was μmoles p-nitrophenol/min. per mg. protein and μmoles P_i/10 min. per mg. protein, respectively. The number within parentheses is the percent of control. All data represent the mean of 2-3 experiments.

On the other hand, the activity of $Mg^{++}+Ca^{++}$-stimulated ATPase decreased slightly in the presence of 10^{-7} M cadmium, and in the presence of 10^{-5} M cadmium this activity was 12 % of the control. Cadmium inhibited the increase in ATPase activity stimulated by calcium ions. Melancon et al. (8) investigated the effect of many inhibitors on ATPase activity in the chick intestinal brush border but they did not examined the effect of cadmium. In this experiment, cadmium has been shown to be a potent inhibitor of ATPase.

The starting body weight of the control and experimental group was 225±12 and 228±21 (M±SD) g, respectively. During the experimental period, the increase of the body weight in the experimental group was lower than that in the control group, but this difference was not significant. During the experimental period there was no difference in the serum calcium and phosphorus between the control and experimental group.

TABLE 2 shows the activity of ALPase and ATPase in the control and experimental group. As shown in TABLE 2, the activities of all enzymes of the experimental group decreased significantly to about one-half of the control, respectively. The brush borders of the experimental group were washed with 5 mM EDTA solution in the procedures of preparing this, but the final brush border suspension was contained cadmium in the level of $12.56\pm2.56\times10^{-2}$ μg/mg protein (M±SD). However, cadmium was not detected in the control group final suspension by the technique of an atomic absorption. Thus a assay tube of ALPase in the experimental group was contained cadmium in the level of 3.2×10^{-8} M and a assay tube of ATPase was contained cadmium in the level of 1.2×10^{-7} M. The cadmium concentration of these assays was lower than that in the assay in vitro (TABLE 1).

TABLE 2 Effect of Cadmium, in vivo, on ALPase and ATPase

Group	ALPase	ATPase	
		$Mg^{++}+Ca^{++}$	Mg^{++}
control	2.01±0.51	6.86±1.38	0.96±0.26
experimental	*1.01±0.64	*2.72±1.95	*0.45±0.13

Unit of ALPase and ATPase activity was μmoles p-nitrophenol /min per mg protein and μmoles P_i/10 min per mg protein, respectively. All data is represented by M±SD and analyzed according to the t-test (*$p<0.01$).

Melancon et al. (8) reported that addition of calcium ions had little effect on ATPase of brush borders from vitamin D_3 deficient chick but it had a marked stimulatory effect on that from chicks which had received the vitamin D_3. Their results appeared possible that the vitamin might induce calcium-dependent ATPase which in turn might be related to well-known action of the vitamin in increasing intestinal calcium transport. And in this experiment the activity of ATPase in the brush borders from rats administered cadmium was decreased as compared with that of the control. It might seem that cadmium affected preventively on the metabolism of vitamin D_3 or on the action of the vitamin on ATPase.

Martin and DeLuca (9) gave evidence to support the existence of a vitamin D-dependent factor in the intestinal brush border region which was necessary for calcium uptake. And Norman et al. (5) reported that the increase in ALPase activity exactly parallels a simillar increase in calcium transport activity. From their results, the decrease of ALPase activity of brush borders from rats received cadmium in this study suggests that cadmium decreases presumably the absorption of calcium from the rat intestine.

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