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Short Communication

**DECREASED PHOSPHODIESTERASE ACTIVITY IN CHOLERA
TOXIN-INDUCED HYPERSECRETION IN SUCKLING RATS**

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SUMMARY: During cholera toxin (CT)-induced hypersecretion in suckling rats, the rise in the intestinal cAMP concentration was found to be accompanied by a decrease in the cAMP-phosphodiesterase activity. The results suggest the involvement of phosphodiesterase (PDE) as one of the factors governing the rise of cAMP.

Adenosine 3',5'-cyclic monophosphate (cAMP) has been shown to mediate the intestinal secretion in cholera (1-3). Intestinal adenylate cyclase activity and the cAMP level have been found to be elevated in clinical and experimental cholera, suggesting the possibility that cholera enterotoxin (CT) exerts its effect through the activation of adenylate cyclase and the subsequent elevation of cAMP (4,5). Cyclic nucleotide phosphodiesterases are the physiological enzymes that also govern the levels of cyclic nucleotide. This area has been little studied in CT-induced hypersecretion. In this paper, we report that the cAMP-phosphodiesterase (PDE) activity is also reduced in CT-induced hypersecretion in suckling rats.

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The procedure for experimental secretory diarrhea in the rat model has been described previously (6). In brief, 14 suckling Wistar rats as controls were given 0.1 ml of intragastric injections of 0.8% (w/v) Evans blue dye in physiological saline. Thirteen Wister rats which showed positive toxin reaction, that is, the infected group were given 0.1 ml of CT (25 μ g/ml) prepared in the same dye solution. The milk-filled stomach of the animal was identified below the left lung and between the liver and the spleen as a white patch. After 3 hr, the animals were sacrificed by neck/head dislocation and the stomachs were examined to confirm the existence of dye in the gut lumen. The intestines were removed, and the weights of the gut and the remaining carcass were measured. For each rat, the ratio of gut to carcass weight was calculated and the ratio of higher than 0.065 was taken as showing a positive effect (i.e., demonstrating a toxic intestinal secretory diarrhea), according to Dean et al. (7) and Baselski et al (8). [The mean ratio for the CT-treated group was $0.077 \pm (\text{SE}) 0.003$]. All animals in the control group showed the ratio lower than 0.065. [The mean ratio for control group was $0.042 \pm (\text{SE}) 0.001$]. The intestines were washed with chilled 0.25 M sucrose containing 0.5% of Tris-HCl buffer (pH 7.4), blotted, weighed and then homogenized in 0.25 M sucrose ($20 \times$ the weight of tissue) with a Potter-Elvehjem homogenizer. Each homogenate was divided into two portions. One portion was frozen and thawed five times to lyse the cells and then used for PDE measurement. The other portion which was used for measuring cAMP level was heated in a boiling water bath for 2 min to destroy the PDE activity and then immediately frozen and stored at -20°C until the assay.

PDE (EC 3.1.4.1) was assayed according to the method of Lin et al (9). The inorganic phosphate released after addition of nucleotidase was measured by the method of Aye-Kyaw et al (10). cAMP was assayed with the assay kit supplied by Amersham, UK. Protein was measured by the method of Miller (11).

PDE activities were expressed as units (U) per mg protein or per g tissue, where 1 U is equal to 1 μ mol of inorganic phosphate produced per hour. cAMP levels were also expressed as picomoles per mg protein or per g tissue. Statistical analyses were performed by Student's unpaired *t* test.

PDE activity showed a linear function with the enzyme protein concentration in a homogenate. Subsequent PDE assays were done in the linear region to ensure the validity of the enzyme assays.

As shown in Table I, PDE activities were significantly ($p < 0.05$ and $p < 0.001$) decreased in the CT-treated group when compared with the control in

Table I. Intestinal PDE and cAMP levels in control and CT-treated animals

Group	PDE		cAMP	
	Units per mg protein	Units per g tissue	pmoles per mg protein	pmoles per g tissue
Control (C) n=14	0.605±0.028 (100) ^a	21.68±1.09 (100)	1.092±0.089 (100)	38.86±2.94 (100)
Infected (I) n=13	0.503±0.029 (83)	13.61±1.51 (63)	3.379±0.222 (309)	80.69±7.94 (208)
Significance C-I	p<0.05	p<0.001	p<0.001	p<0.01

Each value represents the mean ± SEM.

'n' represents the number of animals. ^aPercent of control.

the enzyme activity per protein or wet tissue weight. The levels of cAMP significantly increased in the CT-treated group in comparison with the control.

There are many studies on the mechanism of intestinal secretion caused by cholera (1-3). It is known that the mechanism involves elevation of cAMP concentration in intestinal mucosa accompanied by an increase in the activity of adenylate cyclase, which catalyzes the cAMP synthesis (4,5). However, Schaffer et al. (12) suggested that the rise in the cAMP level may also involve direct or indirect effects on cyclic-nucleotide phosphodiesterase. cAMP-phosphodiesterase is the enzyme which catalyzes the conversion of 3'-5' cAMP to 5'AMP and this enzyme has been little studied in CT-induced hypersecretion. Thus, intestinal cAMP and PDE levels were measured in the present study. cAMP concomitantly increased and PDE activity was consistently decreased per protein and wet tissue weight in the CT-treated group when compared with their respective controls.

It may, therefore, be concluded that during CT-induced hypersecretion, the rise in cAMP is accompanied not only by the increase in adenylate cyclase activity (4,5) but also by the decrease in PDE activity. It is not yet clear whether the decrease in PDE activity occurs due to direct inhibition by the subunits of the toxin which is transported into cells or due to decreased *de novo* synthesis of PDE.

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