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EFFECT OF CALCIUM ON CYCLIC NUCLEOTIDE PHOSPHODIESTERASE IN PARATHYROID TISSUE

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SUMMARY: The hydrolysis of cyclic AMP and cyclic GMP by homogenates of normal bovine parathyroid gland and human parathyroid adenomas was decreased by EGTA. When supernatants were chromatographed on DEAE-cellulose it was found that sheep brain calmodulin in the presence of calcium stimulated cyclic AMP and cyclic GMP phosphodiesterase activity. The response to calmodulin in two human parathyroid adenomas was less than that in normal bovine parathyroid. Calmodulin was detected in heat-treated supernatants of 11 parathyroid adenomas by its ability to activate calmodulin-free sheep brain phosphodiesterase. The results suggest a role for calcium in the hydrolysis of cyclic nucleotides in parathyroid tissue.

In an *in vitro* study of normal bovine parathyroid glands, Abe *et al.* (1) proposed that cyclic AMP mediated the effects of calcium on parathyroid hormone (PTH) secretion. Addition of dibutyryl cyclic AMP *in vitro* to normal bovine parathyroid slices (2) or human parathyroid adenomas in organ culture (3) increased the secretion of PTH even in the presence of high medium calcium concentrations. The secretory response to calcium of abnormal human parathyroid tissue is variable (4-6) and may involve adenylate cyclase and cyclic nucleotide phosphodiesterase. Calcium inhibits the adenylate cyclase activity of normal horse (7) and human parathyroid adenomas (8, 9); in addition, Habener *et al.* (10) using the calcium ionophore A 23187 suggested a direct intracellular involvement of calcium ions in hormone secretion in the parathyroid gland. In many tissues multiple forms of cyclic nucleotide phosphodiesterase have been reported (11), some shown to be activated by calcium. Cheung (12) and Kakiuchi *et al.* (13) isolated from brain a heat-stable calcium-dependent regulatory protein, now called calmodulin (14), which activates the enzyme from the same source. Chromatography on DEAE-cellulose separates calmodulin from

Abbreviations: EGTA, ethylene-bis-(oxyethylene-nitrilo)-tetraacetic acid; cyclic GMP, guanosine 3′, 5′ cyclic monophosphoric acid; cyclic AMP, adenosine 3′, 5′ cyclic monophosphoric acid.

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phosphodiesterase (12,15,16). The aim of the present investigation was to examine the role of calcium ions in the regulation of cyclic nucleotide degradation in parathyroid tissue and to compare normal bovine parathyroid and human parathyroid adenomas.

MATERIALS:

Cyclic AMP, cyclic GMP and snake venom (*Ophiophagus hannah*) were obtained from the Sigma Chemical Company, U.S.A. [^{3}H] cyclic AMP, [^{3}H] cyclic GMP, [^{3}H] guanosine and [^{3}H] adenosine were obtained from the Radiochemical Centre, Amersham, U.K. EGTA was obtained from the Aldrich Chemical Company, U.S.A., AGI x 2 resin 200-400 mesh from BioRad Laboratories, U.S.A., DEAE-cellulose from Brown and Co., U.S.A. and Sephadex G-100 from Pharmacia, Sweden.

METHODS:

Phosphodiesterase activity was measured by a modification (17) of the method of Thompson *et al.* (18) using [^{3}H]-labelled substrates in 40 mM tris-HCl, 5 mM $MgCl_2$, 1 mM β-mercaptoethanol, pH 8, at 30° C. Corrections were made for recovery using [^{3}H] adenosine and [^{3}H] guanosine. Calmodulin was purified from sheep cerebral cortex by the method of Lin *et al.* (19) with slight modification. The preparative electrophoresis step was replaced by gel filtration on Sephadex G-100. Calmodulin levels in heat-treated tissue homogenates were measured by the method of Smoake *et al.* (20) using a calmodulin-free phosphodiesterase purified from sheep cerebral cortex (21). Inorganic phosphate was estimated by the method of Goldenberg and Fernandez (22). The amount of protein (μg) required to produce a 50% activation of the phosphodiesterase was defined as 1 unit of activator. Specific activity was expressed as units per mg protein (20). Protein concentration was estimated by the method of Schacterle and Pollack (23).

Parathyroid tissue was homogenized in 40 mM tris-HCl, 5 mM $MgCl_2$, 1 mM β-mercaptoethanol, pH 7.4 (Buffer A) using a teflon glass homogenizer. Homogenates (4 mL) were sonicated for 2 min and centrifuged for 60 min at 100,000 x g. The supernatant was applied to a column of DEAE-cellulose equilibrated in Buffer A. The column was then washed with 0.05 M NaCl in Buffer A and the phosphodiesterase eluted with a linear 0.05 to 0.6 M NaCl gradient. Fractions were assayed with 1 μM cyclic AMP and cyclic GMP in the presence and absence of purified sheep brain calmodulin and 0.1 mM $CaCl_2$. The area under the curve was measured with a compensating planimeter.

RESULTS:

Addition of the calcium chelator EGTA to parathyroid homogenates decreased the hydrolysis of cyclic AMP and cyclic GMP (Table I). The hydrolysis of cyclic AMP was reduced to 80% of control in the bovine parathyroid and to 82 ± 1.9% (mean ± S.E.) of control in the four human parathyroid adenomas studied. The hydrolysis of cyclic GMP was reduced to 46% of control in the bovine parathyroid and to 57 ± 9% of control in the adenomas.

Chromatography on DEAE-cellulose of 100,000 x g supernatants is shown in Figures 1-3. From a comparison of the area under the curves, purified sheep brain calmodulin in the presence of calcium stimulated the hydrolysis of cyclic GMP and cyclic AMP to 457 and 244% respectively of

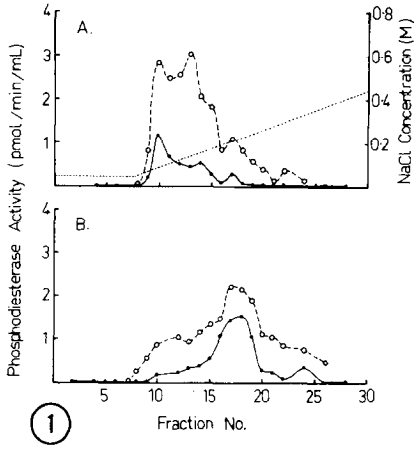
TABLE I

Effect of EGTA on phosphodiesterase activity of parathyroid homogenates

Tissue		Cyclic AMP*		Cyclic GMP*	
		-EGTA	+EGTA**	-EGTA	+EGTA**
parathyroid adenoma	S.P.	0.28	0.22	0.28	0.12
	T.K.	0.49	0.41	0.80	0.31
	V.C.	1.49	1.19	1.66	1.28
	V.P.	1.06	0.92	1.59	1.06
bovine parathyroid		0.74	0.59	0.81	0.37

* pmole per min per mg protein using 1 μM cyclic AMP and cyclic GMP
** EGTA concentration 125 μM

unstimulated activity in the bovine parathyroid (Figure 1). The stimulation by calmodulin for the two human parathyroid adenomas was much less. Cyclic GMP hydrolysis was stimulated 204% by adenoma S.P. (Figure 2) and 162% by adenoma T.E. (Figure 3). Cyclic AMP hydrolysis was stimulated 135% and 114% by adenomas S.P. and T.E. respectively.



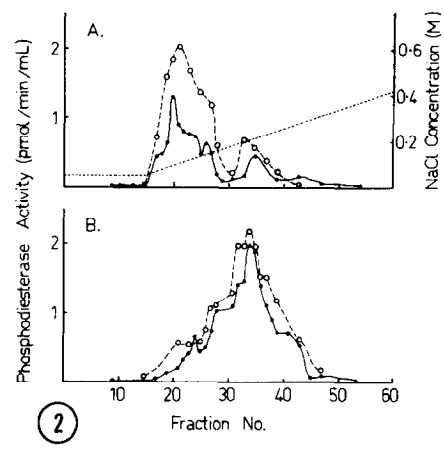


Figure 1. DEAE-cellulose column profile of phosphodiesterase activity in bovine parathyroid gland. Supernatant (100,000 x g, 5.1 mg protein) was applied to a DEAE-cellulose column (1.5 x 30 cm) equilibrated in Buffer A. Phosphodiesterase was eluted with a 200 mL 0.05 to 0.6 M linear NaCl gradient (- - - - -). Fractions were assayed with 1 μM cyclic GMP (A) and 1 μM cyclic AMP (B) in the absence (•) and presence (○) of 5.2 μg sheep brain calmodulin and 0.1 mM $CaCl_2$.

Figure 2. DEAE-cellulose column profile of phosphodiesterase activity in a human parathyroid adenoma (S.P.). Supernatant (100,000 x g, 17 mg protein) was applied to a DEAE-cellulose column (2 x 40 cm) equilibrated in Buffer A. Phosphodiesterase was eluted with a 600 mL, 0.05 to 0.6 M linear NaCl gradient (- - - -). Fractions were assayed with 1 μM cyclic GMP (A) and 1 μM cyclic AMP (B) in the absence (•) and presence (○) of 2.2 μg sheep brain calmodulin and 0.1 mM $CaCl_2$.

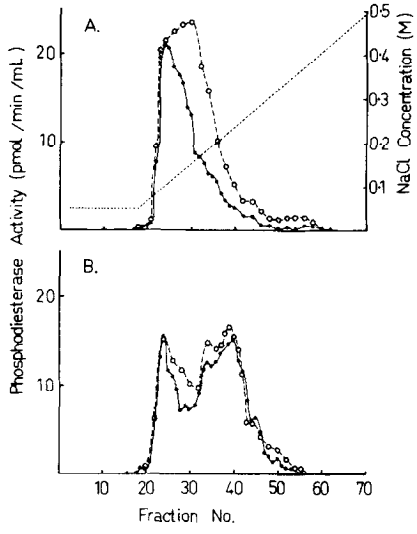


Figure 3. DEAE-cellulose column profile of phosphodiesterase activity in a human parathyroid adenoma (T.E.). Supernatant (100,000 x g, 19 mg protein) was applied to a DEAE-cellulose column (2 x 40 cm) equilibrated in Buffer A. Phosphodiesterase was eluted with a 600 mL 0.05 to 0.6 M linear NaCl gradient (- - - -). Fractions were assayed with 1 μm cyclic GMP (A) and 1 μM cyclic AMP (B) in the absence (•) and presence (○) of 5.2 μg sheep brain calmodulin and 0.1 mM $CaCl_2$.

Calmodulin was assayed in eleven parathyroid adenomas using a calmodulin-free phosphodiesterase from sheep brain. The specific activity ranged from 17 to 88 units per mg protein with a mean of 46 ± 8. The specific activity of calmodulin in the bovine parathyroid was 58 units per mg protein.

DISCUSSION:

Removal of calcium from parathyroid homogenates by the addition of EGTA decreased the hydrolysis of cyclic GMP and to a lesser extent cyclic AMP (Table I). Chromatography on DEAE-cellulose removes calmodulin from phosphodiesterase (12,15,16). Addition of calmodulin plus calcium to column fractions from bovine parathyroid increased the hydrolysis of cyclic GMP and cyclic AMP (Figure 1), the greater effect again being on cyclic GMP hydrolysis. Three distinct phosphodiesterase activities were found (24) when rat liver homogenates were chromatographed on DEAE-cellulose. Other mammalian tissues have different profiles on DEAE-cellulose and two or more forms of the enzyme are generally found (11). The substrate specificities of the column fractions from the bovine parathyroid were different and could be explained by different forms of phosphodiesterase. Fraction 10, for example, hydrolysed cyclic GMP at 6.6 times the rate of cyclic AMP, and fraction 18 hydrolysed cyclic AMP at 21 times the rate of cyclic GMP. The two

human parathyroid adenomas studied on DEAE-cellulose showed lower activation by sheep brain calmodulin than the normal bovine parathyroid. Cyclic GMP phosphodiesterase activity was increased to a greater extent than cyclic AMP activity. Our experiments also indicate the presence of calmodulin in parathyroid tissue. Although no attempt was made to isolate calmodulin in our samples, heat-treated homogenates activated the calmodulin-deficient phosphodiesterase from sheep brain in the presence of calcium ions. There have been several reports (25-27) of phosphodiesterase inhibitory proteins, including calmodulin binding protein, which may not be separated from the enzyme on DEAE-cellulose. It is therefore possible that the presence of these inhibitory proteins could reduce the calmodulin sensitivity of the phosphodiesterase.

In parathyroid tissue, increased extracellular calcium decreases cyclic AMP levels and secretion of PTH (1,28). The precise mechanism by which calcium reduces AMP levels has not been elucidated; however, calcium inhibits the adenylate cyclase activity of parathyroid membrane preparations (7,8,9). An intracellular role for calcium has been suggested as the calcium ionophore A 23187 has been found to reduce PTH secretion (10); such a role could involve calmodulin. Our experiments indicate the presence of a calmodulin-dependent degradation pathway for cyclic nucleotides in the parathyroid. The effects of calmodulin on phosphodiesterase activity are greater in the normal bovine parathyroid than in the human adenomas, which is consistent with the observation that the secretion of PTH by human parathyroid adenomas is suppressed only by higher than normal concentrations of calcium (6).

In conclusion, we report the presence of calmodulin sensitive phosphodiesterase activity in normal bovine parathyroid glands and human parathyroid adenomas and provide evidence for the presence of calmodulin in these tissues. The decreased calmodulin activation of the phosphodiesterase in the human parathyroid adenomas compared to the normal bovine parathyroid indicates a role for this enzyme in the regulation of cyclic nucleotide levels and PTH secretion. The hydrolysis of cyclic GMP is more sensitive to calcium than that of cyclic AMP. However, to our knowledge, a role for cyclic GMP in the parathyroid gland has not been investigated.

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