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Parathyroid Hormone Activation of Adenylate Cyclase in Liver (38343)

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Kidney and bone are two generally recognized target organs for parathyroid hormone (PTH). Increased transport of calcium across the intestinal epithelium was once considered a direct effect of PTH, but current evidence indicates that this effect is mediated by the action of PTH on the metabolism of vitamin D (1).

Over the years there has been abundant evidence to suggest an effect of PTH on other tissues. Toverud and Munson (2) showed that parathyroidectomy of lactating rats increased the calcium content of the milk despite prevailing hypocalcemia. Tenenhouse (3) and Borle (4) and their associates have reported PTH stimulation of calcium transport in Hela cells and in Ehrlich ascites cells.

Whitfield and his colleagues (5–8) demonstrated that the administration of PTH increases mitogenic activity in bone marrow and thymus cells. Conversely, parathyroidectomy causes a drastic reduction of cell proliferation in these tissues. The resulting atrophy of the thymus and depressed erythropoiesis of bone marrow can be alleviated by PTH or calcium therapy. More recently, these workers have reported that the normal proliferative response of liver parenchymal cells to partial hepatectomy requires the presence of parathyroid glands and is preceded by a transient hypocalcemia (9). PTH stimulates DNA synthesis and mitogenic activity in the hepatocyte as a prelude to liver regeneration.

This *in vivo* hepatic action of PTH complements extensive work done *in vitro* showing an effect of PTH on liver mitochondria. Rasmussen (10) and DeLuca (11) and their co-workers showed increased respiration and specific ion transport in mitochondria isolated from liver as well as from kidney and intestinal mucosa. It has also been demonstrated that PTH can stimulate an influx of calcium into liver cells (12).

This background led us to an investigation of the liver as a possible end organ for PTH action. In addition, during studies of activation of skeletal adenylate cyclase by PTH, Chase *et al.* (13) noted some stimulation of this enzyme in liver. They considered the response to PTH as minimal in comparison to that elicited by glucagon and did not explore the phenomenon further. In the present paper, we present in detail the effect of PTH on liver adenylate cyclase.

Materials and Methods. Preparation and assay of hepatic adenylate cyclase. Rats and cats were anesthetized with pentobarbital, 25–35 mg/kg intraperitoneally, and the livers were rapidly removed. Approximately 200 mg of tissue was homogenized in 4.5 ml of cold 0.25 *M* sucrose. The homogenate was centrifuged at 12,000*g* for 10 min at 4°, and the supernatant fluid was decanted; the particles were washed with cold 0.25 *M* sucrose, resuspended and recentrifuged at 12,000*g* for 10 min. The washed particles were then resuspended and rehomogenized in cold 0.25 *M* sucrose. Protein was determined by the method of Lowry *et al.* (14). Adenylate cyclase was assayed by the method of Krishna *et al.* (15). The particulate fraction containing 0.04–0.06 mg of protein was incubated at 37° for 10 min in a total volume of 60 μl with 2.5 m*M* ATP; α-^{32}P-ATP, 3.0–3.5 $\times$ 10^6 cpm; theophylline, 8 m*M*; $MgCl_2$, 3.2 m*M*: Tris–HCl, 21 m*M*, pH 7.7; bovine PTH[2],

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[2] Highly purified bovine PTH was obtained from Wilson laboratories in Chicago, IL. Specific activity of this material, based on the Munson rat bioassay, is 2000–2500 MRC units/mg.

the *N*-terminal of bovine PTH (1-34 peptide)[3], glucagon, epinephrine, or sodium fluoride at concentrations noted in the text. The diluent for PTH and the peptide 1-34, 0.01 *N* HAc, was added to control incubations. Hormone solutions were prepared fresh for each experiment. The cAMP produced was determined as previously described (16).

Results. Effect of bovine parathyroid hormone and synthetic 1-34 peptide on liver adenylate cyclase. The effect of bovine PTH and the synthetic *N*-terminal peptide 1-34 on liver adenylate cyclase is shown in Fig. 1. Bovine PTH increased the accumulation of cAMP over the concentration range $6 \times 10^{-8} M - 1 \times 10^{-6} M$ with half-maximal activation occurring at about $1 \times 10^{-7} M$. The 1-34 peptide increased the accumulation of cAMP over a concentration range of $6 \times 10^{-9} - 6 \times 10^{-7} M$: half maximal activation occurring at about $6 \times 10^{-8} M$. The sensitivity of adenylate cyclase appeared to be greater for the synthetic peptide as compared to bovine PTH with significant differences occurring at 6×10^{-8} *M* ($P < 0.05$) and at 1×10^{-7} *M* ($P < 0.05$). No significant differences were observed at maximal concentrations of the hormones in which the synthetic peptide produced approximately a 70–80% increase in cAMP accumulation as compared to 60–70% for bovine PTH.

[3] Synthetic *N*-terminal (1-34 amino acids) of bovine PTH was obtained from Beckman Laboratories, Palo Alto, CA. Specific activity of this material, based on *in vitro* activation of adenylate cyclase from the rat renal cortex, is 3100 IU/mg.

The activation of the liver adenylate cyclase by bovine PTH and the synthetic peptide 1-34 was linear for the first 10 min of the incubation and over a range of protein from 15 to 75 μg per incubation.

In Fig. 2 are compared the maximal increases in cAMP achieved with known stimulators of liver adenylate cyclase, including glucagon, epinephrine, and sodium fluoride. Glucagon is most effective, producing about a threefold increase in cAMP accumulation. Although glucagon produced a much greater stimulation of adenylate cyclase than PTH, half-maximal activation with glucagon occurred at the same concentration as with the synthetic PTH peptide, $5 \times 10^{-8} M$ (17, 18).

In order to determine the specificity of the activation of liver adenylate cyclase by PTH, we examined the effect of several other polypeptide hormones (Table I). Calcitonin, thyrotropin, and gastrin did not activate the enzyme in the same preparation in which the 1-34 peptide produced approximately a twofold increase in cAMP accumulation.

Bitensky *et al.* demonstrated separate adenylate cyclase systems in liver for glucagon and epinephrine (19). In order to test which system is activated by PTH, we examined the effects of various peptides, singly and in combination (Table II). Maximal stimulatory concentrations of each were used. The 1-34 peptide was fully additive with epinephrine, but only partially additive with glucagon. It is possible that the reason for the lack of additivity is due to the fact that the glucagon stimulation of adenylate cy-

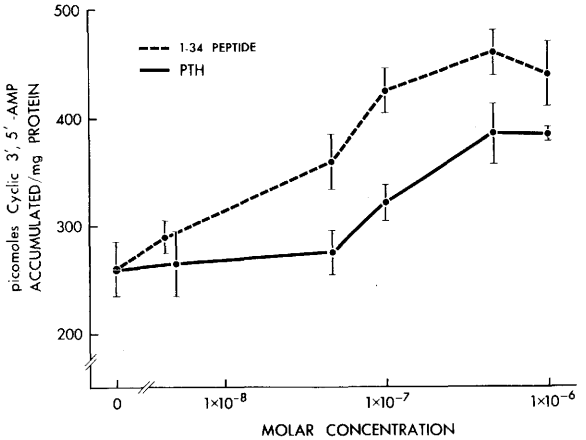

FIG. 1 *Concentration response curves for bovine PTH and synthetic peptide 1-34.* Each value represents the mean ± SEM of 5–7 experiments, each experiment having been performed with either triplicate or quadruplicate samples.

TABLE I. Effect of Other Polypeptide Hormones on Liver Adenylate Cyclase.[a]

		Picomoles cyclic 3′,5′-AMP accumulated/10 min/mg protein
Control		180 ± 20
1–34 Peptide	$2.0 \times 10^{-6} M$	330 ± 10
Calcitonin	$5.0 \times 10^{-6} M$	200 ± 20
TSH	$6.0 \times 10^{-7} M$	220 ± 10
Gastrin	$2.0 \times 10^{-6} M$	180 ± 4.0

[a] Each value is the mean ± SE of six samples from two rats.

TABLE II. Effect of Combined Maximal Concentrations of Hormones in Liver Homogenates.

	Picomoles cyclic 3′, 5′-AMP accumulated/10 min/mg protein[a]
Control	33 ± 8.0
1–34 Peptide $6 \times 10^{-7} M$	89 ± 9.0
Epinephrine $1 \times 10^{-4} M$	75 ± 6.0
Epinephrine + 1–34 peptide	125 ± 6.0
Glucagon $1 \times 10^{-5} M$	345 ± 9.0
Glucagon + 1–34 peptide	378 ± 12.0
Glucagon + Epinephrine	398 ± 4.0

[a] Each value is the mean ± SE of eight samples from two rats.

clase alone is at or near the maximum for the enzyme.

The beta-adrenergic blocking drug propranolol at $1 \times 10^{-6} M$ did not abolish the activation of adenylate cyclase by $6 \times 10^{-7} M$ 1-34 peptide. Thus, the 1-34 peptide does not appear to be mediated through release of membrane-bound catecholamines.

Species specificity. In order to determine the species specificity of these observations, we examined the effect of bovine PTH and synthetic peptide 1-34 on cat liver adenylate cyclase (Table III.) At $1 \times 10^{-6} M$, bovine PTH produced approximately a twofold increase in cAMP accumulated and synthetic peptide about a 2.5-fold increase.

Discussion. It has become evident that the binding of many polypeptide hormones to their target organs is followed by activation of the membrane bound enzyme adenylate cyclase (20). The subsequent increase in intracellular cAMP is thought to mediate the effects of the hormone on its target tissue. Furthermore, activation of the enzyme in a particular tissue by a hormone is considered strong evidence that the tissue is a target organ for the hormone. According to the present data, liver is a target organ for PTH. In comparison with two hormones for which liver is a well established target, glucagon and epinephrine, the activation of PTH was less than that of glucagon but greater than that of epinephrine. These results are in accord with the preliminary report of Moxley *et al.* (21).

Bone and kidney, the recognized end organs for PTH, share with liver the following attributes: (a) Each of these tissues have been reported to have enzymes selectively metabolizing PTH (22–25) . (b) The hormone increases calcium entry into the cells of each tissue (12, 26–28). (c) For all three, a maximal stimulation

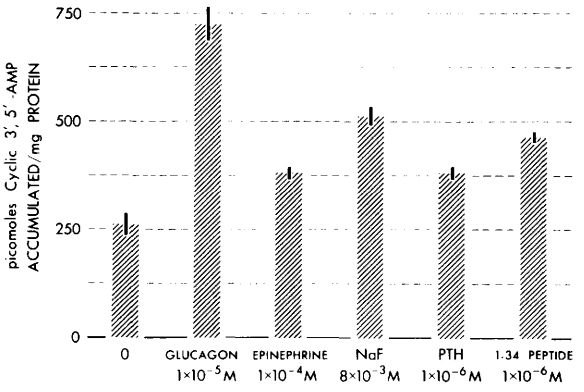

FIG. 2. *Comparison of various activators of liver adenylate cyclase.* Each value represents the mean ± SEM of 4 experiments, each experiment having been performed with either triplicate or quadruplicate samples.

TABLE III. Activation of Cat Liver Adenylate Cyclase by Bovine PTH and Synthetic Peptide 1–34.

	Picomoles cyclic AMP accumulated/mg protein/10 min[a]
Control	91 ± 17
PTH	174 ± 29
1–34 Peptide	215 ± 27
Glucagon	349 ± 10

[a] Each value represents the mean ± SE of four samples.

of adenylate cyclase is achieved at roughly equal molar concentrations of PTH (13, 29). However, while the distinctive physiological effects of PTH mediated in bone and kidney by cAMP are well known, no such actions have been discerned in the liver. The most attractive possible role for PTH on hepatic cell function is the regulation of the conversion of cholecalciferol to its 25-hydroxy derivative, but this possibility has not yet been explored systematically.

Summary. The effect of parathyroid hormone (PTH) on the membrane bound enzyme adenylate cyclase in rat and cat liver tissue homogenates was studied. Bovine PTH increased the accumulation of cyclic AMP (cAMP) over a range of $6 \times 10^{-8} - 1 \times 10^{-6} M$. The synthetic *N*-terminal peptide (1-34 amino acids) of the hormone increased the accumulation of cAMP over a range of $6 \times 10^{-9} - 6 \times 10^{-7} M$. Compared to well studied stimulators of liver adenylate cyclase, glucagon and epinephrine, PTH produced a greater stimulation than epinephrine but less than glucagon. Calcitonin, thyrotropin, and gastrin did not stimulate the liver adenylate cyclase. Maximal concentrations of the 1-34 peptide and epinephrine were additive but maximal concentrations of the 1-34 peptide and glucagon were only partially additive.

The concentration of PTH showing maximal stimulation of liver adenylate cyclase is equivalent to that reported for bone and kidney, the two well studied end organs for the hormone. Although the data suggest that the liver is an end organ for PTH, there is no known physiologic role for this hormone in the liver except for a possible requirement for PTH in liver regeneration.

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