The Effect of Pyrophosphate Infusion on the Response of the Thyroparathyroidectomized Rat to Parathyroid Hormone and Adenosine-3',5'-cyclic Monophosphate

Allyn DeLong, Joel Feinblatt, and Howard Rasmussen

Received November 2, 1970, accepted January 1, 1971

The infusion of inorganic pyrophosphate at a rate of 15 μmoles/h into thyroparathyroidectomized rats blocked the effects of parathyroid hormone infusion upon plasma calcium and phosphate, and upon the renal excretion of calcium, phosphate, hydroxyproline-containing peptides, and cyclic 3'5'AMP. In contrast, pyrophosphate infusion caused no significant change in the effects of cyclic 3'5'AMP infusion upon plasma calcium and phosphate, or upon the urinary excretion of calcium, phosphate, and hydroxyproline-containing peptides.

Key words: Pyrophosphate – Cyclic 3'5'-AMP – Parathyroid – Adenyl cyclase – Bone – Resorption.

There have been several recent hypotheses concerning the ionic and hormonal mechanisms regulating bone mineral accretion and resorption in mammals [5, 9, 21, 23]. Fleisch et al., (1966) have proposed that pyrophosphate plays a central role in the regulation of these processes. These authors have shown that pyrophosphate inhibits both mineral crystal growth [11], and dissolution [8] in vitro. They have also shown that diphosphonates, analogs of pyrophosphate, inhibit parathyroid hormone induced bone resorption in vivo [10]. Based on the results of their in vitro experiments, it has been suggested that pyrophosphate acts in vivo as a crystal poison.

An alternative hypothesis for the mechanism of regulating bone resorption has been proposed by Chase and Aurbach [6]. They suggest that parathyroid hormone
causes enhanced bone resorption by stimulating the production of cyclic 3′5′ AMP.\(^1\) The enzyme adenyl cyclase, which controls cyclic 3′5′ AMP synthesis, catalyzes the following reaction [22]:

\[
\text{ATP} \xrightleftharpoons[\text{Mg}^{2+}]{\text{3′5′AMP + PPi}}.
\]

Two aspects of this reaction are important: 1) pyrophosphate is a product of the reaction; and 2) the reaction is reversible, at least in bacterial systems [13]. These facts suggest a possible alternative for the effects of pyrophosphate upon parathyroid-hormone-induced bone resorption. Instead of acting by a direct effect upon mineral crystal dissolution, pyrophosphate could act by inhibiting cellular function, specifically cyclic 3′5′ AMP synthesis, and thereby influence the response of the animal to parathyroid hormone infusion. In order to test this alternative possibility we have examined the effects of pyrophosphate infusion upon the response of thyroparathyroidectomized rats to parathyroid hormone and cyclic 3′5′ AMP.

**Material and Methods**

The techniques of animal preparation, animal perfusion, and chemical analysis of blood and urine were performed as previously described [1, 2, 17]. Cyclic 3′5′ AMP was measured in the urine by the method of Goldberg et al., (1969). Parathyroid hormone, having an activity of 2500 units/mg, was prepared by the method of Hawker et al., (1966). Sodium pyrophosphate was obtained from Merck and Co. (Rahway, New Jersey) and cyclic AMP from Schwartz Bioresearch, Inc. (Mt. Vernon, New York). The protocol for the experiments was: 1), thyroparathyroidectomy, and insertion of catheters in jugular vein and urinary bladder under light ether anesthesia; 2), mounting of animal in Cotlove cage; 3), initiation of perfusion via the jugular vein with a solution containing 10 mM glucose, 20 mM NaCl, 2.5 mM MgCl\(_2\), and 2.5 mM KCl, pH 7.4 at a rate of 3 ml/h; 4), an initial period of 16–22 h perfusion for stabilization of ion and water excretion; 5), collection of hourly urine samples for a period of 8–12 h; 6), the addition of sodium pyrophosphate, pH 7.4, at a concentration of 5 mM to the perfusate of some animals and its infusion for a period of 4 h before adding to the perfusate either parathyroid hormone or cyclic 3′5′ AMP. During these infusions the rates of perfusion of the various substances were: glucose-30, Na\(^+\)-60, Mg\(^{2+}\)-7.5, K\(^+\)-7.5, and pyrophosphate 15 \(\mu\)moles/h cyclic 3′5′ AMP-2 mg/h; and PTH-5 \(\mu\)g/h. In the case of parathyroid hormone, the infusion was continued for several hours after pyrophosphate infusion was stopped. Urine was collected at hourly intervals, and analyzed for its content of calcium, inorganic phosphate, and hydroxyproline-containing peptides, and in some cases cyclic 3′5′ AMP. Blood samples were collected at the end of each experiment and analyzed for calcium and phosphate. The data plotted are the mean values obtained from 4–5 animals perfused under each type of experimental circumstance.

**Results**

**PTH and Pyrophosphate**

During initial perfusion studies with pyrophosphate, it was found that if parathyroid hormone and pyrophosphate were infused simultaneously, the hormone exerted its initial effects, but these effects were not sustained [19]. However, it was found that if pyrophosphate was infused several hours before hormone infusion was begun, most of the initial, hormonally-induced changes were not observed. Therefore, the standard protocol adopted for the remainder of the studies

---

1 Abbreviations: PTH = parathyroid hormone; CT = calcitonin; HOP = hydroxyproline; PPi = inorganic pyrophosphate cyclic 3′5′ AMP — cyclic 3′5′ adenosine monophosphate.