REGULATION OF PARATHYROID HORMONE SECRETION BY ADENYL CYCLASE

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Received June 14,1972

SUMMARY

In a well-studied physiologically functioning in vitro system for the study of parathyroid hormone (PTH) secretion, both theophylline and dibutyryl cyclic adenosine monophate (dAMP) caused significant stimulation of PTH secretion. The quantity of PTH in the medium paralleled the release of cyclic 3', 5'-AMP (CAMP) from incubated tissues. These studies suggest strongly that adenyl cyclase is the mediator of the known effects of calcium ion on PTH secretion.

INTRODUCTION

The membrane-bound enzyme adenyl cyclase and its product CAMP have been implicated as intermediates in both the end organ effects and the regulation of secretion of a number of polypeptide hormones (1). While it has recently been shown that PTH produces its physiologic action on both bone and kidney through the intermediate action of CAMP (2, 3), the potential relationship of this cyclic nucleotide to actual secretion of the hormone has not yet been determined. In order to investigate the relationship of adenyl cyclase to PTH secretion, a functioning in vitro system described earlier was utilized (4, 5).

MATERIALS AND METHODS

Fresh bovine parathyroid glands were obtained immediately after slaughter of the animals, kept on ice until use, and then stripped of fat and surrounding connective tissue. The glands were cut into 1-2mm^3 pieces and incubated in 25 ml Erlenmeyer flasks in a metabolic shaker at 37°C in an atmosphere of 5% carbon dioxide - 95% oxygen. The tissue was preincubated for 2 hours in Krebs-Ringer bicarbonate buffer (8ml at pH 7.4) containing 3 mg per ml egg white lysozyme (Worthington), 1mg/ml glucose and 3.0mM calcium. The magnesium ion concentration was 0.75mM in all experiments. Following the preincubation period,

the tissue was rinsed twice with fresh buffer and placed in fresh medium containing different concentrations of calcium ion as well as varying concentrations of dAMP (Calbiochem) or theophylline (Calbiochem). Sequential samples of medium were removed from each flask at different times during the chase period, and similar flasks were run in triplicate. PTH released into the medium was measured by a sensitive and highly specific radioimmunoassay for bovine PTH described earlier (6). At the end of each experiment, the tissue was homogenized in 8M urea, 0.2N hydrochloric acid and the protein content of the tissue determined by the method of Lowry (7). The amount of hormone released was expressed in terms of ng bovine PTH per mg tissue protein. In similar fashion, quantities of CAMP released into the medium during the incubation were measured by a modification of the radioimmunoassay method of Steiner et al (8). The assay for CAMP was sensitive to less than 1 picomole of the nucleotide, and the ^{125}I-labeled tyrosine methyl esther of succinylated CAMP (Collaborative Research) was used. Bound and free nucleotide were separated by the double antibody method. In some experiments, the tissue was pulsed during the preincubation stage with uniformly labeled leucine-^{14}C (New England Nuclear, 3μc/flask) and the tissue then chased with cold medium during the incubation period. Radioactive hormone released into the medium was separated on columns of Biogel P-10 and counted after pooling of the fractions.

RESULTS AND DISCUSSION

As shown in Fig. 1, the release of PTH with time was inversely related to the concentration of calcium, with smaller amounts being released at 2.0mM than at 0.5mM. When theophylline and dAMP were added at concentrations less than 10^{-4}M, there was no significant stimulation of hormone release. When the two agents were added in combination at 10^{-4}M in the presence of 0.5mM calcium (Fig. 1), there was significant stimulation of PTH release one, two and three hours after incubation. At higher concentrations of each agent ($5 \times 10^{-4} - 1 \times 10^{-3}$), both dAMP and theophylline alone caused a one-and-a-half to two fold or greater increase in PTH release (Figures 2A and 2B). The effects of the two agents were significant in the presence of both low (0.5mM) and high (3.0mM) calcium solutions. Release of hormone in the 0.5mM calcium solution in the presence of theophylline or dAMP was equivalent to the maximum stimulation of PTH secretion that could be produced by concentrations of calcium as low as 0.1mM. In similar fashion, more radioactive PTH was released into the medium in the pulse-chase experiments

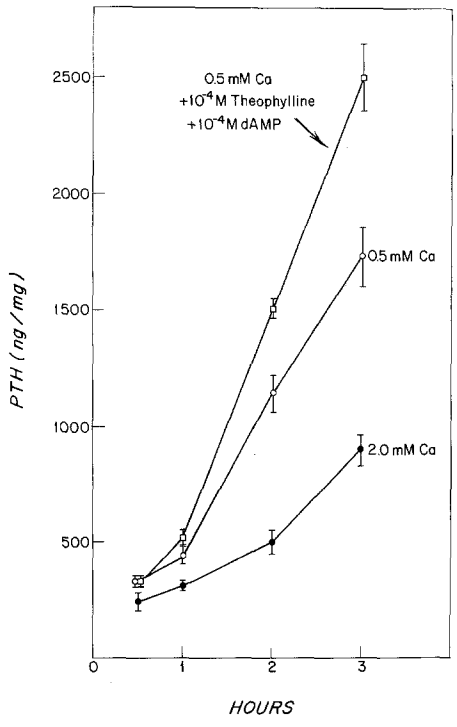

Figure 1. Release of immunoreactive PTH (ng/mg tissue protein) at different calcium concentrations and in presence of 10^{-4}M dAMP and theophylline. All differences at 1, 2 and 3 hours significant at $p < 0.01$.

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in the presence of theophylline and dAMP (see Table I).

In order to be certain that the release of PTH was correlated with the generation of spontaneous CAMP by the tissue, the concentrations of CAMP released into the medium were measured directly. As shown in Fig. 3, there was excellent correlation between the release of immunoreactive PTH and that of CAMP at concentrations of calcium varying from 3.0mM down to 0.25mM. Tissue levels of CAMP as well as adenyl cyclase and phosphodiesterase activity are currently being investigated in the same studies, but, because of the occasional presence of adipose cells in bovine parathyroid glands, it is more difficult to measure CAMP concentrations in parathyroid epithelial cells alone.

The current report describes the effects of dAMP and theophylline on the secretion of PTH in vitro by normal parathyroid glands and provides strong evidence that CAMP is an intermediate in the regulation of parathyroid secretory activity. Since calcium ion is a known regulator of parathyroid gland activity both in vivo

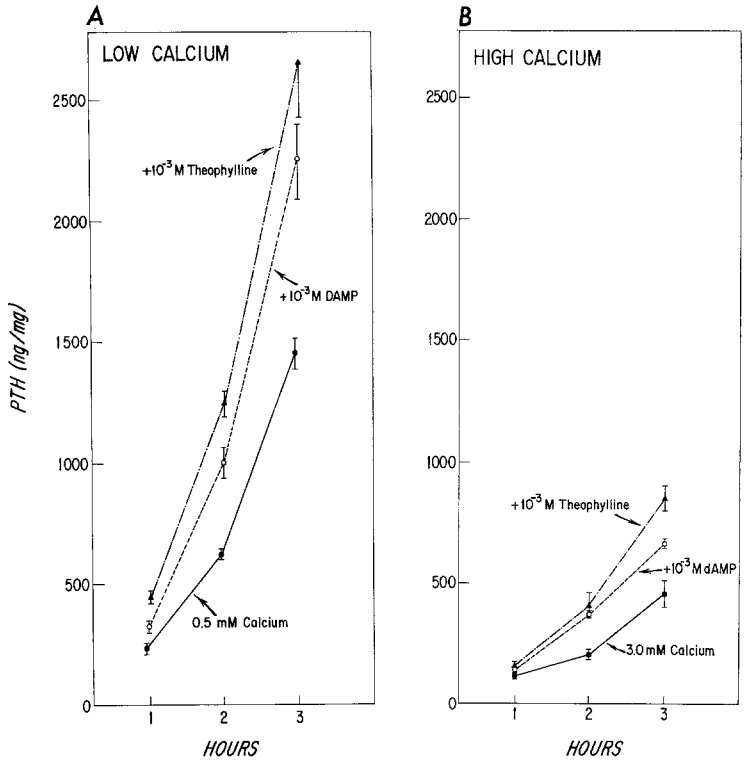

Figure 2. Release of PTH by 10^{-3}M dAMP and theophylline in presence of 0.5mM (2A) and 3.0mM (2B) calcium. All differences after 1 hour significant at $p < 0.01$.

(9, 10) and in vitro (4, 5), it is tempting to postulate that the effects of calcium ion on hormone secretion might be mediated through the cyclic nucleotide. Both dAMP (an analogue of CAMP as well as an inhibitor of the phosphodiesterase enzyme that breaks down CAMP) and theophylline (a phophodiesterase inhibitor) markedly stimulate PTH secretion independently of calcium concentration. Calcium ion is known to inhibit adenyl cyclase activity in other tissues, and the relationship between calcium ion and the release of PTH is first-order in nature (11). Since adenyl cyclase is a membrane-bound enzyme, binding of calcium ion to the cell membrane could provide a plausible mechanism for the regulation of secretory activity. Further support for this hypothesis may be found in the recent identification by Dufresne and Gitelman (12) of a highly calcium-sensitive adenyl cyclase enzyme in dog parathyroid tissue and our own observation showing that the release of CAMP into the medium varies in parallel with PTH secretion.

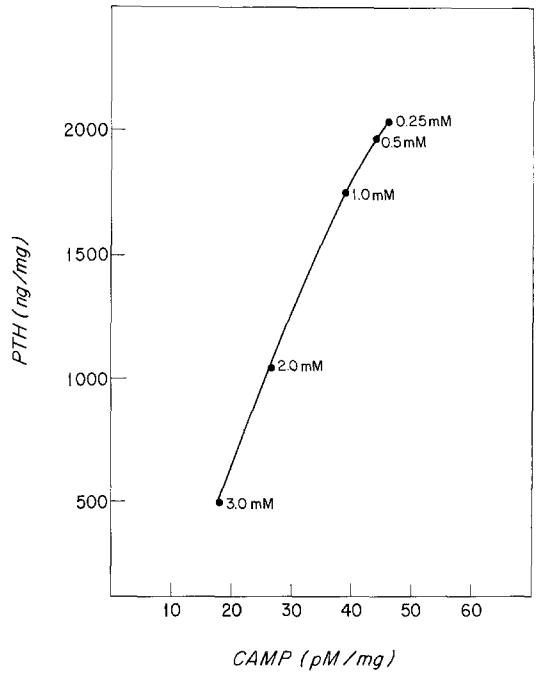

Figure 3. Release of immunoreactive PTH and CAMP (pM/mg tissue protein) at calcium concentrations varying from 3.0mM to 0.25mM (3 flasks per point).

TABLE I

Release of Radioactive PTH (by gel filtration) after 2 hour pulse with ^{14}C-leucine in 3.0mM calcium followed by addition of 10^{-3}M dAMP in 0.5 and 3.0mM calcium.

	TIME (HOURS)			
Experiment	1	2	3	4
0.5mM calcium	1450 ± 80	1330 ± 150	5550 ± 135	2660 ± 65
+ 10^{-3}M dAMP	2660 ± 140*	4850 ± 250*	10100 ± 300*	12300 ± 450*
3.0mM calcium	950 ± 50	940 ± 50	1300 ± 56	1660 ± 95
+ 10^{-3}M dAMP	1067 ± 70	2100 ± 75*	3100 ± 150*	4330 ± 150*

* $p < 0.01$ compared with value above

Although CAMP is known to be involved in the secretion of insulin, of a number of pituitary hormones, and of calcitonin (13), this is the first evidence that the secretion of PTH may involve this mechanism. These observations provide a useful basis for further studies of the regulation of PTH secretion by normal and abnormal parathyroid glands.

ACKNOWLEDGEMENT

This work was supported in part by grants from the United States Public Health Service (HD-03388) and the John A. Hartford Foundation, Inc. Dr. Sherwood is the recipient of a research career development award (1 KO4 AM 46426) from the Public Health Service.

REFERENCES

1. Sutherland, E.W., J. Amer. Med. Assoc., 214, 1281 (1970).
2. Chase, L.R. and Aurbach, G.D., J. Biol. Chem., 245, 1520 (1970).
3. Chase, L.R. and Aurbach, G.D., Proc. Nat. Acad. Sci., 58, 518 (1967).
4. Sherwood, L.M., Herrman, I., and Bassett, Nature, 225, 1056(1970).
5. Sherwood, L.M., Lundberg, W.B., Targovnik, J.H., Rodman, J.S., and Seyfer, A., Amer. J. Med., 50, 658 (1971).
6. Berson, S.A., Yalow, R.S., Aurbach, G.D., Potts, J.T., Jr., Proc. Nat. Acad. Sci., 49, 613 (1963).
7. Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J., J. Biol. Chem., 193, 265 (1951).
8. Steiner, A.L., Kipnis, D.M., Utiger, R., and Parker, C., Proc. Nat. Acad. Sci., 64, 367 (1969).
9. Sherwood, L.M., Potts, J.T., Jr., Care, A.D., Mayer, G.P., and Aurbach, G.D., Nature, 209, 52 (1966).
10. Sherwood, L.M., Mayer, G.P., Ramberg, C.F., Jr., Kronfeld, D.S., Aurbach, G.D., and Potts, J.T., Jr., Endocr., 83, 1943 (1968).
11. Targovnik, J.H., Rodman, J.S., and Sherwood, L.M., Endocr., 88, 1477 (1971).
12. Dufresne, L.R., Andersen, R., and Gitelman, H.J., Clinical Research, 19, 529 (1971).
13. Robison, G.A., Butcher, R.W., and Sutherland, E.W., Cyclic AMP (Academic Press, New York, New York, 1971).