EFFECTS OF PARATHYROID HORMONE AND CALCITONIN ON ADENYLATE CYCLASE IN MURINE MONONUCLEAR PHAGOCYTES

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Summary: Peritoneal mononuclear phagocytes elicited by thioglycollate demonstrate responsiveness to parathyroid hormone (PTH) and calcitonin (CT) which differs from that seen in the normal resident population. PTH causes a twofold stimulation of adenylate cyclase activity in elicited cells but inhibits this activity in resident cells. CT causes a greater stimulation of adenylate cyclase in elicited than in resident cells. Both CT and PTH cause an increase in cyclic AMP accumulation in cultures of elicited mononuclear phagocytes. These results indicate that cells of the mononuclear phagocyte lineage have functional receptors for both PTH and CT. This is the first biochemical evidence to support the hypothesis that mononuclear phagocytes are precursors of the bone resorbing osteoclast.

INTRODUCTION

Cells of the mononuclear phagocyte lineage have been demonstrated to possess a high degree of functional heterogeneity (1) and play a central role in host defense mechanisms as well as in the digestion of damaged cells and tissue debris. It has been postulated that mononuclear phagocytes are precursors of the osteoclast, a multinucleated bone cell intimately involved in the process of bone resorption (2,3,4). The evidence for this has been obtained primarily from sequential morphologic observations of [^{3}H]-thymidine labeled cells. There is no information available on the physiology or biochemistry of the transition from mononuclear phagocyte to osteoclast. Thus, it has been difficult to provide unambiguous evidence for this relationship and to design critical experiments to explore the cellular mechanisms

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whereby mononuclear phagocytes become bone resorbing cells.

Parathyroid hormone (PTH) and calcitonin (CT) are hormones intimately involved with the regulation of bone remodeling. Recent experiments using enzymatically dissociated bone cells have resulted in the isolation of a functionally defined population of mononuclear cells which possess "osteoclast-like" properties. These cells initially respond to PTH and CT by increased accumulation of cyclic AMP (5,6). If mononuclear phagocytes are osteoclast precursors they may also demonstrate alterations in cyclic nucleotide metabolism in response to PTH and CT. Such responsiveness would provide a physiological and biochemical basis for investigating the relationship of mononuclear phagocytes to the process of osteoclast differentiation and bone resorption.

MATERIALS AND METHODS

Cell Harvest and Culture. Murine mononuclear phagocytes, either resident (RMϕ) or thioglycollate-elicited (EMϕ), were obtained from peritoneal washings of male Swiss-Webster mice (25-30 gm) by established procedures (7). Cells were plated (4×10^5 per cm^2) and allowed to adhere to plastic Petri dishes (Lux Scientific) by incubation at 37°C in medium RPMI-1640 supplemented with 10% heated fetal calf serum (FCS). After 2 hours of culture, plates were rinsed vigorously with several changes of medium and adherent cells were then harvested. Cytocentrifuge smears and routine histologic analysis were performed and the remainder of the cell suspension was either prepared as a homogenate for adenylate cyclase assay (8) or resuspended to an appropriate concentration for subsequent incubation and cyclic AMP assay.

Adenylate Cyclase Assay. Adenylate cyclase assays were conducted using a modification of the method of Krishna et al. (9). The reaction was initiated by adding a 20 µl aliquot of cell homogenate (containing 5 to 60 µg of protein) to 30 µl of incubation medium. The final composition of the reaction mixture was 2 mM ATP, 5 mM $MgCl_2$, 10 mM caffeine, 10 mM phosphoenol pyruvate, 10 µg pyruvate kinase in 50 mM Tris HCl buffer, pH 7.5 containing 0.1% heat inactivated essential fatty acid free fraction V bovine albumin. NaF when present was 10 mM; [α^{32}P]-ATP (20-25 Ci/mMole - ICN Pharmaceuticals) was present at 1-2 x 10^6 cpm per assay. Incubations were conducted at 37°C and terminated by the addition of 50 µl of Tris-$MgCl_2$ stopping solution containing 20 mM ATP and 2 x 10^4 cpm of [^{3}H]-cAMP (as recovery marker), followed immediately by boiling for 3 minutes. One ml of water was added to each tube followed by centrifugation and the supernatants were chromatographed as described by Salomon et al. (10). Eluates were analyzed and corrected for recovery using liquid scintillation counting. Buffer and boiled enzyme blanks were included and samples were corrected accordingly.

Cyclic AMP assay. Cyclic AMP assays were performed using a double antibody radioimmunoassay (New England Nuclear Corp.) based upon methods of Steiner et al. (11). Cell incubations containing usually 0.5-1.0 x 10^6 adherent EMϕ were conducted in glass culture tubes (10 x 75 mm) in RPMI-1640

TABLE 1

ADENYLATE CYCLASE ACTIVITY IN RESIDENT AND ELICITED MOUSE PERITONEAL MONONUCLEAR PHAGOCYTES

	Basal	NaF
	pMoles cAMP formed/mg protein/10 min.	
Resident Cells (RMϕ)	72	823
Elicited Cells (EMϕ)	170	975

containing 10% FCS, 10 mM thiophylline and appropriate hormonal additions. Incubations were terminated by placing tubes in a boiling water bath after adding [^{3}H]-cyclic AMP recovery marker, and the cells were further disrupted by either ultrasonication or several cycles of freezing and thawing. The particulate material was removed by centrifugation and the supernatant was used in the radioimmunoassay following standard procedures outlined by the supplier. An acetylation step was included to increase sensitivity. Data were corrected both for percent recovery and the added recovery marker (usually 0.1 picomoles of cAMP per culture).

Reagents. All reagents were from Sigma Chemical Co. unless otherwise stated. Culture media and sera were from Grand Island Biological Co. PTH was of bovine origin prepared to the G-100 stage (1300 units/mg) and was obtained through NIAMMD. Synthetic salmon calcitonin (2700 MRC units/mg) was provided by Armour Pharmaceutical.

RESULTS

Adenylate cyclase in cell homogenates. Homogenates of adherent EMϕ and RMϕ were prepared and assayed for adenylate cyclase activity. A positive linear relationship was observed between enzyme activity and incubation time (from 0 to 20 minutes) as well as homogenate protein concentration (from 5 to 60 micrograms). The basal level of enzyme activity obtained from EMϕ was higher than that from RMϕ (Table 1). Sodium fluoride caused a six to ten-fold stimulation of enzyme activity.

When EMϕ homogenates were incubated in the presence of PTH (10^{-8}M) or CT (10^{-9}M), we observed a twofold increase in cyclic AMP formation when compared to basal levels (Table 2). Epinephrine had a similar effect. In contrast, the adenylate cyclase of RMϕ homogenates responded quite

TABLE 2

EFFECT OF HORMONE TREATMENT ON ADENYLATE CYCLASE ACTIVITY OF Mϕ HOMOGENATES: DIFFERENTIAL EFFECT IN ELICITED AND RESIDENT CELLS[1]

	Basal[2]	PTH	CT	EPI
Resident Cells (RMϕ)	100	69 ± 7*	112 ± 3*	89 ± 28
Elicited Cells (EMϕ)	100	188 ±11**	195 ±16**	207 ± 15**

[1] Adenylate cyclase activity is expressed as a percent of basal activity within individual experiments and $\bar{x}$ ± SEM were calculated from 5 separate experiments. Student's t-test was used to test for significance
* - $p<0.05$, ** - $p<0.01$

[2] Basal activity was stimulated 6 to 10 fold in the presence of 10mM NaF in either RMϕ or EMϕ preparations.

differently to hormonal treatment. PTH caused an apparent inhibition of cyclic AMP formation; CT caused only a slight increase in enzyme activity, and epinephrine had no effect (Table 2). The enzyme in these RMϕ homogenates was shown to be functional as demonstrated by the stimulation of activity in the presence of sodium fluoride (Table 1).

Cyclic AMP in intact cells. Further studies were conducted with the objective of demonstrating hormonal stimulation of adenylate cyclase in intact adherent cell preparations. Cyclic AMP levels were determined in cell cultures incubated in the presence of either PTH (10^{-10}M) or CT (10^{-10}M) for 1,3,5 and 10 minutes, and compared to control cultures having no hormone added. Both PTH and CT caused a significant transient increase in cyclic AMP levels in these cells (Table 3). The effect of CT was observed at 1 minute while that of PTH occurred at 3 minutes of incubation.

DISCUSSION

The composition and kinetics of the elicited peritoneal cell population have been carefully studied and they represent mostly young cells which have recently replicated and arrived in the peritoneal cavity (7). In

TABLE 3

EFFECT OF HORMONE TREATMENT ON CYCLIC AMP ACCUMULATION IN INTACT EMϕ *in vitro* AS A FUNCTION OF TIME

Time	Control	PTH	CT
	pMoles cAMP/5 x 10^5 cells[1]		
1	0.303 ± 0.05	0.283 ± 0.04	0.400 ± 0.03**
3	0.232 ± 0.08	0.397 ± 0.05*	0.350 ± 0.01*
5	0.295 ± 0.04	0.296 ± 0.03	0.311 ± 0.05
10	0.270 ± 0.03	0.287 ± 0.01	0.204 ± 0.02*

[1] Data are presented as $\bar{x}$ ± SEM four replicate incubations at each time point. Since there was no significant difference among control groups at the various times, the control values were pooled (n = 16) and their mean (0.275 ± 0.02 pMoles cAMP per 5 x 10^5 cells) was used to test for significant difference from treatment groups using student's t-test. * - $p<0.05$, ** - $p<0.01$.

contrast, the normal resident population of the peritoneal space are more mature macrophages which do not appear to undergo replication.

The differences which we have observed in adenylate cyclase activity and hormonal responsiveness between elicited and resident cells may be a function of this younger cell population. In the resident cells basal enzyme activity was low and only CT caused a slight stimulation. However, in the elicited cells there was a significant increase in basal activity and each of the hormones caused a similar significant stimulation of enzyme activity. These observations suggest that the elicited cell population contain a subclass(es) of mononuclear phagocytes (presumably a young cell) possessing receptors for PTH, CT and epinephrine.

The results using cell homogenates are further supported by the experiments on intact cells. Both PTH and CT cause a transient but significant increase in cyclic AMP. Recent studies on the role of prostaglandin F2α and 1-methyl-3-isobutyl xanthine in the differentiation of 3T3 cells to adipocytes have set forth the notion that increased intracellular cyclic AMP levels result in the induction of phosphodiesterase (12). Thus, in

our studies the transient increase in cyclic AMP followed by a return to control levels in response to hormonal stimulation may have been caused by a cyclic AMP induction of phosphodiesterase. Furthermore, if such a mechanism exists in these cells, the decrease below control levels of cyclic AMP seen in CT treated cells at 10 minutes (Table 3) may reflect the longer duration of cyclic AMP stimulation observed with CT as compared to PTH.

PTH receptors have been reported to exist in bone, kidney and liver, and they have been shown to be target organs and/or sites of hormone metabolism (13-16). CT receptors have been found in bone and kidney which are known target organs, and in lymphoid cell lines where the hormone is postulated to regulate lymphoproliferation (13). It appears that young peritoneal mononuclear phagocytes may also be classed as cells which possess PTH and CT receptors.

The evidence, per se, does not prove that a mononuclear phagocyte is an osteoclast precursor or is itself a bone resorbing cell. However, when considered in the context of the body of literature which supports this hypothesis (3), such an interpretation is reasonable. Moreover, the observations reported here provide a potentially useful functional biochemical parameter with which to explore the differentiation of mononuclear phagocytes and their relationship to osteoclast formation and bone resorption.

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REFERENCES

1. Walker, W.S. (1976). In: Immunobiology of the Macrophage (Nelson, D.S., ed.) Academic Press, New York, pp. 91-110.
2. Fishman, D.A., and Hay, E.D. (1962). Anat. Rec. 143, 329-333.
3. Hall, B.K. (1975). Anat. Rec., 183, 1-12.
4. Jee, W.S.S., and Nolan, P. (1962). Anat. Rec. 142, 310-315.

5. Luben, R.A., Wong, G.L., and Cohn, D.V. (1976). Endocrin. 99, 526-534.
6. Luben, R.A., Wong, G.L., and Cohn, D.V. (1977). Nature 265, 629-630.
7. Vassalli, J., Hamilton, J. and Reich, E. (1976). Cell, 8, 271-281.
8. Remold-O'Donnell, E. (1974). J. Biol. Chem., 24a, 3615-3621.
9. Krishna, G., Weiss, B., and Brodie, B.B. (1968). J. Pharmacol. Exp. Ther., 163, 379-385.
10. Salomon, Y., Londos, C., and Rodbell, M. (1974). Anal. Biochem., 58, 541-548.
11. Steiner, A.L., Parker, C.W., and Kipnis, D.M. (1972). J. Biol. Chem., 247, 1106-1110.
12. Russell, T.R., and Ho, R. (1976). Proc. Nat. Acad. Sci. USA, 73, 4516-4520.
13. Marx, S.J., Auerbach, G.D., Gavin, J.R., and Buell, D.W. (1974). J. Biol. Chem. 249, 6812-6816.
14. Malbon, C.C. and Zull, J.E. (1975). Biochem. Biophys. Res. Commun. 66, 179-187.
15. Fang, V.S., and Tashjian, A.H. (1972). Endocrin. 90, 1177-1182.
16. Chu, L.L.H., Forte, L.R., Anast, C.S., and Cohn, D.V. (1975). Endocrin., 97, 1014-1023.