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Actions of Calcitonin, Parathyroid Hormone, and Prostaglandin E_2 on Cyclic AMP Formation in Chicken and Rat Osteoclasts

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The effects of calcitonin, parathyroid hormone, and prostaglandin E_2 on cyclic AMP production were studied in osteoclast-rich cultures derived from medullary bone of laying hens and from the long bones of newborn rats. Cyclic AMP was assayed biochemically in replicate cultures, and furthermore, changes in cytoplasmic fluorescence were sought by indirect immunofluorescence with rabbit anti-cyclic AMP and FITC-labelled goat anti-rabbit IgG. Treatment of rat osteoclasts with calcitonin increased cyclic AMP formation as measured biochemically, and this was confirmed by the immunofluorescence method. No such increase took place in chick osteoclasts. Prostaglandin E_2 increased cyclic AMP production in both rat and chick osteoclasts as determined by both methods. Since the immunofluorescence method failed to detect a response to parathyroid hormone either in chick or rat osteoclasts, its variable biochemical effects were concluded to be due to actions on contaminating osteoblasts in the cultures. Thus it has been possible with a combined biochemical and immunocytochemical approach to define the cyclic AMP responses to the calcium-regulating hormones in rat and chick osteoclasts. The failure of calcitonin to increase cyclic AMP in chick osteoclasts identifies a need to investigate the nature of calcitonin action on avian osteoclasts, which may contribute to understanding of its actions on mammalian cells.

Key words: calcium-regulating hormones, bone cells

The osteoclast, the major bone-resorbing cell, is derived from a bone marrow stem cell [1–4]. Its activity is regulated by circulating calciotrophic hormones and interactions with other cells in bone [5]. The osteoblast, derived from primitive mesenchymal cell precursors [6], has been amenable to study either in cultures established from fetal or newborn rodents [7] or in cloned osteogenic sarcoma cells of the osteoblast phenotype [8]. Osteoclasts have presented more difficulty. Small

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numbers have been obtained from short-term cultures derived from newborn rat long bones [9–11] and from embryonic chick tissue [12], but pure cultures of osteoclasts have never been obtained. Osteoclast-like cells and progenitors have been grown among other cells from feline bone marrow [13,14]. Zambonin-Zallone et al [15] have also developed methods to grow cultures enriched in viable osteoclasts derived from the medullary bone of laying hens and have shown these cells to be capable of resorbing vital or devitalized bone in vitro [16].

In the present experiments, osteoclasts derived from hen medullary bone have been cultured by the method of Zambonin-Zallone et al [15,16] and from newborn rat long bones by the method of Chambers and colleagues [2,10]. The actions of calcitonin and other calciotrophic hormones upon cyclic AMP formation have been studied by the use of biochemical and immunocytochemical methods, and the conclusion was reached that calcitonin stimulates cyclic AMP formation in rat but not in avian osteoclasts. Parathyroid hormone (PTH) has no effect on cyclic AMP formation in osteoclasts from either species.

MATERIALS AND METHODS

Chemicals, Hormones, Culture Media, and Antisera

Culture media, fetal calf serum (FCS), and phosphate-buffered saline were obtained from Flow Laboratories Inc. (Glen Waverley, Australia). Naphthol ASTR phosphate, naphthol ASMX phosphate, fast blue RR salt, and leupeptin were obtained from Sigma Chemical Co. (St. Louis, MO), p-rosanoline from Tokyo Kasai (Tokyo), Percoll from Pharmacia (South Seas, Australia), and Aquamount from Lerner Laboratories (New Haven, CT). Thermanox discs were obtained from Miles Scientific (Naperville, IL). Synthetic salmon calcitonin (SCT), potency 4,400 units/mg, was a gift from the Armour Pharmaceutical Co. (Kankakee, IL). Prostaglandin E_2 (PGE_2) was purchased from Upjohn Co. (Kalamazoo, MI) and human parathyroid hormone (1–34) [hPTH(1–34)] from Beckman Pty. Ltd. (Palo Alto, CA). Rabbit antiserum to cyclic AMP for use in immunocytochemical experiments was prepared against 2′-O-succinyl cyclic AMP and was a generous gift from Dr. A.L. Steiner (Houston, TX) [17]. A similarly prepared antiserum for use in cyclic AMP radioimmunoassay was a gift from Dr. N.H. Hunt (Canberra, Australia). Fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit IgG, Na[^{125}I], 2,8-[^{3}H]-adenine, [^{32}P]-cyclic AMP, and [^{125}I]-cyclic AMP tyrosine methyl ester were purchased from the Radiochemical Centre (Amersham, Bucks, UK). All other chemicals were of reagent grade and obtained from standard suppliers.

Chicken Osteoclast Preparation

The method was adapted from that of Zambonin-Zallone et al [15] with minor modifications. White Leghorn-cross hens aged 8–12 mo were obtained from the Animal Research Institute, Department of Agriculture, Victoria. At peak lay they were fed a low calcium diet (0.13% calcium) for 7 days. They were sacrificed by decapitation, the femora and tibiae removed and rapidly cooled to 4°C. The shafts of the bones were then split longitudinally, the marrow lifted out, and the bone fragments washed in three changes of phosphate-buffered saline (PBS) with 0.1% bovine serum albumin (BSA). The medullary bone was gently scraped with 14-gauge needles while immersed in PBS/BSA. The resulting cell suspension was then sequentially filtered

through 350 μm, 105 μm, and 53 μm polypropylene mesh and the final filtrate was centrifuged at 400g for 5 min. The cell pellet was resuspended in 10 ml PBS/BSA and overlaid on a 7-step discontinuous Percoll gradient with densities from 1.08 to 1.02 g/ml. Gradients were centrifuged at 400g for 20 min at 4°C. The Percoll fractions 1.03 to 1.06 g/ml were collected, diluted in 10 volumes of PBS/BSA, and centrifuged at 400g for 5 min. The cell pellets were resuspended in minimal essential medium (MEM) and the cells were plated in 6- or 12-well tissue culture plates with or without glass coverslips. For those experiments in which cyclic AMP generation was to be measured biochemically, purer cultures were required. Therefore a further purification step of unit gravity sedimentaiton in 20% FCS [15] was used before cells were plated out. The cultures were maintained at 37°C in 5% CO_2, 95% air in MEM with 20% FCS, penicillin-streptomycin (100 U and 100 μg/ml). Leupeptin (2 μg/ml) was added to the PBS/BSA and (for the first 2 days) to the culture medium. The medium was changed daily initially by gentle aspiration, although as soon as the multinucleate cells were firmly adherent (at 2 or 3 days) the plates were vigorously rinsed to remove nonadherent or less strongly adherent contaminating cells. Five percent FCS was substituted for 20% FCS 24 hr before the experiments.

Newborn Rat Osteoclast Preparation

A published method [9] was used and is briefly described. One or 2-day-old Sprague-Dawley rats were sacrificed by decapitation. The femora, tibiae, and humeri were cleaned of soft tissue, cut across at the diaphysis, split longitudinally and curetted with a scalpel into Medium 199 with 5% FCS (1 ml per 4 bones). The cell suspension was triturated with a siliconized pasteur pipette and 330 μl aliquots placed on 19 mm round glass coverslips in 12-well tissue culture plates, which were then incubated at 37°C for 20 min. The coverslips were then vigorously washed with medium to remove the majority of nonadherent cells and incubated with fresh Medium 199 plus 5% FCS for 2 hr before the experiments were commenced.

Histochemistry

For acid phosphatase detection, cells were fixed for 10 min in 4% formaldehyde, 0.1 M calcium acetate, 1 M sucrose at room temperature, and washed in three changes of PBS. Tartrate resistant acid phosphatase was identified by a method [18] employing naphthol ASTR phosphate and p-rosanaline in 0.1 M acetate buffer pH 5.0 with 10 mM sodium tartrate. Activity was identified by intense red granules. For alkaline phosphatase, cells were fixed as for acid phosphatase. Alkaline phosphatase was identified by the method of Ackermann [19] with naphthol ASMX phosphate 0.01% (w/v), pH 8.6, and fast blue RR salt. Activity was identified by blue granules.

Electron Microscopy

For examination in situ by transmission electron microscopy, cells were grown on Thermanox discs until confluent, then fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer for 4 hr. Discs were washed in buffer and transferred to processing bottles, then postfixed with 2% uranyl acetate in 70% acetone. After dehydrating through graded acetones, cells were infiltrated and embedded in Spurr embedding media [20] by inverting the discs over troughs filled with resin. Blocks were polymerized at 60°C overnight and the Thermanox discs were peeled from the surface, leaving a layer of cultured cells. Thin sections were cut directly from the block

surface (without trimming) on an LKB Ultratome III, stained with 5% uranyl nitrate and Reynolds lead citrate on a LKB ultrastainer, and viewed on a Siemens 102 at 60 kV.

Immunocytochemistry

Cells on glass coverslips were prepared as described above. Cell preparations from hen medullary bone were used after 4–5 days in culture and rat cell preparations within 2–3 hr of plating. Coverslips in 6- or 12-well plates were treated with appropriate hormone diluted in 100 μl of medium 199 with 5% FCS. Following incubation, medium was aspirated, coverslips were washed twice in PBS at 37°C and fixed in 2% formaldehyde (electron microscopy grade) in PBS at room temperature for 4 min. Following three washes in PBS, cells were permeabilized in acetone (−20°C) for 2 min and washed a further three times in PBS. Coverslips were then blocked for nonspecific protein adhesion by incubation with 3% BSA in PBS for 15 min. Specific first antibody to cyclic AMP [17] was added in 25 μl of PBS-0.1% BSA to the surface of coverslips and incubated at 37°C in a humidified chamber for 45 min. Following three washes in PBS, FITC-conjugated second antibody was added in 25 μl of PBS plus 2% gelatin and 1% BSA and incubated for 30 min. Coverslips were then washed three times in PBS, mounted in Aquamount, and viewed with a Nikon Diaphot-TMP fitted with epifluorescence. Photography used Ilford XPI 400 film ASA set at 200 and processed with C41 chemistry. For studies with hormone effects on cyclic AMP, photographs were taken in memory mode to ensure constant exposure, and prints were developed similarly.

Cyclic AMP Generation

The generation of [^{3}H]-cyclic AMP was measured in hen osteoclast cultures in which the ATP pool had been labeled with [^{3}H]-adenine [21,22]. Forskolin (10^{-7} M) was added to all incubations at the same time to increase the amplitude of hormone responses [23]. Cyclic AMP production in rat osteoclast cultures was measured by radioimmunoassay with rabbit anti-cyclic AMP. Osteoclasts growing on glass coverslips were treated for 15 min at 37°C in the presence of 1 mM isobutylmethylxanthine, after which media were removed and 0.5 ml 95% ethanol, pH 3.0, was added. After standing for 2 hr, the acid-ethanol was removed and dried in a vacuum centrifuge. Samples were reconstituted for assay, acetylated and assayed as described [24,25]. Specificity of the antiserum has been previously reported [25].

RESULTS

Chick Osteoclasts

The cell yields and morphology were very similar in these experiments to those reported by Zambonin-Zallone et al [15,16]. After initial purification, the yields of multinucleate osteoclasts from medullary bone were from 5 to 10 × 10^6 cells per hen. However, many of these cells failed to adhere to the substratum, and following rinsing of the plates, 1–2 × 10^6 osteoclasts were routinely obtained. Immediately after isolation, hen osteoclasts appeared as large, markedly granular and pleomorphic cells, which within 24 hr rounded up and began to adhere to the substrate. Cell spreading then began and was completed in 3–5 days to produce very large (50–200 μm diameter) circular profiles. Generally the central area of the cells contained nuclei

and other organelles and was thicker than the extremely thin outer area, which appeared devoid of visible structures. However, by the use of phase contrast microscopy, a "band" was usually visible just inside the outer margin of the cells (Fig. 1). The cells tended to maintain circular profiles, with centrally located nuclei, although complex profiles with groups of eccentrically placed nuclei were also observed. Undulating membranes and other indicators of intensely motile cells were only rarely encountered.

After 3–5 days, multinucleate osteoclasts composed variably 50–90% of cells in these cultures. However, because of the much greater size, the percentage of the total area covered by osteoclast-like cells was higher. There were two major types of contaminating mononuclear cells. The first type had a similar phase contrast appearance to osteoclasts but had a single eccentrically placed nucleus and probably belonged to the monocyte-macrophage lineage. The second type had a rhomboid or fusiform shape and occasionally proliferated and overgrew the cultures after 5 days.

Histochemistry

All multinucleated cells in the cultures showed intense cytoplasmic staining for tartrate-resistant acid phosphatase, particularly in the perinuclear areas. Monocyte-macrophage cells also showed some acid phosphatase activity but other contaminating cells did not. Alkaline phosphatase activity was not detected in osteoclasts but was strongly positive in some of the rhomboid-shaped cells, suggesting that these cells were osteoblasts. The percentage of alkaline phosphatase-positive cells varied in different preparations and was occasionally very low.

Electron Microscopy

Transmission electron microscopic examination of cells cultured on plastic (Thermanox) coverslips for 5 days demonstrated multinucleate cells with many

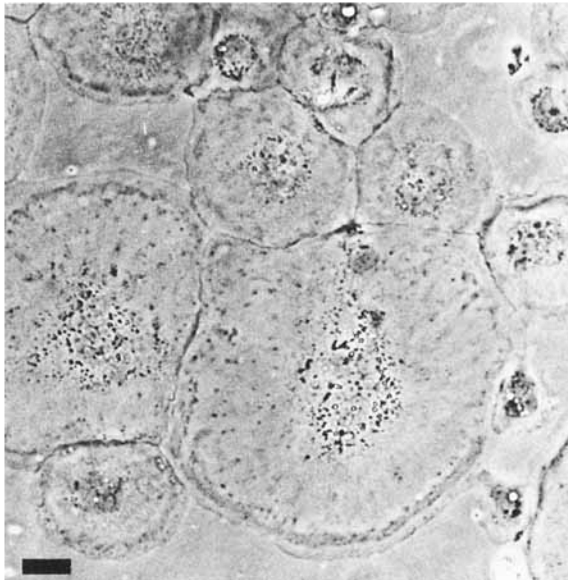

Fig. 1. Chick osteoclasts growing on plastic dishes at 5 days (as in Materials and Methods) (phase contrast × 300). Bar = 20 μm.

mitochondria and lysosomal bodies (Fig. 2). Typical ruffled membrane borders were not seen, and this is consistent with the absence of bone-resorbing activity.

Cyclic AMP Production

[^{3}H]-cyclic AMP production was measured in the hen medullary bone cell cultures, prelabelled with [^{3}H]-adenine. The results of experiments are summarized in Table I, in which the mean values are given as treated to control ratios. Apart from a small effect in one experiment at high hormone concentration (3×10^{-9} M), SCT had no significant effect on [^{3}H]-cyclic AMP formation. The major stimulator of cyclic AMP production was PGE_2, the amplitude of the response being consistently 20- to 50-fold. hPTH(1–34) usually increased [^{3}H]-cyclic AMP production, but the response in different cultures was variable and always less than that to PGE_2. The main purpose in summarizing the data from several experiments in Table I is to draw attention to the consistently high cyclic AMP response to PGE_2, the variable response to PTH, and the lack of response to SCT in the chick osteoclast cultures.

Immunocytochemistry

Immunocytochemistry with an anti-cyclic AMP antiserum [17,24] was used to determine single cell responses to SCT, PGE_2, PTH, and forskolin. A consistent increase in cytoplasmic immunofluorescence in chick osteoclasts was demonstrated in response to PGE_2 and forskolin (Fig. 3). This was in contrast to the lack of effect of SCT or PTH in any of many experiments carried out with chick osteoclast cultures, by the use of a wide range of hormone concentrations and incubation times up to 1

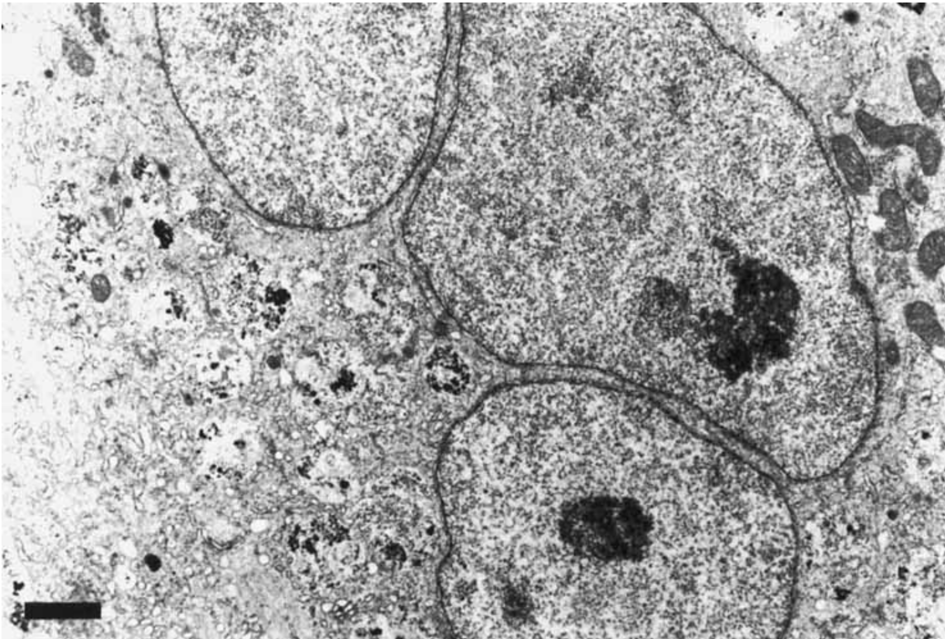

Fig. 2. Chick osteoclast showing nuclei, prominent mitochondria, and lysosomal bodies. Cells grown on Thermanox discs for 5 days and processed as described in Materials and Methods (electron microscopy $\times$ 6,000). Bar = 2 μm.

TABLE I. The Effects of Calcitonin, Parathyroid Hormone and Prostaglandin E_2 on Cyclic AMP Formation in Osteoclast-Rich Chicken Cell Cultures

Experiment[a]	SCT (3×10^{-9} M)	hPTH (1–34) (3×10^{-9} M)	PGE_2 (10^{-7} M)
1	1.5	1.6	18.9*
2	1.8**	5.3*	30.2*
3	1.1	2.5*	17.8*
4	1.3	3.4*	23.1*
5	1.3	26.3*	48.3*
6	1.5	12.7*	
7	1.4	8.6*	16.6*

[a]Summary of data from seven separate experiments in which generation of [^{3}H]-cyclic AMP in response to hormones was assayed in chick osteoclasts prelabelled with [^{3}H]-adenine (see Materials and Methods). Treated/control ratio = cAMP in treated cells/cyclic AMP in control cells. In each case the concentration of agonist used was that required for the maximum response. Each point is the mean of triplicate observations, with SEMs less than 10% of mean in all cases.
* $p < 0.001$.
** $p < 0.05$.

hr. While no attempt was made to quantitate cytoplasmic fluorescence, interpretations are based on the relative intensity of fluorescent labelling in a large number of treated and untreated osteoclasts. In interpreting the immunocytochemical experiments, it should be noted that nuclear fluorescence was constant and unrelated to treatment. This might reflect nonspecific interactions.

RAT OSTEOCLASTS

As reported by Chambers et al [10] 70–90% pure cultures of osteoclasts were isolated from newborn rat long bones, with a yield of approximately 600 per animal. These were studied after adherence to culture surfaces and always within 12 hr of isolation. The rat osteoclasts behaved as reported [9,10,26] in that they were motile, and their motility rapidly ceased upon exposure to calcitonin or PGE_2, but not to PTH. Calcitonin treatment resulted in a rapid and sustained contraction of the osteoclasts, and PGE_2 produced a similar response, although of shorter duration (data not shown) [26]. It should be noted that the chick osteoclasts in culture were not motile, and shape change in response to calcitonin was much less evident.

Cyclic AMP Formation

Since relatively few rat osteoclasts were available, a sensitive radioimmunoassay for cyclic AMP was used rather than the [^{3}H]-adenine prelabeling method. Replicate cultures of rat osteoclasts growing on glass coverslips were treated with hormones and cyclic AMP measured after sample acetylation. The results (Fig. 4)showed that calcitonin treatment resulted in a substantial increase in cyclic AMP production, a response not seen in the chick osteoclast cultures. Responses were observed also to PGE_2 and PTH. Although in the experiment of Figure 4 the amplitude of response to PTH was greater than that to PGE_2, in repeated experiments the reverse was usually found to be the case. Since even quite minor (less than 5%)

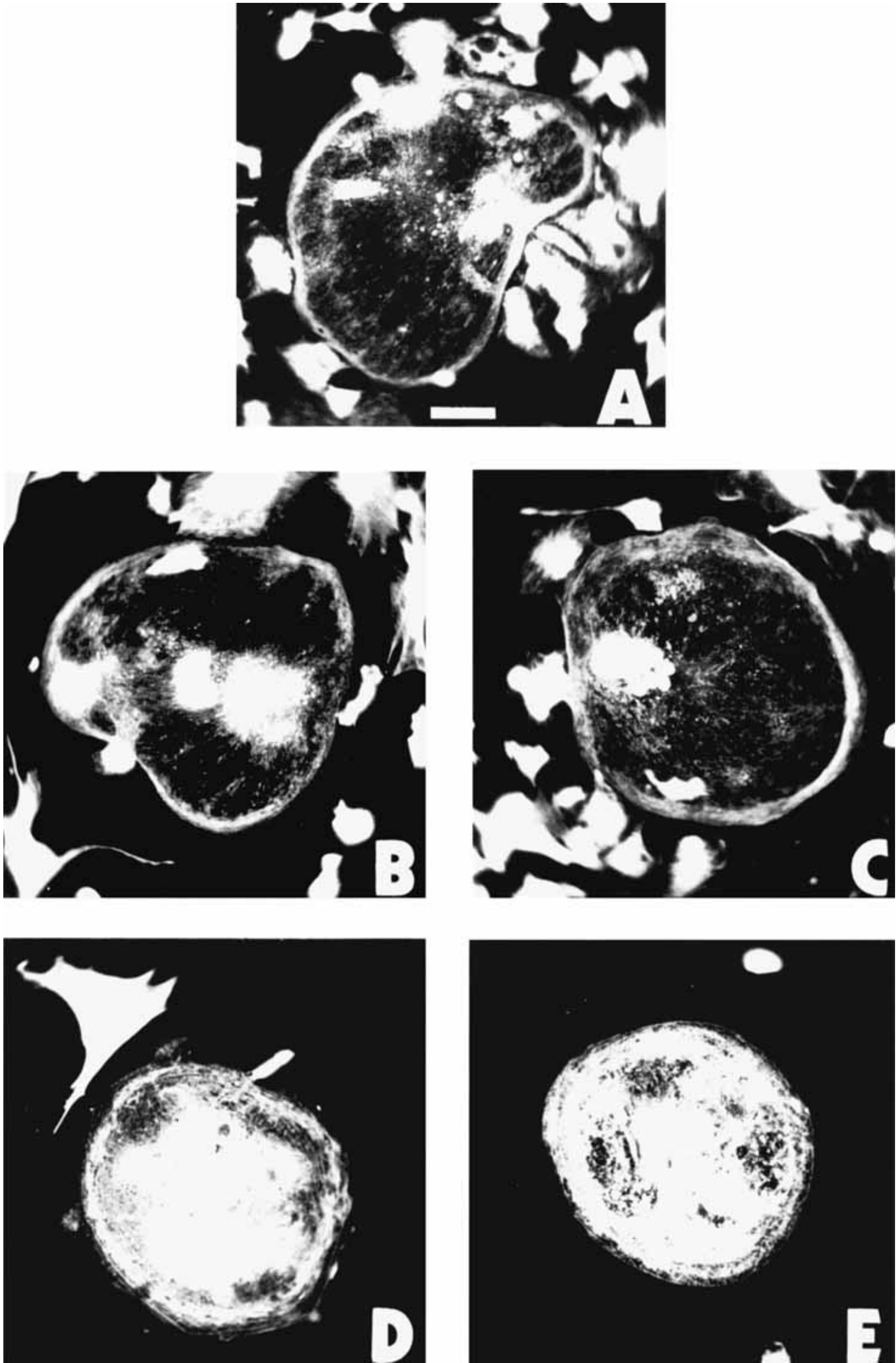

Fig. 3

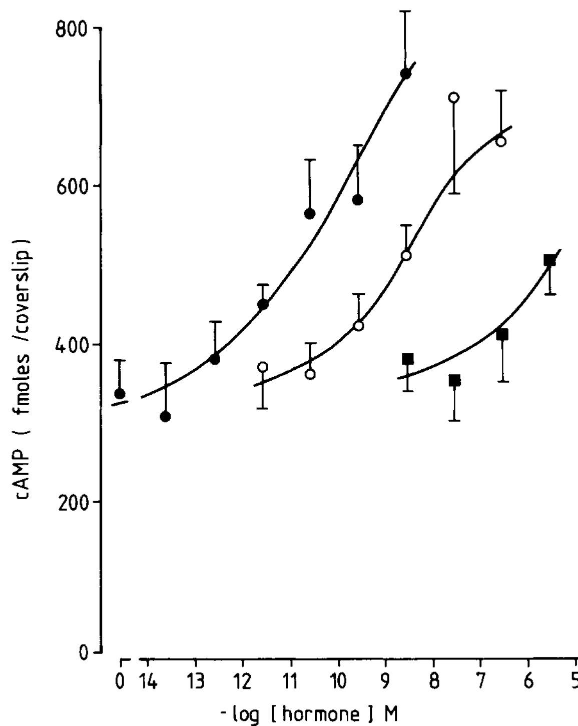

Fig. 4. Cyclic AMP response in isolated rat osteoclasts. Osteoclasts were grown as replicates on coverslips as described in Materials and Methods, and cyclic AMP was assayed in response to SCT (●), hPTH(1-34) (○), and PGE_2 (■). Points are means ± SEM of triplicate observations.

contamination with responsive mononuclear cells could account for a hormone response in these experiments, it was necessary to use the immunocytochemical approach to determine which hormones influenced rat osteoclast cyclic AMP content.

Immunocytochemistry

Using anti-cyclic AMP and FITC-labeled second antibody, consistent increases in cytoplasmic fluorescence were obtained in rat osteoclasts in response to calcitonin, PGE_2, and forskolin, with no change in response to PTH (Fig. 5).

DISCUSSION

We have used a combination of immunocytochemistry and biochemistry to examine directly for the first time the cyclic AMP responses to hormones in chick and rat osteoclasts. The immunochemical approach was used because we considered it mandatory to assess responses at the single cell level. The limitations of the immunocytochemical method and the importance of using an antiserum of high

Fig. 3. Hormone effects on cytoplasmic cyclic AMP in chick osteoclasts. Cells in culture were treated for 5 min, fixed, incubated with rabbit anti-cyclic AMP and FITC-labelled goat anti-rabbit IgG, and prepared for fluorescence microscopy as described in Materials and Methods (× 750). Bar = 20 μm. A) control; B) salmon calcitonin (10^{-7} M); C) PTH (10^{-7} M); D) PGE_2 (10^{-6} M); E) forskolin (10^{-6} M).

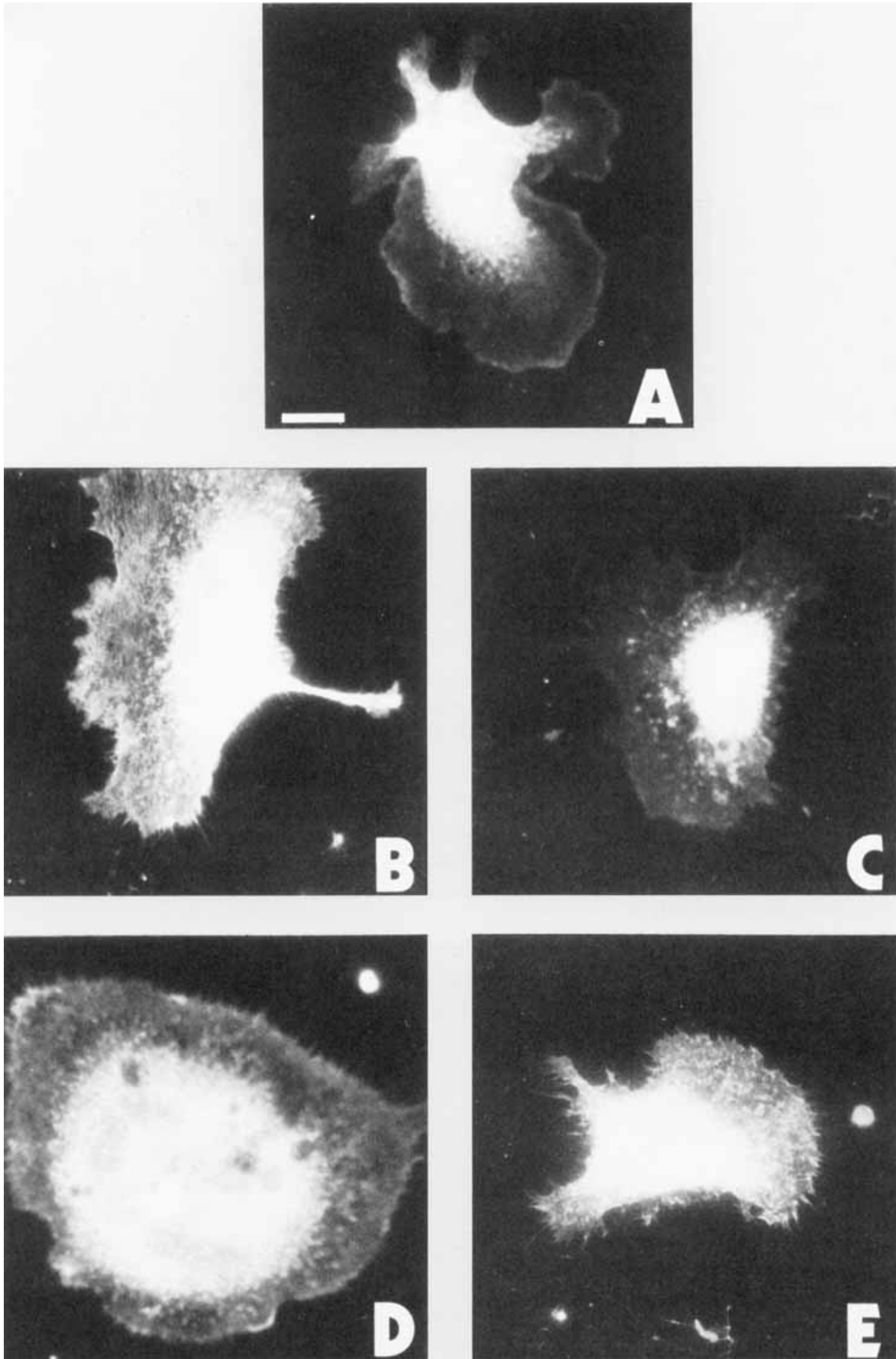

Fig. 5

specificity have been stressed previously [17,27]. In selecting an anti-cyclic AMP antiserum, for example, it is especially important that the reagent does not recognize cyclic GMP or ATP [17]. The antiserum used in the present experiments was selected especially for immunocytochemistry and was used in earlier studies [17,27]. Therefore, provided that appropriate controls are used, these two approaches allow certain conclusions to be drawn. These are that calcitonin increases cyclic AMP formation in rat, but not in chick osteoclasts, whereas PGE_2 does so in both species. PTH has no effect on cyclic AMP formation in osteoclasts of either origin.

The multinucleate cells cultured from hen medullary bone in the present experiments reproduce very closely the features of the cells that have been described by Zambonin-Zallone et al [15,16,28–31] and designated osteoclasts. Indeed, the similarities include even the fact that some multinucleate cells were noted in all cultures in the present experiments to have multiple organizing centres of tubulin structure detected by antitubulin antibodies [28] (results not shown). The cells isolated in this way possess the important features ascribed to osteoclasts, including the facts that they form a ruffled border and resorb vital and devitalized bone [16] and are rich in acid phosphatase. Osteoclasts have proven difficult to grow in culture, and the development of this system has provided a useful approach to studying hormone action and osteoclast biology. In the present work we have shown that the chick osteoclast does not respond to calcitonin with a rise in cyclic AMP. In view of the role ascribed to cyclic AMP in calcitonin action on osteoclasts [32,33], this seemed a surprising finding. However, it is consistent with preliminary data obtained by others with chick cells prepared in a similar way [34], with studies of calcitonin effects on avian bone [35] and on freshly isolated osteoclasts from 15- to 24-day-old chick metatarsi [36].

There are several possible explanations for the lack of a cyclic AMP response to calcitonin. First, despite the fact that chick calcitonin is very similar in structure and specific biological activity to that of salmon [37], it is possible that the hormone does not inhibit osteoclast function in birds. Although circulating levels of calcitonin are readily measurable in avian species, it has not been possible to demonstrate a hypocalcemic effect of calcitonin in birds [35,38] and calcitonin failed to inhibit bone resorption induced by freshly disaggregated embryonic chick osteoclasts [39]. However, in preliminary experiments we have shown that calcitonin treatment of cultured chick osteoclasts results in rapid disassembly of the tubulin network [40]. The functional significance of this for calcitonin effects on avian osteoclast function is not known.

Second, even though our observations are in agreement with those made on chick bone [35] and freshly isolated chick osteoclasts [36], the possibility should be considered that the multinucleated cells prepared by the present method and studied after 5 days in culture might not be mature osteoclasts but a mixture of osteoclasts and polykaryons formed by a fusion of mononuclear cells in culture. We have no evidence that this is the case, but it is known that fusion does take place in these cultures [30], and therefore the possibility cannot definitely be excluded. In our experiments the cells were uniformly rich in acid phosphatase. Zambonin-Zallone et al [15,16,28–31] have gathered much evidence that the chick cells cultured in this

Fig. 5. Hormone effects on cytoplasmic cyclic AMP in rat osteoclasts. Experiment carried out as described in legend to Figure 3 ($\times$ 750). Bar = 20 μm. A) control; B) salmon calcitonin (10^{-7} M); C) PTH (10^{-7} M); D) PGE_2 (10^{-6} M); E) forskolin (10^{-6} M).

way are indeed osteoclasts, a finding confirmed by Blair et al [41], who showed the cells to be capable of resorbing a mineralized matrix. If any of the multinucleated cells in our experiments had responded to calcitonin with a cyclic AMP increase, this should have been detected at least with the immunocytochemical approach. If calcitonin does have an action on avian osteoclasts that does not use cyclic AMP as a second messenger, it will be important to characterize the mechanism, which could help understanding of calcitonin action in other species.

The studies with freshly isolated rat osteoclasts pointed clearly to a species difference in initial events in hormone action. There is ample evidence that at least some cells in rodent bone respond to calcitonin with increased cyclic AMP formation [7,42–45], although direct effects on osteoclast cyclic AMP have not previously been demonstrated. The inhibition of bone resorption by dibutyryl cyclic AMP [32] is thought to mimic calcitonin action. The freshly isolated rat osteoclasts in the present experiments clearly responded to calcitonin with a rise in cyclic AMP, detected biochemically and immunocytochemically. An interesting feature of the action of calcitonin and PGE_2 on rat osteoclasts is that they both increase cyclic AMP and both cause osteoclasts to cease motility and to contract [26]; the calcitonin effect is much more prolonged than the PGE_2effect as shown by Chambers and Dunn [26] and confirmed in our own experiments (results not shown). Such a difference could be explained by a second action of calcitonin in the osteoclast not shared with PGE_2. If cyclic AMP action in osteoclasts contributes towards inhibition of their function, it might be predicted that PGE_2 would inhibit osteoclast activity in the chick as it has been shown to do in rat and rabbit osteoclasts [46,47]. This could be the case, even though a stable analogue of PGE_2 (16,16-dimethyl PGE_2) has been shown to elevate serum calcium in the young chick [48], since in the latter experiments the predominant mechanism of hypercalcemia was shown to be by inhibition of calcium exit from extracellular fluid pools rather than by resorption of bone.

Finally, the results with PTH warrant comment. The fact that both PTH and calcitonin increase cyclic AMP production in bone has been explained by the PTH effect taking place in osteoblasts and the calcitonin effect in osteoclasts [33], but this has never been demonstrated directly until the present study. A biochemical response to PTH was noted in impure osteoclast cultures derived from both rat and chick, but PTH did not influence cyclic AMP formation in osteoclasts from either species as detected by immunocytochemistry. Ito et al [36] studied the PTH effect closely in chick osteoclast cultures and found that greater cyclic AMP responses to PTH occurred in cultures with greater degrees of contamination by alkaline phosphatase-rich cells. Our conclusion from that work [36] and our own data is that PTH does not increase cyclic AMP formation in either rat or chick osteoclasts; it is consistent with the view that the PTH response occurred in osteoblast-like cells and that the target cells in bone for the effect of PTH on cyclic AMP are exclusively cells of the osteoblast lineage [5,33].

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