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CALCITONIN RECEPTORS IN A CLONED HUMAN BREAST CANCER CELL LINE (MCF 7)

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SUMMARY: A human breast cancer cell line, MCF 7, is shown to possess a specific calcitonin receptor and calcitonin responsive adenylate cyclase, and calcitonin treatment results in activation of cyclic AMP-dependent protein kinase. Studies with several analogues of calcitonin show that the receptor and adenylate cyclase response preserve the ability to discriminate among the structure-function relationships of the calcitonin molecule. The same cell line has been shown recently to possess a receptor for the steroid hormone, $1,25(OH)_2$-vitamin D. Coexistence in MCF 7 cells of receptors for two calcium-regulating hormones may be related to the osteoclast-like properties of these cells.

INTRODUCTION: Several human breast cancer cell lines, including the widely studied MCF 7 line, have been shown to be capable of eroding bone *in vitro*, independently of the action of osteoclasts (1). Together with the evidence that metastatic cancers in bone can resorb bone by a direct action of the tumor cells (2,3) this suggests that breast cancer cells possess the means of transporting calcium and phosphorus. The recent discovery (4) that the MCF 7 cell contains a classical cytosol receptor for $1,25(OH)_2$-vitamin D (the active hormonal form of vitamin D) indicates a possible hormonal control of this function. Skeletal metastases in breast cancer are frequently accompanied by hypercalcaemia, which responds at least temporarily to calcitonin (5,6). It is reported here that MCF 7 cells also possess a specific cell surface receptor for calcitonin which is associated with activation of adenylate cyclase and of cyclic AMP-dependent protein kinase.

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METHODS:

Binding: MCF 7 cells were routinely cultured in RPMI 1640 medium containing 10 per cent fetal calf serum with 10^{-7} M-insulin (Novo crystalline) and 10^{-7} M-hydrocortisone hemisuccinate. The latter hormones were omitted for 24 hours before experiments. Cells were subcultured by incubating at 37°C for 2 mins with 0.0125% trypsin in 0.02% EDTA-saline, followed by addition of serum and two washes in buffered saline. Binding experiments were carried out at 20°C for 1 hour in a buffered salt solution, using incubation and separation conditions exactly as described for studies in other calcitonin responsive cells (7), except that in the present experiments bacitracin (1 mg/ml) was included in incubations. Synthetic salmon calcitonin (SCT, Armour Pharmaceutical Co., Kankakee, Illinois, USA) was labelled with I^{125} as previously described (7,8) to specific activities of 150 to 250 μCi per μg. Human calcitonin and its analogues were provided by Dr. W. Rittel, Ciba Geigy, Basel, and [$Asu^{1,7}$] eel calcitonin was provided by Dr. J. Murase, Toyo Jozo Co. Ltd., Ohito, Shirynoka, Japan. Synthetic human parathyroid hormone (1-34) was provided by the Armour Pharmaceutical Co., prostaglandin E_2 by Dr. John E. Pike, Upjohn Co., Kalamazoo, Michigan, glucagon by Eli Lilly & Co., Indianapolis, Indiana.

Adenylate cyclase: MCF 7 cells were washed, scraped off the surfaces of culture vessels in 0.25 M sucrose, 25 mM Tris pH 7.4, 1 mM dithiothreitol, 1 mM EDTA and homogenized in an all glass homogenizer. Adenylate cyclase assay was carried out by incubating at 37°C for 10 mins, using 0.1 mM ATP^{32} as substrate, and other conditions and product purification as previously described (8).

Cyclic AMP-dependent protein kinase: Replicate confluent cultures in 12 cm^2 dishes were washed with buffered saline and incubated with 2 ml buffer (Eagle's Minimal Essential Medium with 20 mM HEPES, 1 mg/ml bovine serum albumin, and 10^{-5} M isobutyl methylxanthine). Cells were removed at indicated times by scraping into 2 ml extraction buffer (20 mM morpholino-ethanesulphonic acid, 0.4 mM EDTA, 0.2 mM dithiothreitol, 2% Triton X-100, pH 6.8). After centrifugation for 20 secs at 8000 g, 20 μl samples were incubated for 5 mins at 30°C with [γ-P^{32}] -ATP (1 mM) and 156 μM synthetic peptide substrate (phosphorylation site sequence of porcine hepatic pyruvate kinase) (9). Incubations were terminated by precipitation onto Whatman P81 phosphocellulose paper as described (9,10). Activities in the absence and presence of 6.5 μM cyclic AMP were measured to calculate activity ratios.

RESULTS:

Suspensions of MCF 7 cells bound labelled calcitonin, reaching saturation after 45 mins at 20°C. Specificity of binding is indicated in Fig. 1, showing competition by unlabelled SCT and three analogues of human calcitonin, with no effect of very high concentrations of unrelated peptides. Scatchard analysis of binding of SCT to MCF 7

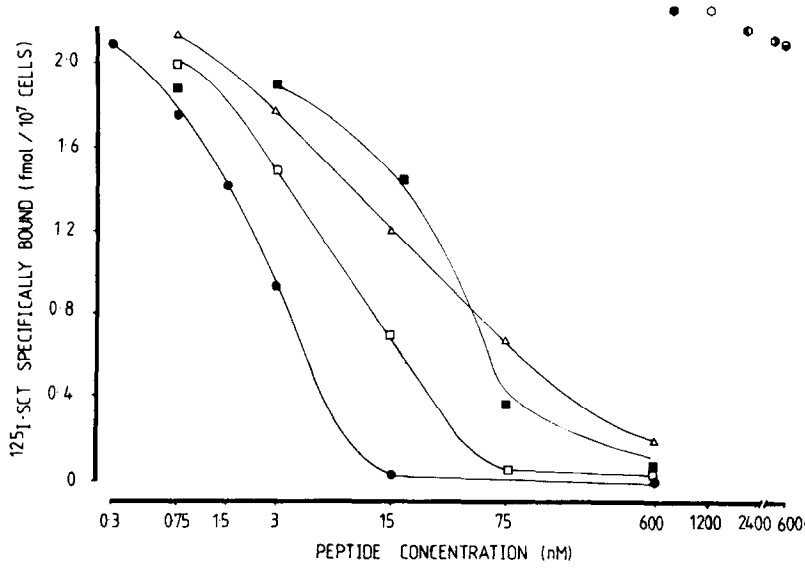

Fig. 1. Binding of I^{125}-labelled salmon calcitonin (SCT) to intact MCF 7 cells, and competition by various unlabelled calcitonins.
● : SCT; □ : $Thre^{27}$ HCT; △ : Leu^{12} HCT;
■ : Arg^{24} HCT; ◖ : human growth hormone;
○ : insulin; ◐ : human PTH (1-34);
◑ : ACTH (1-24); ◖ : glucagon.

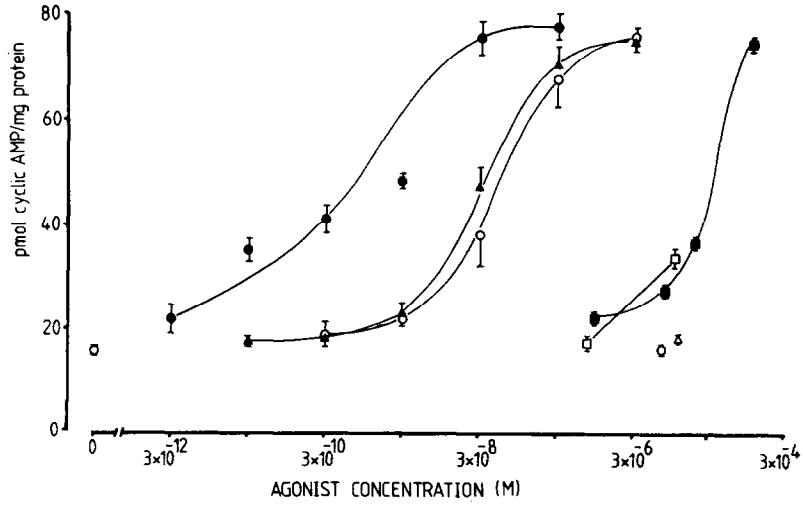

Fig. 2. Effects of calcitonin and other hormones on adenylate cyclase activity in MCF 7 cell lysates. Each point is the mean ± SEM of three observations.
● : synthetic salmon calcitonin; □ : synthetic human parathyroid hormone (1-34); ○ : human calcitonin; ▲ : Val^8 human calcitonin;
■ : prostaglandin E_2; △ : glucagon;
◖ : insulin and human growth hormone.

cells yielded linear plots (data not shown) providing mean K_d and receptor number per cell in six separate experiments of 1.6 ± 0.2 x 10^{-9} M and 4700 ± 1300 respectively (means ± SEM). Furthermore calcitonin activated adenylate cyclase in a concentration-dependent fashion (Fig. 2). Of the several other agents tested, only prostaglandin E_2 was capable of stimulating adenylate cyclase activity fully at near physiological concentrations. Synthetic human PTH had a small effect at very high concentrations. The adenylate cyclase responses to a range of calcitonin analogues were compared with their ability to compete for binding to MCF 7 cells, and close agreement was found between the two systems (Table 1). Finally, calcitonin treatment

TABLE 1: Comparison of efficacies of various calcitonins in stimulating adenylate cyclase activity in MCF 7 cell lysates, and in binding to intact MCF 7 cells.

Hormone	Adenylate Cyclase	Binding
Salmon calcitonin	100	100
[$Asu^{1,7}$] Eel calcitonin	150	100
Human calcitonin (HCT)	3	5
Val^{8} HCT	6	8
Leu^{12} HCT	12	12
$Leu^{12,16,19}$ HCT	30	10
Asn^{26} HCT	10	12
$Thre^{27}$ HCT	36	33
$Asn^{26}Thre^{27}$ HCT	45	50
Arg^{24} HCT	27	10

The potencies of several calcitonins were assessed in adenylate cyclase activation (as in Fig. 2) and in competition for binding (as in Fig. 1). The half maximally effective concentration of each preparation was compared with that of salmon calcitonin, which was assigned an arbitary value of 100 units in each system. At least two preparations were assayed at several concentrations in each experiment, against a standard of salmon calcitonin. Superscript numerals indicate the position of the amino acid substitution.

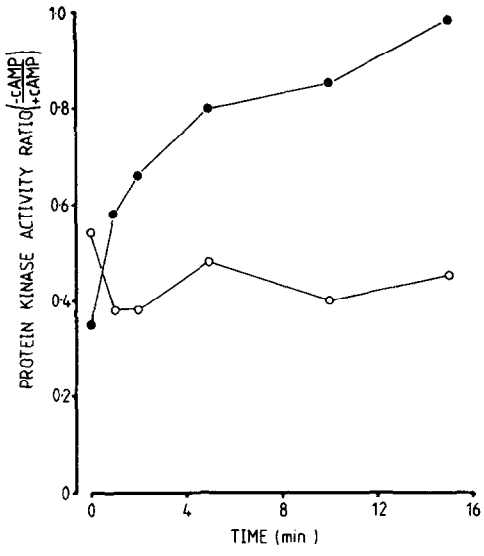

Fig. 3. Activation of cyclic AMP-dependent protein kinase by SCT in cultured MCF 7 cells. Results are expressed as the ratio of the activity in the absence to that in the presence of 6.25 μM cyclic AMP, in control cells (○) and those treated with SCT (●), 10^{-9} M.

of cultured MCF 7 cells led to rapid activation of cyclic AMP-dependent protein kinase (Fig. 3).

DISCUSSION:

The results indicate that MCF 7 cells, used extensively in the study of human breast cancer as a model for receptors of oestrogen, progesterone and androgens (11-13), possess a specific receptor for the peptide hormone, calcitonin. A link between receptor binding and adenylate cyclase activation is indicated by the studies with several calcitonin analogues, which competed for binding with virtually the same relative efficacy with which they stimulated adenylate cyclase. It should be noted also that these same relative potencies were obtained when the analogues were assayed as calcium-lowering agents in the rat (14,15). Thus the binding of calcitonin to MCF 7 cells is closely related to activation of adenylate cyclase, and exhibits

properties which would be expected of calcitonin interacting specifically with a target cell receptor. The activation of protein kinase by calcitonin indicates that the MCF 7 cells are capable of proceeding with post-receptor events in response to calcitonin. It will be important to determine the details of these actions, and to assess the significance for the MCF 7 cells of the effects of calcitonin on cyclic nucleotide metabolism.

The coexistence of receptors for at least two calcium-regulating hormones in MCF 7 cells, and the known ability of these cells to resorb bone (1) points to their osteoclast-like properties. Such properties could be related to the ability of breast cancers to erode bone as metastatic growths. This is a testable possibility which accords with the view that cancer cells growing in different organs as metastases require special properties to allow them to do so (16). Receptors for $1,25(OH)_2$-vitamin D have now been shown to occur commonly in human breast cancers (17).

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