Bone and Kidney Adenylate Cyclase-stimulating Activity Produced by a Hypercalcemic Canine Adenocarcinoma Line (CAC-8) Maintained in Nude Mice

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ABSTRACT

The tumor line, CAC-8, is a serially transplantable adenocarcinoma maintained in nude mice which originated from a hypercalcemic dog. Nude mice with CAC-8 developed a syndrome of humoral hypercalcemia of malignancy. CAC-8 contained a protein factor which stimulated adenylate cyclase of bone and kidney cells in vitro. The adenylate cyclase (AC) of rat osteosarcoma cell lines, ROS 17/2.8 (ROS) and UMR-106, was stimulated by the tumor extract and potentiated by forskolin (0.1 μM). The ROS cells responded to the lowest concentration of CAC-8 extract, but UMR cells responded with a greater increase in AC activity compared to controls following exposure to CAC-8 extract. Pretreatment of ROS 17/2.8 cells with dexamethasone enhanced the response to CAC-8 extract. The opossum kidney cell line (OK) was less sensitive to the AC-stimulating activity of CAC-8 extract, but AC stimulation was increased in the presence of forskolin. Bovine (1-34) parathyroid hormone (BPTH) (10 nM) stimulated AC equally in ROS, UMR, and OK cells. Isoproteonel (1.0 μM) stimulated AC activity in ROS and UMR cells but not in OK cells. The AC-stimulating activity of CAC-8 appeared to bind to the parathyroid hormone receptor of ROS, UMR, and OK cells since addition of the parathyroid hormone receptor antagonist, [1-38]norleucine, [3-34]tyrosine]BPTH (3-34) amide, inhibited CAC-8-mediated cyclic adenosine 5'-monophosphate production and alone did not stimulate AC activity. The AC-stimulating activity of CAC-8 was acid and heat stable. Trypsin digestion reduced BPTH and CAC-8 stimulation of AC to near basal levels and treatment of CAC-8 extract with dithiothreitol reduced AC stimulation in UMR cells by approximately 50%.

Extracts of the hypercalcemic tumor line (CAC-8) contained bone and kidney AC-stimulating activity which was enhanced by forskolin and dexamethasone, inhibited by [1-38]NorLe, [3-34]TyrylBPTH (3-34) amide, heat stable, trypsin sensitive, inactivated by reduction, and had a relative molecular weight of 34,000 by gel exclusion chromatography. Isolation and characterization of the factor(s) produced by CAC-8 that stimulate AC activity will be useful in further understanding the pathogenesis of humoral hypercalcemia of malignancy in animal and human patients.

INTRODUCTION

A serially transplantable tumor line, designated CAC3-8, has been developed from a canine hypercalcemic adenocarcinoma (1) in nude mice that develop an identical clinical syndrome of HHM as reported in human patients (2). Nude mice with CAC-8 developed hypercalcemia, hypophosphatemia, increased serum 1,25-dihydroxycholecalciferol, increased urinary excretion of cAMP, and increased rates of bone formation and resorption without bone metastasis (2). Tumor extracts and conditioned tissue culture medium contained a factor which increased in vitro bone resorbing activity but was not suppressed by indomethacin, stimulated osteoclast hyperplasia and hyper trophy in neonatal mouse calvaria, and had transforming growth factor activity that was not dependent on the presence of epidermal growth factor (3, 4).

Humoral hypercalcemia of malignancy is an important clinical syndrome resulting from the secretion of unidentified substances by tumor cells which results in increased osteoclastic bone resorption, altered renal excretion of phosphorus and calcium, and increased nephrogenic cAMP (5). In HHM of human beings and laboratory animals there is increased total and fractional excretion of phosphorus and increased total and decreased fractional excretion of calcium (6, 7). These features also are a characteristic response to PTH excess (8). The effects on bone and kidney may result from one or more humoral factors released by tumor cells. Prostaglandins, PTH, TGFs, and PTH-like peptides have been suggested as the factors produced by tumor cells that result in the development of HHM (9). Most forms of HHM have been shown not to be the result of excessive secretion of PTH or prostaglandins (10). It has been hypothesized that HHM is due to a combined secretion of TGF and PTH-like peptides by the neoplastic cells (9). Transforming growth factors stimulate in vitro bone resorption and the TGF activity of the rat Leydig cell tumor and the Walker carcinosarcoma coelute on chromatography with bone resorbing activity (11, 12). Tumors associated with HHM in human beings and animals have been reported to contain factors which bind to PTH receptors in target cells and stimulate adenylate cyclase (13-15). It is not known whether the bone-resorbing activity and the adenylate cyclase-stimulating activity of tumors associated with HHM are the same or different proteins (15).

The tumor line CAC-8 contains in vitro bone-resorbing activity (3), TGF activity (4), and activity that stimulates adenylate cyclase in both bone and kidney. This triad of biological activities is typical of the few well-described laboratory animal models of HHM (8). PTH-like activity from a collection of human tumors associated with hypercalcemia correlated well with occurrence of HHM and could be used to classify the patients into "HHM" and "non-HHM" categories (15). In addition, the tumor line CAC-8 is a well-differentiated epithelial malignancy generally similar in type to the forms of cancer that produce HHM in human patients. The tumor doubling rate of CAC-8 in nude mice is approximately 14 days which allows time for morphological changes consistent with HHM to develop in nude mice.

The objectives of this investigation were to (a) determine whether hypercalcemic and normocalcemic canine adenocarcinomas contained activity capable of stimulating adenylate cyclase in ROS 17/2.8 (rat osteosarcoma) cells, UMR-106 (rat osteosarcoma cell line); TGF, transforming growth factor.
材料和方法

材料。牛PTH（1-34）、 forskolin、isoproterenol、dexamethasone、isobutylmethylxantine、phenylmethylsulfonyl fluoride、pepsatin A、ATP、ADP、AMP、cAMP、adenine、adenosine、dithiothreitol、soybean trypsin inhibitor、and calcium- and magnesium-free Hanks' balanced salt solution were obtained from Sigma Chemical Co. (St. Louis, MO); [87Ne, 34Tyr]BPTH (3-34) amide from Bachem, Inc. (Torrance, CA); trypsin treated with TCPK from Cooper Biomedical, Inc. (Malvern, PA); [2,87H]adenine from New England Nuclear (Boston, MA); basal medium Eagle's and F12 medium from Grand Island Biological Co. (Grand Island, NY); and Fractogel TSK HW-50s from Pierce Chemical Co. (Rockford, IL).

细胞培养。ROS 17/2.8 细胞由 Dr. R. Majeska (University of Connecticut, Farmington, CT) 提供，并在 F12 培养基中与 3 个 FBSs 之前作为唯一的 15 (6)。UMR 106 和 OK 细胞系由 Dr. G. Strewler (University of California, San Francisco, CA) 提供，并在basal medium medium's supplemented with 10% FBS, 2 mm L-glutamine, penicillin (50 units/ml), streptomycin (50 pg/ml), and neomycin (100 µg/ml) 中培养。ROS 细胞系是从一个细胞系中选择的，可以对腺苷酸 cyclase phospho-和 isoprotopenol (16)。UMR 细胞是来自一个细胞系，可以对腺苷酸 cyclase phospho-和 prostaglandins (17)。ROS 和 UMR 细胞可能在不同的生命周期或不同的亚细胞群中代表不同的阶段 (18)。OK 细胞是来自一个细胞系，可以对腺苷酸 cyclase phospho-和 prostaglandins (19)。

肿瘤提取的准备。CAC-8 肿瘤组织 (0.5-2.0 g/mouse) 被从hypercalcemic nude 模型 (Life Sciences, St. Petersburg, FL) 和6-70°C (13)。癌和正常组织组由一个腺苷酸 cyclase removed from a normal cell adenocarcinoma removed from a normal mcaclonal cell (negative control, CAC-23) and an adenocarcinoma removed from a hypercalcemic cell (positive control, CAC-22). Tumor tissue was frozen in liquid nitrogen and shattered by compression. The tissue was thawed in a solution (4.0 ml/g of tissue) consisting of 375 ml 95% (vol/vol) ethanol, 7.5 ml concentrated HCl, 33 mg phenylmethylsulfonyl fluoride, and 1.9 mg pepsatin A. Distilled water (2 ml/g of tissue) was added, and the tissue was homogenized in an ice water bath using a Polytron homogenizer (Brinkman Instruments, Inc., Westbury, NY) (three 15-s bursts). The mixture was extracted overnight at 4°C, centrifuged at 15,000 x g for 30 min, and the residue was reextracted for 2 h with 80 ml of a solution consisting of 375 ml 95% ethanol, 105 ml distilled water, and 7.5 ml concentrated HCl. The supernatants were pooled, and the pH was adjusted to 5.2 with concentrated ammonium hydroxide followed by the addition of 1 ml of 2 M ammonium acetate buffer, pH 5.5/85 ml of extract. Two volumes of ice-cold absolute ethanol and 4 volumes of ice-cold anhydrous ether were immediately added. The mixture was cooled to -20°C for 48 h. The resulting precipitate was collected by rapid filtration on Whatman No. 1 paper, redissolved in 1 ml acetic acid (4 ml/g tissue), and centrifuged to remove nonsoluble precipitate. The solution was dialyzed (1:100) against 0.17 M acetic acid for 48 h at 4°C with 4 changes of dialysate (Spectropor tubing, M, 3500 cut-off; American Scientific Products, Columbus, OH). The samples were lyophilized and stored at -20°C (20). One batch of tumor extract (CAC-8 extract) was used in all the experiments described in this investigation, except in the initial experiment in which all tumor extracts (CAC-8 extracts 1 and 2, CAC-22, and CAC-23) were compared in a single AC-stimulating assay using ROS cells. The protein of tumor extracts was determined using the Bradford assay with bovine serum albumin as a standard (21).

腺苷酸 cyclase-stimulating Assay. The AC-stimulating assay was performed as described (13) with minor modifications. Briefly, cells were plated at 10,000 cells/cm² and grown to confluence in either 4 cm²/well or 2 cm²/well plates (Costar, Cambridge, MA). The experiments which compared the 4 tumor extracts and determined the effect of pretreatment with BPTH (1-34) utilized 4 cm²/well plates while all other experiments utilized 2 cm²/well plates. The ROS cells required 8 days and the UMR-106 and OK cells required 4 days to reach confluence in the 2 cm²/well plates. The cells were incubated with 1 ml of F12 medium with 5% FBS containing 1 ug/ml [3H]adenine (100 µCi/ml) for 2 h at 37°C. The wells were washed twice with 1 ml of Hanks' balanced salt solution and incubated with 0.5 ml of F12 medium with 2% FBS and 1 mm isobutylmethylxantine for 10 min at 37°C. Positive control and test substances were added to result in specified concentrations to the wells for a treatment period of 5 min, and the reaction was stopped with the addition of 50 µl of 1.2 M trichloroacetic acid. The medium was transferred to plastic tubes and 50 µl of a carrier solution was added that contained 5 mm ATP, ADP, AMP, cAMP, adenine, and adenosine. The samples were frozen at -20°C overnight, thawed, and centrifuged for 10 min at 1200 x g. The supernatant was neutralized with 4 N KOH (25 µl), the cAMP was separated by the methods of Salomon (22) using serial Dowex X50 (Bio-Rad Laboratories, Richmond, CA) and alumina column chromatography, and the radiation was determined by scintillation spectrophotometry.

The effect of 4 canine tumor extracts (extracts 1 and 2 of CAC-8, CAC-22, and CAC-23) was determined on the AC activity of ROS cells. Bovine PTH (5-20 µM) was the positive control in each experiment. The effect of 2 h pretreatment of BPTH was evaluated on CAC-8- and BPTH-stimulated AC of ROS cells. A time response curve (1-20 min) was developed on the AC stimulation of UMR cells by CAC-8 extract. The dose response of AC stimulation by CAC-8 extract was determined using UMR and ROS cells. The effect of BPTH, isoproterenol (1 µM) (b-adrenergic agonist), and CAC-8 extract (0.5 mg/ml) was compared on the AC stimulation of ROS, UMR, and OK cells. Forskolin (0.1 µM) was added directly to test substances or the 3 cell lines were incubated with medium supplemented with 30 nm dexamethasone for 48 h prior to the AC assay in order to determine the effects of forskolin or dexamethasone on the stimulation of AC in ROS, UMR, and OK cells by BPTH and CAC-8 extract.

The BPTH receptor antagonist, [87Ne, 34Tyr]BPTH (3-34) amide was added to ROS, UMR, and OK cells in addition to BPTH (1-34) or CAC-8 extract to determine the dependence of AC stimulation on binding to the PTH receptor. Bovine PTH and CAC-8 extract were exposed to 60°C for 1 h, 100°C for 3 min, or trypsin-TCPK (50 µg/ml) for 1 h at 37°C to determine the effect of heat treatment and protease digestion on AC stimulation of UMR cells. The trypsin digestion was stopped by the addition of soybean trypsin inhibitor (100 µg/ml). The trypsin control solution contained both trypsin-TCPK and trypsin inhibitor at the same concentrations. CAC-8 extract was treated with dithiothreitol (0.062 M) for 1 h at 25°C and then extensively dialyzed against 0.17 M acetic acid. A paired batch of CAC-8 extract was treated identically without exposure to dithiothreitol.

Ge chromatography. CAC-8 tumor extract (20 mg; 1 ml of sample) was dissolved in 1 N acetic acid, applied to a column (2.5 x 80 cm) of Fractogel TSK HW-50s (25-40 µM), and eluted with 1 N acetic acid at a flow rate of 2 ml/h. Fractions (1 ml) were collected, evaluated for absorbance of light at 280 nm, lyophilized, and stored at -20°C until assayed for AC-stimulating activity on UMR cells in the presence of forskolin (0.1 µM). Chromatographic molecular weight markers included bovine serum albumin (M, 67,000), chymotrypsinogen (M, 25,000), and insulin (M, 6,000).

Statistical Analysis. The AC stimulation assays were performed in triplicate. The data were expressed as cpm/well ± SE. Data consisting of 3 or more groups were analyzed by analysis of variance and the Newman-Keuls multiple range test (23). Data with 2 groups were evaluated with Student's t test. A significant difference was considered to exist when P < 0.05.
RESULTS

Three canine tumor extracts produced significant stimulation of AC in ROS cells (Table 1). The 2 batches of CAC-8 extract were not significantly different and produced approximately 47% the magnitude of stimulation of AC activity compared to BPTH. The extract from CAC-22 (a second anal sac adenocarcinoma from a hypercalcemic dog) significantly stimulated AC but was less active than the extracts from CAC-8 obtained by tumors grown in nude mice. Tumor extract designated CAC-23 (adenocarcinoma of anal sac from a nor-mocalcemic dog) did not significantly stimulate AC activity as compared to CAC-8, CAC-22, and controls despite numerical doubling of AC activity (one-way analysis of variance). The protein content of CAC-8 extracts 1 and 2, CAC-22, and CAC-23 was 0.28, 0.29, 0.20, and 0.27 mg protein/mg dry extract, respectively. Pretreatment of ROS cells with BPTH for 2 h significantly reduced the response of AC to stimulation by both BPTH and CAC-8 extract by 45% (Table 2).

The time response curve (Fig. 1) of AC stimulation by CAC-8 extract demonstrates that cAMP accumulates in UMR cells in a linear pattern during the initial 10 min of incubation and then plateaus from 10–20 min. The point of median stimulation occurs at approximately 5 min. The dose response curve of AC cyclase stimulation in ROS cells (Fig. 2) by CAC-8 demonstrates a sigmoidal pattern with significant stimulation of AC at 0.01 mg of extract/well. In comparison, the UMR cells respond to CAC-8 extract with significant stimulation of AC at 0.035 mg CAC-8 extract/well (Fig. 3).

In Table 3 results are compared of AC activity in ROS, UMR, and OK cells after stimulation by BPTH, isoproterenol, and CAC-8 extract. The concentrations used for the 3 agonists were maximal for AC stimulation of the cell lines and were kept constant to permit comparisons between cell lines. Although the AC of ROS cells was stimulated by BPTH, isopro-

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Table 1 | Effect of canine adenocarcinoma extracts and BPTH on the stimulation of adenylate cyclase in ROS 17/2.8 (bone) cells

<table>
<thead>
<tr>
<th>Treatment</th>
<th>cpm/well</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>486 ± 33*</td>
</tr>
<tr>
<td>BPTH (20 nm)</td>
<td>23,040 ± 1,682*</td>
</tr>
<tr>
<td>CAC-8 (1.5 mg, extract 1)</td>
<td>10,895 ± 691*</td>
</tr>
<tr>
<td>CAC-8 (1.5 mg, extract 2)</td>
<td>10,931 ± 594*</td>
</tr>
<tr>
<td>CAC-22 (1.5 mg)</td>
<td>6,554 ± 470*</td>
</tr>
<tr>
<td>CAC-23 (1.5 mg)</td>
<td>1,011 ± 85'</td>
</tr>
</tbody>
</table>

a Mean ± SE from triplicate wells of a representative experiment.
b Different from control, at P < 0.001.
c Different from control, at P < 0.01.
d Not significantly different from control.

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Table 2 | Effect of pretreatment with BPTH on the stimulation of adenylate cyclase in ROS 17/2.8 (bone) cells by BPTH and CAC-8 extract

<table>
<thead>
<tr>
<th>Treatment</th>
<th>cpm/well</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>524 ± 54*</td>
</tr>
<tr>
<td>BPTH (20 nm)</td>
<td>1,670 ± 58*</td>
</tr>
<tr>
<td>CAC-8 (0.6 mg)</td>
<td>9,313 ± 654</td>
</tr>
</tbody>
</table>

a Mean ± SE from triplicate wells.
b Different from corresponding no pretreatment group, at P < 0.01.
Table 3 Effect of BPTH, isoproterenol, and CAC-8 extract on the stimulation of adenylate cyclase in ROS 17/2.8, UMR-106, and OK cell lines.

<table>
<thead>
<tr>
<th>Cell line (cpm/well)</th>
<th>Treatment</th>
<th>Control</th>
<th>BPTH (20 nM)</th>
<th>Isoproterenol (1 μM)</th>
<th>CAC-8 (0.5 mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ROS 17/2.8 (bone)</td>
<td>Control</td>
<td>237 ± 27</td>
<td>212 ± 9</td>
<td>538 ± 69</td>
<td></td>
</tr>
<tr>
<td>UMR-106 (bone)</td>
<td>BPTH</td>
<td>7211 ± 849</td>
<td>5906 ± 263</td>
<td>6593 ± 210</td>
<td></td>
</tr>
<tr>
<td>OK (kidney)</td>
<td>Isoproterenol</td>
<td>8942 ± 126</td>
<td>1394 ± 211</td>
<td>485 ± 50</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CAC-8</td>
<td>2639 ± 1038</td>
<td>5086 ± 507</td>
<td>771 ± 72</td>
<td></td>
</tr>
</tbody>
</table>

* Mean ± SE from triplicate wells of a representative experiment.
* Different from control, at P < 0.01.
* Different from control, at P < 0.05.
* Different from both BPTH and CAC-8, at P < 0.01.
* Different from both forskolin and isoproterenol, at P < 0.01.

The effects of forskolin and dexamethasone on AC stimulation were also assessed. Table 4 presents the results of these experiments.

Table 4 Effect of forskolin and dexamethasone on the stimulation of adenylate cyclase in ROS 17/2.8, UMR-106, and OK cells by BPTH and CAC-8 extract.

<table>
<thead>
<tr>
<th>Cell line (cpm/well)</th>
<th>Treatment</th>
<th>No treatment</th>
<th>Forskolin (0.1 μM)</th>
<th>Dexamethasone (30 nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ROS 17/2.8 (bone)</td>
<td>Control</td>
<td>238 ± 20</td>
<td>249 ± 15</td>
<td>199 ± 13</td>
</tr>
<tr>
<td></td>
<td>BPTH (0.05 mg)</td>
<td>797 ± 56</td>
<td>1,349 ± 73</td>
<td>1,690 ± 85</td>
</tr>
<tr>
<td></td>
<td>BPTH (10 nM)</td>
<td>3,721 ± 344</td>
<td>5,774 ± 91</td>
<td>10,595 ± 684</td>
</tr>
<tr>
<td>UMR-106 (bone)</td>
<td>Control</td>
<td>295 ± 35</td>
<td>310 ± 27</td>
<td>479 ± 49</td>
</tr>
<tr>
<td></td>
<td>CAC-8</td>
<td>3,074 ± 160</td>
<td>4,063 ± 91</td>
<td>2,168 ± 214</td>
</tr>
<tr>
<td></td>
<td>BPTH (10 nM)</td>
<td>5,918 ± 664</td>
<td>7,947 ± 780</td>
<td>6,567 ± 635</td>
</tr>
<tr>
<td>OK (kidney)</td>
<td>Control</td>
<td>449 ± 51</td>
<td>275 ± 27</td>
<td>497 ± 69</td>
</tr>
<tr>
<td></td>
<td>CAC-8</td>
<td>881 ± 93</td>
<td>1,412 ± 126</td>
<td>712 ± 112</td>
</tr>
<tr>
<td></td>
<td>BPTH (10 nM)</td>
<td>3,672 ± 215</td>
<td>9,957 ± 868</td>
<td>3,130 ± 265</td>
</tr>
</tbody>
</table>

* Mean ± SE from triplicate wells of a representative experiment.
* Different from control, at P < 0.05.
* Different from control, at P < 0.01.
* Different from no treatment group, at P < 0.01.
* Different from no treatment group, at P < 0.05.

The production of OK cells with or without forskolin, but the AC stimulation was increased significantly compared to controls only in the presence of forskolin. Forskolin, at 0.1 μM, did not stimulate the AC activity of the cell lines in the absence of another agonist. Preincubation of the cell lines for 2 days in medium with 30 nM dexamethasone significantly increased cAMP production by ROS cells in response to BPTH and CAC-8 extract. The AC stimulation of UMR and OK cells by BPTH was not potentiated by dexamethasone pretreatment, and cAMP production of UMR cells induced by CAC-8 extract was significantly reduced compared to control cells. Dexamethasone pretreatment of the cell lines did not alter cAMP production in the absence of an AC agonist.

The PTH receptor competitive antagonist [1143Nle, 34Tyr]-BPTH (3-34) amide significantly reduced AC stimulation of ROS, UMR, and OK cells by BPTH (1-34) and CAC-8 extract (Table 5). The [1143Nle, 34Tyr]BPTH (3-34) amide did not stimulate AC activity of the 3 cell lines.

Heat treatment of CAC-8 extract (Table 6) demonstrated that the AC-stimulating activity of CAC-8 or BPTH in UMR and CAC-8 extract.
The bone and kidney alterations seen in human patients with hyperparathyroidism that are not present in patients with HHM which develops in nude mice with transplanted CAC-8 does include increased serum 1,25-dihydroxycholecalciferol, and increased bone formation (9). However, the syndrome of HHM which develops in nude mice with treated CAC-8 extract did not stimulate the AC complex of the 3 cell lines to the same degree as did BPTH, when the tumor extract was compared to BPTH by other physical and chemical means they resulted in parallel AC stimulation. Pretreatment of ROS cells with BPTH for 2 h prior to exposure to CAC-8 extract or BPTH did result in desensitization of the AC stimulation similar to cultured chick kidney cells which undergo homologous desensitization to PTH after 1 h of preincubation with PTH (24). AC stimulation in response to CAC-8 extract and BPTH was enhanced in all 3 cell lines by the presence of forskolin (0.1 μM). This low concentration of forskolin alone did not stimulate cAMP production. Forskolin is a potent activator of the PTH-responsive adenylyl cyclase system in a number of cell lines and can directly stimulate AC activity in cells at concentrations as low as 1.0 μM (25). The site of action of forskolin is unknown but may be the nucleoside regulatory unit of the AC catalytic system (25, 26). Preculture with dexamethasone enhanced AC stimulation by either CAC-8 extract or BPTH only in ROS cells (27). The mechanism of dexamethasone enhancement of AC in ROS 17/2.8 cells is unknown, but it has been reported that dexamethasone increased the guanine nucleotide regulatory protein of the AC catalytic system (28). The antagonist of the PTH receptor, [1,23P]BPTH (3-34) amide significantly reduced AC stimulation induced by CAC-8 extract or BPTH in ROS, UMR, and OK cells. The PTH receptor antagonist did not stimulate AC alone. We concluded that CAC-8 contains a protein factor which stimulated the AC of bone and kidney cells by binding to the PTH receptor, since the response to CAC-8 extract paralleled the response to BPTH in AC desensitization, potentiation of AC with forskolin or dexamethasone, and inhibition of AC by the PTH receptor-antagonist.

Heat treatment of CAC-8 extract and BPTH at 60°C or 100°C did not reduce AC-stimulating activity. The cause of the small increase in AC-stimulating activity of CAC-8 extract after heating at 100°C is not known but may represent a direct enhancement of the AC-stimulating activity or removal of an inhibitory factor by denaturation. Bovine PTH and CAC-8 AC-stimulating activity were deactivated by exposure to trypsin indicating that both substances are proteins that were susceptible to trypsin-induced proteolysis. The AC-stimulating activity of CAC-8 extract was decreased significantly by reduction with dithiothreitol, suggesting that disulfide bonds are important structural components of the AC-stimulating protein.

Gel chromatography of CAC-8 extract demonstrated a peak of AC-stimulating activity at a relative molecular weight of 34,000 (Fig. 4).
nomas associated with hypercalcemia. PTH-like peptides have been purified from a human squamous cell carcinoma and the rat Leydig tumor (H-500) (31). Peptides which were active in AC-stimulating assays had molecular weights of 9000 and 9500 by amino acid analysis. In addition, the rat Leydig cell tumor contained an active peptide of Mr, 28,000. These findings suggest that the approximate molecular weights of AC-stimulating activities from gel exclusion chromatography experiments may be overestimated due to possible protein glycosylation and the lack of denaturing conditions. Two classes of AC-stimulating activity have been isolated from a murine squamous cell carcinoma model of HHM (32). Class I consisted of closely related peaks observed in reverse phase high-performance liquid chromatography fractions that stimulated AC in disrupted and intact cells and were inhibited by [3H-Nle, 34Tyr]BPTH (3-34) amide. It was postulated that Class II AC-stimulating activity acts by a postreceptor mechanism and potentiates the activity of the Class I factor. The CAC-8 tumor-related peptide described in our study appears to more closely resemble Class I activity of the murine squamous cell carcinoma.

In summary the tumor line CAC-8 contains a protein factor(s) which stimulates the AC of bone and kidney cells by binding to the PTH receptor. The tumor-related activity was enhanced by forskolin and dexamethasone, was heat and acid stable, was inactivated by reduction, and had a relative molecular weight of approximately 34,000. Future purification and characterization of the AC-stimulating activity in CAC-8 will be useful in understanding the pathogenesis of HHM.

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