Vol. 149, No. 1, 1987 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
November 30, 1987 Pages 227-232

SYNTHETIC TUMOR-DERIVED HUMAN HYPERCALCEMIC FACTOR EXHIBITS PARATHYROID HORMONE-LIKE VASORELAXATION IN RENAL ARTERIES

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Received October 15, 1987

SUMMARY: Synthetic human tumor hypercalcemic factor (1-34, hHF) was compared with parathyroid hormone (human sequence, 1-34; hPTH) for vasorelaxant activity in isolated rabbit renal artery segments. The hHF exhibited a potent (IC_{50} = $1.3x10^{-9}M$) and profound (98%) relaxation which was significantly greater in magnitude than that obtained for hPTH (IC_{50} = $4.5x10^{-9}M$; maximal relaxation = 78%). The relaxations to both peptides were concentration-dependent and not associated with changes in cyclic AMP levels. These results demonstrate a parathyroid hormone-like response, independent of adenylate cyclase activation, in isolated renal arteries. Renal vasodilation may be important for the effects on renal function shared by these two peptides.

The hypercalcemia associated with malignant disease is believed to be caused by a tumor-derived circulating factor that has biological actions similar to those of parathyroid hormone (PTH). Both extracts from excised tumors (1) as well as a protein released from a cultured tumor cell line (BEN; 2) derived from these patients are active in stimulating adenylate cyclase in bone-derived cell lines which are responsive to PTH. Although partial amino terminal acid sequence analysis of the isolated peptide factor disclosed significant homology with PTH (2), the tumor factor cannot be detected by PTH radioimmunoassays (3) implying other structural distinctions between the circulating factor and PTH. Recently, using BEN cells, Suva et al (4) successfully isolated and characterized a complementary DNA clone which was predicted to encode the full length PTH-like tumor factor. Following expression of the corresponding protein in a mammalian cell line PTH-like

Abbreviations: hHF (synthetic human hypercalcemic factor (1-34)); hPTH (synthetic human parathyroid hormone (1-34)).
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0006-291X/87 $1.50

activity was documented by stimulation of cyclic AMP formation in an osteoblast-like cell line (UMR106). The disclosure of the primary sequence by Suva et al (4) will allow for elucidation of the physiological functions of this hypercalcemia factor (hHF).

We have recently found that PTH exerts a potent and profound vasorelaxant response on isolated renal artery segments (5) which appears to be among the most sensitive assays for the known vasodilator actions of PTH (6-8). Renal vasodilation to PTH, which is typically prominent in laboratory animals (8), may act in concert with the known tubular effects of PTH on phosphate and calcium absorption. Therefore, we compared the vasorelaxant effects of synthetic hHF (1-34) with hPTH (1-34) using isolated renal artery ring segments. In addition, since the vasodilation elicited by PTH may be associated with activation of adenylate cyclase (7), we measured tissue levels of cyclic AMP in renal arteries exposed to these peptides.

MATERIALS AND METHODS

Male New Zealand White rabbits (2.2 to 2.6 Kg) were killed by asphyxiation and the renal arteries were rapidly removed and placed in physiological salt solution (PSS) of the following composition (in mM): NaCl (130); KCl (4.7); KH_2PO_4 (1.18); $MgSO_4 \cdot 7H_2O$ (1.17); $NaHCO_3$ (14.9); $CaCl_2$ (1.6); dextrose (11.0); Na_2EDTA (0.026). The pH was corrected to 7.3 with 1N NaOH and the PSS was continually gassed with 95% O_2 and 5% CO_2. Renal arteries were cleaned of extraneous tissue taking care not to perturb the luminal surface. 4mm ring segments were carefully mounted on fine (0.0085) stainless steel wires which were held in Plexiglas holders suspended in jacketed organ baths (50 ml) kept at 38°C. Renal artery rings were connected via surgical silk (4-0) to Grass force-displacement transducers (FT.03) for monitoring changes in isometric force. Rings were stretched (2.0 grams-force) to an optimal level of resting force as determined by maximal responsiveness to a test concentration of a contractile agent (methoxamine). Following a 1 hour equilibration period, renal artery rings were contracted to a 50% maximal response (EC_{50}) by methoxamine which averaged approximately 1.5 grams-force. Tissues were then washed several times over a 30 minute period after which the same concentration of methoxamine was reapplied. A cumulative concentration-response experiment with either hHF or hPTH was begun once the contractile response to methoxamine reached an equilibrated plateau. Each renal artery was used for only one dose-response experiment. Concentrations of peptides eliciting 50% relaxation of the methoxamine contraction (i.e. IC_{50}) were obtained by linear regression using log transformation of the concentrations. Statistical evaluation of the data was performed by the Student's t test for unpaired samples using geometric means of the IC_{50} values and the 0.05 level of probability as the level of significance ($P \leq 0.05$).

A synthetic sequence encompassing residues 1-34 of the amino-terminus of hHF (4) was used and is described by Horiuchi N. et al (9). hPTH (1-34) was purchased from Bachem. The purity of each peptide as well as the documentation of stock solution concentrations was accomplished by amino acid analysis.

Measurements of cyclic AMP were made in ring segments exposed to varying concentrations of hHF, hPTH or forskolin for 5 minutes. Isobutylmethylxanthine (IBMX, 10^{-6}M) was included in the PSS during these experiments. Tissues were immediately frozen in liquid nitrogen and homogenized in 1 ml of cold 6% trichloroacetic acid. Homogenates were centrifuged and the supernatants extracted with water-saturated ether and lyophilized overnight. The dried samples were assayed for cyclic AMP using a non-acetylated RIA (New England Nuclear). TCA insoluble total protein was measured by the method of Lowry et al (10) following solubilization in 0.4N NaOH.

Methoxamine was supplied as a gift from Burroughs Welcome (Research Triangle Park, NC). IBMX was purchased from Sigma Chemical Company (St. Louis, MO), and forskolin from Calbiochem (San Diego, CA).

RESULTS

Both hHF and hPTH caused concentration-dependent decreases of the contractile response to methoxamine in isolated rabbit renal artery rings (Fig. 1). The hHF elicited significantly larger relaxations than hPTH at the middle to high concentrations tested (i.e. 10^{-9}M to $3x10^{-8}$M, Fig. 1). The calculated IC_{50} for hHF ($1.3x10^{-9}$M) was slightly but not significantly less than that for hPTH ($4.5x10^{-9}$M). The maximal relaxation of the methoxamine contraction obtained for hHF (98±1%) was significantly greater than the maximal relaxation with hPTH (78±5%). The levels of methoxamine contractile force did not differ between the two groups prior to the relaxation experiments (hHF = 1.46±0.07 grams-force; hPTH = 1.51±0.08 grams-force).

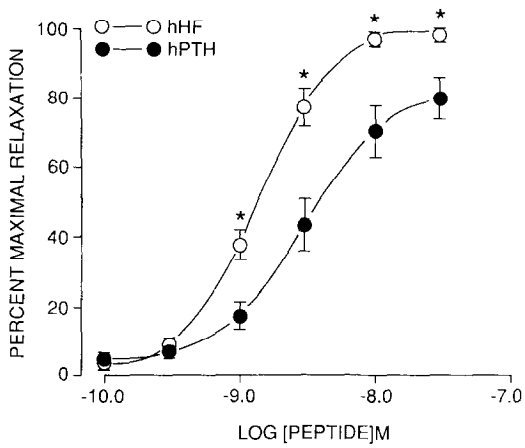

Figure 1. The relaxation response to hHF (n=4) and hPTH (n=7) in isolated rabbit renal artery rings contracted by methoxamine. The ordinate is expressed in terms of precent maximal relaxation of the methoxamine contraction while the abscissa is log concentration of the particular peptide in molarity. Values are means ± standard error of the means. An asterisk designates a significantly greater relaxation to hHF compared to hPTH ($P \leq 0.05$).

TABLE I

	hHF cAMP (fmole/mg)	hPTH(1-34) cAMP (fmole/mg)	Forskolin cAMP(fmole/mg)
Control	10226 ± 1108	10226±1108	10226±1108
[Peptide]M			
$1x10^{-9}$	7538 ± 916	6726 ± 211	
$1x10^{-8}$	5713 ± 849	6652 ± 247	
$1x10^{-7}$	6239 ± 461	7087 ± 226	
Forskolin*			113742 ± 792**

Cyclic AMP levels were measured by radioimmunoassay as described in Methods. Protein was measured by the method of Lowry et al (10). *Final concentration of forskolin was 10^{-5}M. Numbers represent the mean of 3 independent determinations ± S.E.M. **$p \leq 0.05$.

Incubation of renal artery ring segments with several concentrations (up to 10^{-7}M) of both hPTH and hHF failed to significantly alter tissue levels of cyclic AMP (Table 1). Forskolin, (10^{-5}M) which is a direct activator of adenylate cyclase was added as a positive control and caused a large significant increase in the levels of cyclic AMP ($P \leq 0.05$).

DISCUSSION

The biochemical similarities between humoral hypercalcemia of malignancy (HHM) and hyperparathyroidism (e.g. 1,3) suggested that a PTH-like factor circulates and is responsible for the HHM syndrome. This was borne out with the recent isolation, purification and molecular cloning (2,4) of the hHF which has significant amino terminal homology with hPTH but, possibly due to structural differences residing in the carboxy terminal portion of hHF does not interact with PTH antisera (e.g. 3). However, the amino terminal homology appears sufficient for hHF to interact with the PTH receptor which is coupled to adenylate cyclase in bone-derived cell lines and renal cortical membranes (4,9).

We have now shown that hHF also exhibits PTH-like activity in relaxing isolated renal arteries (Fig. 1). The hHF was more effective than hPTH at several concentrations and produced almost total relaxation of the contracted arteries which was a significantly larger response than that obtained with hPTH. It is of interest to note that hHF is 6-10 times more effective than

PTH in producing hypercalcemia in thyroparathyroidectomized rats (9). The reason for this enhanced response to hHF in renal arteries is not at present clear since neither peptide caused significant changes in cyclic AMP levels. Therefore, differences in the level of this cyclic nucleotide appear not to be responsible for the observed relaxation. This is in contrast to forskolin which at a concentration of 10^{-5}M elicited an 80-100% relaxation (data not shown) and an approximate 10 fold increase in the level of cyclic AMP (Table I).

As with the results obtained in bone derived cell line assays, we feel that the hHF is eliciting vasorelaxation via a direct interaction with the PTH receptor in vascular tissue. The synthetic hHF used in this study has been shown to possess comparable PTH-like activity in a variety of in vitro and in vivo assays (9). We have not examined the relaxation to hHF in the presence of antagonist analogs of PTH (e.g. PTH (7-34) amide (12)) since these peptides display partial agonist (i.e. relaxation) activity and fail to alter the relaxation to hPTH in renal arteries (Winquist, et al., unpublished observations). The failure of hPTH or hHF to elevate cyclic AMP levels in renal arteries may indicate a different subclass of PTH receptor and/or coupling mechanism in this particular tissue. PTH-induced increases in cyclic AMP in vascular tissue preparations, and vascular smooth muscle cells have been reported by some (6,7) but not all (11) investigators which may indicate regional differences in the mechanism of vascular relaxation induced by this peptide. The relaxation to PTH, and ostensibly to hHF, in renal arteries occurs independent of the particular spasmogen (e.g. methoxamine, serotonin, histamine) utilized and appears to require the presence of an intact endothelium for full efficacy (5).

Our results demonstrate a functional, PTH-like response of hHF in isolated renal arteries. Renal vasodilation if associated with enhanced glomerular filtration in response to hHF as well as to PTH may play an important role in the effects of these peptides on renal function such as either lowering renal phosphate threshold or enhancing phosphate excretion (e.g. 3). Conversely,

the renal vasodilation may be a necessary response to both mitigate any renal vasoconstrictor effect of the calcium load which is reabsorbed and thus ensure delivery of the calcium to systemic sites.

ACKNOWLEDGMENTS

We would like to thank Drs. M. Caulfield, M. Rosenblatt, R. Nutt and Mr. Jay Levy for providing the synthetic hHF (1-34) peptide, Lori-Anne Wassel for amino acid analysis, and Carole Hannan for her competent secretarial support.

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