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# RESPONSE OF HUMAN AND CHICK KIDNEY ADENYLATE CYCLASE TO BIOLOGICALLY ACTIVE SYNTHETIC FRAGMENTS OF HUMAN PARATHYROID HORMONE †

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Summary: The 34-amino acid $NH_2$-terminal fragment of human parathyroid hormone synthesized according to the sequence described by Niall et al. (1) is approximately 140 times more potent than the fragment synthesized according to Brewer et al (2) in activating human renal cortex adenylate cyclase. The potencies of the two peptides, relative to the effect of MRC standard bovine parathyroid hormone preparation 67/342 in this system, were 5600 ± 600 (S.E.M.) units/mg and 40 ± 5 units/mg respectively. The potencies of the more active peptide and the corresponding bovine parathyroid hormone sequence were similar in this system and also in assays based upon the production of cyclic AMP by chick kidney both in vivo and in vitro.

Two sequences have been described for the 34 $NH_2$-terminal amino acid residues of human parathyroid hormone (1,2). The biological activity of intact parathyroid hormone (84 amino acids) can be reproduced by the $NH_2$-terminal one-third of the molecule. Thus it is important to establish the correct amino acid sequence of this fragment, both for the synthesis of biologically active fragments and for the preparation of antisera to the biologically active portion of the hormone molecule.

Parathyroid hormone promotes the urinary excretion of cyclic AMP in man (3) and stimulates the activity of adenylate

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cyclase (ATP-pyrophosphate-lyase (cyclizing), E.C. 4.6.1.1) in particulate preparations of human renal cortex (4,5). By analogy with other species it seems likely that the action of the hormone upon human kidney is mediated, at least in part, via increased intracellular accumulation of cyclic AMP. The effects upon human kidney adenylate cyclase activity of $NH_2$-terminal fragments of human parathyroid hormone prepared according to both reported sequences have not previously been studied and are reported here.

Methods: Tetratriacontapeptide fragments of human parathyroid hormone were gifts of Dr R. Colescott, Armour Pharmaceutical Co., Kankakee, Illinois. The hormone fragment prepared according to the sequence of Brewer et al. (2) (henceforth referred to as "peptide B") was lot no. K691258, and that prepared according to Niall et al. (1) ("peptide N") was lot no. K744206-13. The corresponding synthetic fragment of the bovine parathyroid hormone sequence was a gift of Dr G.W. Tregear, Howard Florey Institute for Experimental Physiology and Medicine, University of Melbourne, Australia. Standard bovine parathyroid hormone preparation 67/342 was obtained from the Division of Biological Standards, Medical Research Council, U.K. Radiochemicals, biochemicals and reagents were from the sources previously reported (6).

Human renal cortex was obtained from the uninvolved tissue surrounding renal cell carcinoma. Histology and ultrastructure were normal (4). After excision the tissue was placed in cold (4°C) 25mmol/l Tris-HCl buffer, pH7.8, containing 130µg bovine serum albumin/ml and transported to the laboratory. Preparation of particulate fractions was completed in less than 2h after excision. The tissue was minced in 25mmol/l Tris-HCl buffer, pH7.8, containing 0.25mol/l sucrose and 1mmol/l EDTA, homogenized (6 strokes, Dounce glass-glass homogenizer with loose-fitting pestle) and transferred into centrifuge tubes. The homogenate was subjected to 2-step differential centrifugation as previously described (6). The particulate preparation was stored as aliquots at -70°C in Tris-Sucrose-EDTA buffer containing 10g dimethylsulfoxide/100ml. The properties of the adenylate cyclase did not change during storage for up to 3 months.

Adenylate cyclase activity was measured by the conversion of [$\alpha$-$^{32}$P] ATP to [$^{32}$P] cyclic AMP as described previously (6). Protein was measured as described (6).

Results: The optimal assay conditions for human renal cortical adenylate cyclase were established. Basal and hormone-stimulated activity were proportional to particulate protein concentration in the range 50-200µg/incubation. Protein concentration was

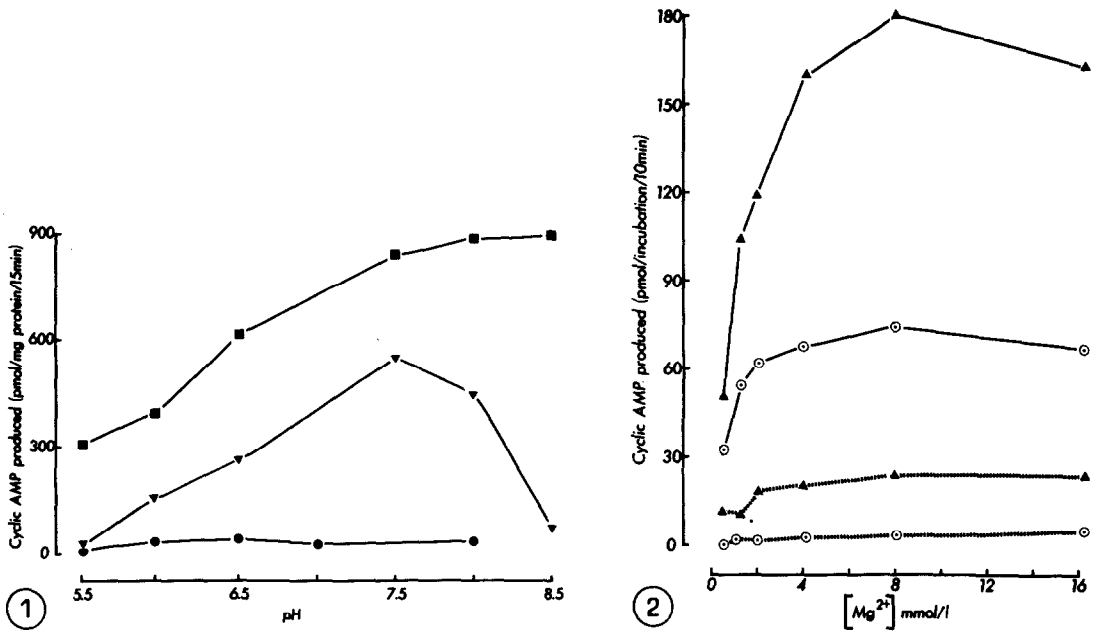


Figure 1. Effect of pH upon adenylate cyclase activity. Adenylate cyclase activity was measured in incubations containing 1mM ATP and 4.5mM $Mg^{2+}$ (see Fig.2) ●, basal activity; ▼, bovine parathyroid hormone (MRC preparation 67/342) 10U/ml; ■, NaF 10mmol/l. Points are means of two determinations.

Figure 2. Effect of ATP and $Mg^{2+}$ concentration on adenylate cyclase activity. Basal activity (broken lines) and bovine parathyroid hormone-stimulated activity (solid lines) in the presence of: ⊙, 0.1mM ATP; ▲, 1mM ATP, with various $Mg^{2+}$ concentrations. The pH of the medium was 7.8 (see Fig.1). Points represent means of 2-3 determinations.

80-150µg in subsequent experiments. Basal and parathyroid hormone-stimulated cyclic AMP accumulation were proportional to time for up to 20 min (data not shown) and therefore subsequent incubations were for 10 or 15 min. Basal adenylate cyclase activity was unaffected by varying medium pH between 6.0 and 8.0, but optimal stimulation by parathyroid hormone was obtained at pH7.5 or 8.0 (Fig. 1). Consequently, buffer pH7.8 was used in later studies. Both basal and hormone-stimulated enzyme activities were influenced by substrate (ATP) and co-factor

Table 1: Potencies of synthetic $NH_2$-terminal peptides of human parathyroid hormone.

$ED_{50}$ and potency (in comparison with MRC bovine parathyroid hormone standard preparation 67/342) were assessed in the human renal cortical adenylate cyclase system. Four effective concentrations of peptides N and B and of the corresponding bovine fragment were assessed in each of three assays. Values are mean with range in parenthesis.

| Hormone | $ED_{50}$ (ng/ml) | Potency (units/mg) |
|---|---|---|
| Peptide N | 66 ( 21 - 120 ) | 5600 (3500 - 9000) |
| Peptide B | 7800 (4800 - 11000) | 40 ( 24 - 48 ) |
| Bovine peptide | 51 ( 36 - 64 ) | 3000 (2700 - 4200) |

($Mg^{2+}$) concentrations (Fig.2). 1mmol/l ATP and 4.5mmol/l $MgSO_4$ were used in subsequent experiments.

Peptides B and N stimulated adenylate cyclase activity in particulate preparations of human renal cortex (Fig.3). The concentrations of the peptides required for half-maximal activation of adenylate cyclase ($ED_{50}$) and their potencies (by comparison with the activity of MRC bovine parathyroid hormone standard preparation 67/342) were determined in three experiments with particulate preparations from two patients (Table 1). Peptide N was approximately 140 times more potent than peptide B and twice as potent as the corresponding fragment of bovine parathyroid hormone.

The potencies of peptide N and the bovine hormone fragment in the chick kidney adenylate cyclase assay (7) were similar, although the maximal enzyme activation produced by peptide N was 20-25% less than that produced by the bovine hormone fragment (Fig.4). Peptide B was approximately 200 times

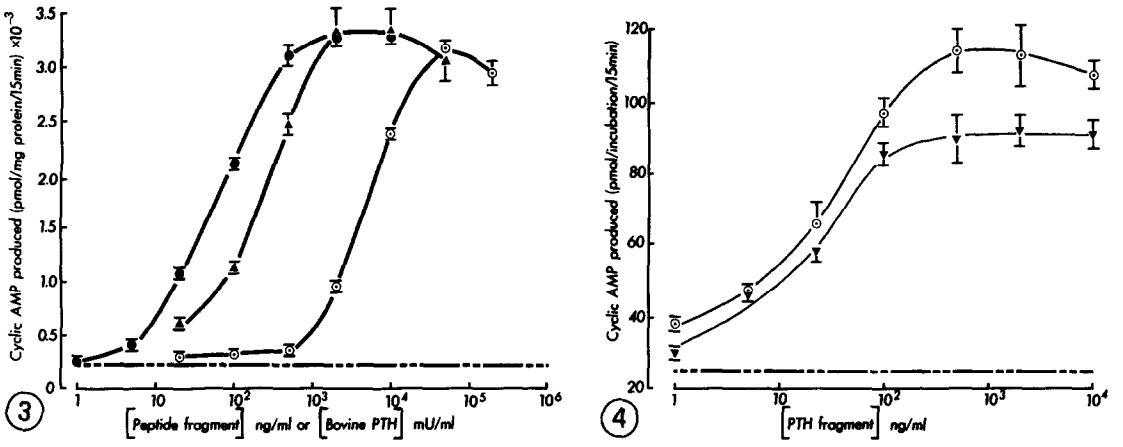


Figure 3. Effects of human parathyroid hormone peptide fragments and bovine parathyroid hormone on human kidney adenylate cyclase activity. ●, peptide N; ⊙, peptide B; ▲, MRC standard bovine parathyroid hormone (PTH) preparation 67/342. Horizontal broken line indicates basal activity. Points and vertical bars are means±S.E.M. from 4 determinations.

Figure 4. Effects of peptide fragments of human and bovine parathyroid hormone (PTH) on chick kidney adenylate cyclase activity. ⊙–⊙, peptide N; ▼–▼, bovine hormone $NH_2$-terminal fragment. Horizontal broken line indicates basal activity. Points and vertical bars are means±S.E.M. from 4 determinations.

less potent, although a full dose-response curve was not examined. The effects of the parathyroid hormone fragments upon chick kidney cyclic AMP content were also studied in vivo, using microwave irradiation for tissue fixation (8). The potencies of peptide N and the bovine fragment were again similar, while peptide B was approximately 100 times less potent.

Discussion: With the exception of that of the rat, mammalian renal cortex adenylate cyclases are generally sensitive to concentrations of parathyroid hormone in the range $10^{-9}$ to $10^{-7}$ mol/l and thus these systems are useful as bioassays for the hormone. The potency of peptide N for activation of human adenylate cyclase is similar to that of the corresponding bovine hormone fragment in the chick (7), rat (7,9) and bovine (V.P.

Michelangeli and N.H. Hunt, unpublished observations) kidney adenylate cyclase systems. Peptide B was much less potent in the human kidney assay, although it behaved as a full agonist (Fig. 3). The full bovine and human hormones are equipotent in activating human kidney adenylate cyclase (5), and the potencies of the $NH_2$-terminal fragment of the bovine hormone and peptide N were similar in the present studies (Table 1). The reported sequences for the $NH_2$-terminal portion of the human hormone differ at positions 22, 28 and 30, which are stated to be glutamic acid, leucine and aspartic acid (1) or glutamine, lysine and leucine (2) respectively. The latter structure contains an extra three nett positive charges within a sequence of nine amino acids and clearly this results in a much reduced potency (Table 1). A synthetic human peptide containing lysine and leucine at positions 28 and 30 has been shown to have greatly reduced potency for activation of bovine and porcine kidney adenylate cyclases (10).

Peptide N and the bovine hormone fragment had similar potencies in *in vivo* and *in vitro* chick kidney assays, confirming previous observations (7). The reportedly lower activity of the human peptide fragment in the chick kidney adenylate cyclase assay (5) presumably is a consequence of the use of peptide B in that study. It should also be emphasized that freshly-prepared chick kidney particulate preparations should always be employed in these assays, as storage may lead to loss of hormone responsiveness (T.J. Martin, unpublished observations).

Although the results do not permit the conclusion that the sequence for human parathyroid hormone reported by Niall *et al.* (1) is correct, nevertheless this seems likely in view of the very low potency of the other reported sequence (2) in a target tissue which is appropriate for the human hormone.

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