# IN VITRO RELATIVE BIOLOGICAL ACTIVITIES OF (1–34) N-TERMINAL SYNTHETIC FRAGMENTS OF HUMAN PARATHYROID HORMONE IN THE HUMAN RENAL CORTICAL ADENYLATE CYCLASE ASSAY

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## 1. Introduction

Two sequences differing in the amino acids at 3 positions have been reported for the (1–34)N-terminal fragment of human parathyroid hormone by Brewer et al. [1,2] and subsequently by Niall et al. [3,4] (fig.1). These two structures have been shown to be biologically active in both in vivo and in vitro assay systems [5–9]. Nevertheless due to the different assay conditions used in these studies, it is at present difficult to assess the relative biological activities of the two sequences. We have reported a comparative study of their relative biological activities in the in vitro renal cortical adenylate cyclase activation assay system [10]. The Niall sequence was shown to be more active than the Brewer sequence on bovine and porcine kidney membranes. Nevertheless this study as well as others [9,11,12] showed also that the biological response depends on the species origin of the membrane. Thus in order to further assess the relative biological activities of these two hormone sequences we studied their relative biological potency in the same, isologous in vitro assay system using human kidney membranes and compared them with a glandular extract of human parathyroid. Our results show unambiguously that the behaviour of the Niall sequence is very close to that of the natural glandular extract and suggest that this sequence is more likely the active natural hormone.

## 2. Materials and methods

### 2.1. *Hormones*

#### 2.1.1. Synthetic fragments

Solid phase synthesis of (1–34)N-terminal fragment of the human parathyroid hormone according to the sequence of Brewer et al., hPTH(1–34)B, and of Niall et al., hPTH(1–34)N1, was carried out by the Merrifield procedure [13]. Benzylhydrylamine

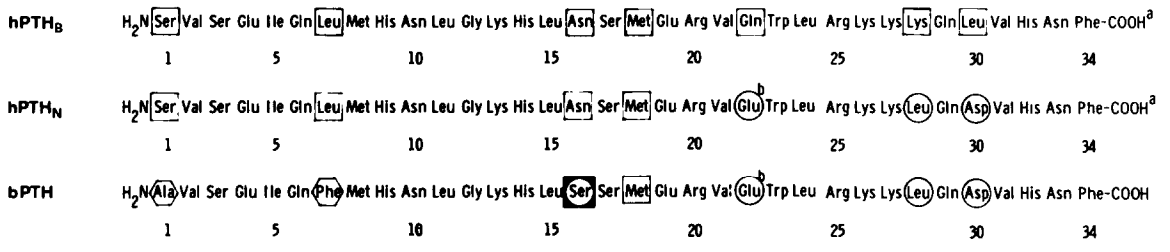


Fig.1. Amino acid sequences of the (1–34)$NH_2$-terminal residues of parathyroid hormone: hPTH$_B$ = human sequence of Brewer et al.; hPTH$_N$ = human sequence of Niall et al.; bPTH = bovine sequence. (a) Our synthesized hPTH$_B$ and hPTH$_N$ were analogues with carboxyl-terminal amide at position 34. (b) Brewer revised sequence· Gln [2].

resin was used giving a terminal amide in the final peptide [14,15]. Details of the synthetic procedure and purification steps have been reported [10]. Fragments were shown to have the theoretical composition by amino acid analysis and the peptides were shown to be homogeneous by complete sequence analysis and repetitive Edman degradation. Purity of the synthesized fragments was estimated to be >80%. In addition a synthetic fragment corresponding to the Niall sequence, hPTH(1–34)N2, was obtained from Armour (lot no. K80017-F). (1–34)N-terminal synthetic fragment of bovine parathyroid hormone, bPTH(1–34)*, was obtained from Beckman-Bioproduct (lot no. 10824).

2.2.2. Native hPTH

Native hPTH was prepared from pooled human parathyroid adenomas obtained from surgical procedures. Due the limited availibility of these tissues, it was not possible to carry out an extraction procedure followed by a complete purification process. Thus we prepared a crude extract containing hPTH. Nevertheless, in order to eliminate as far as possible interference in our assay system by chemical interactions and/or due to the heterogeneity of our preparation which could confuse interpretation of the data, we prepared glandular extracts by two extraction procedures: the urea–HCl method [1], hPTH extract I, and the phenol procedure [16], hPTH extract II.

2.3. *Human cortical kidney adenylate cyclase assay*

Human cortical membranes were isolated from normal human kidneys using the procedure in [10]. The final membrane preparations showed an 8–10-fold increase in the specific activity of adenylate cyclase. Total adenosine triphosphatase activity was enriched 5–7-fold. Results of other enzymatic marker studies specific for various intracellular organelles showed that these were largely eliminated by the purification procedure.

Adenylate cyclase assay was performed using a standard procedure with an ATP regenerating system [17] but with $2.5 \times 10^{-3}$ M ATP and $4.5 \times 10^{-3}$ M $Mg^{2+}$. In order to obtain full activation of adenylate cyclase, $10^{-4}$ M GTP final concentration was added [18] giving a 2-fold increase over the whole range of hPTH concentrations as well as of the basal activity.

Assay of basal and newly formed cyclic AMP was performed by a competitive protein binding assay [19].

Comparisons of hormone products were studied in the same experiments and under identical conditions.

## 3. Results

3.1. *Biological activity of hPTH extract I and II*

The two hormones extracted from glandular tissues give the same dose–response curves (fig.2). Although hPTH extract I gives activation which is slightly higher than obtained with hPTH extract II, fig.2 shows that the 2 extraction procedures give products whose activity is similar. The differences in biological activity could reflect differences in purity of these 2 extracts which are both at only a very early stage in the purificatin procedure. Thus hPTH extract I and II are products which are both acceptable for comparing the potency of hPTH(1–34)B and hPTH(1–34)N.

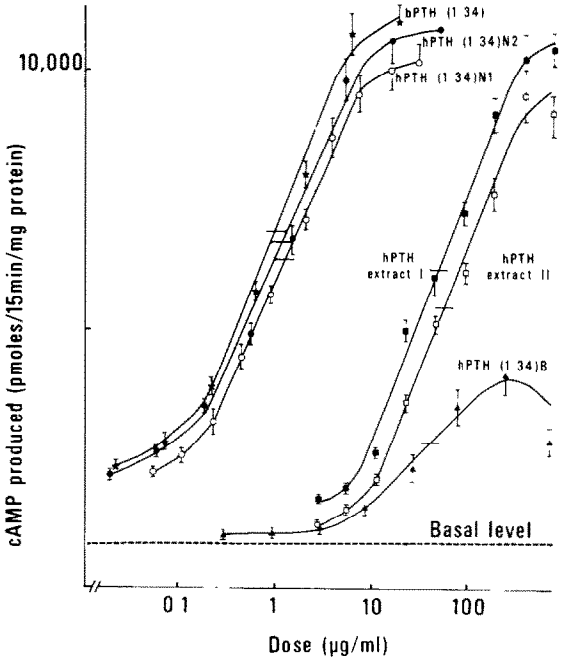


Fig.2. Dose–response curves for hPTH(1–34)B and hPTH(1–34)N in comparison with glandular extracts of hPTH and bPTH(1–34) in the human renal cortical adenylate cyclase assay. Each point represents the mean of 4–6 determinations ± SEM. ⟩——⟨ denotes half-maximal response.

* This fragment contains glutamic acid at sequence position 22

### 3.2. *Relative biological activity of hPTH(1–34)B and hPTH(1–34)N*

hPTH(1–34)N whether synthesized in our laboratory (N1) or obtained from Armour (N2) is able to activate human renal cortical adenylate cyclase and gives the same pattern of activation as hPTH extract I or II (fig.2). Maximal activities are similar (9500–10 500 pmol cyclic AMP produced in 15 min/mg protein) and the linear part of the dose–response curves are parallel.

In the same experimental conditions hPTH(1–34)B also activates human kidney membranes (fig.2). Nevertheless the dose–response curve is not parallel to and the maximal activity is very inferior (4000 ± 150 pmol cyclic AMP produced in 15 min/mg protein) to that obtained with hPTH extracts. To eliminate the possible presence of a contaminant in our hPTH(1–34)B which could act as an inhibitor of the adenylate cyclase activation process, both hPTH(1–34)N and bPTH(1–34) were incubated in the presence and absence of hPTH(1–34)B. The presence of hPTH(1–34)B did not significantly diminish the cyclic AMP produced (table 1).

Half-maximal stimulations (app. $K_m$ values) were, respectively, 1.28 μg/ml ($3.1 \times 10^{-7}$ M) for hPTH(1–34)N1 and 40 μg/ml ($9.8 \times 10^{-6}$ M) for hPTH(1–34)B. Results obtained with hPTH(1–34)N1 and hPTH(1–34)N2 were very similar; the $K_m$ value for N2 was 1.20 μg/ml ($2.9 \times 10^{-7}$ M).

Figure 2 clearly shows that bPTH(1–34) behaves in a manner much more similar to hPTH(1–34)N than to hPTH(1–34)B.

## 4. Conclusions

Previous studies [9–12] have shown that in vitro biological activity as measured by activation of renal cortical adenylate cyclase membranes depends on the species origin of the membranes. In the present study using human kidney membranes and glandular extracts containing hPTH, we show that the Niall sequence is, at least in this in vitro assay system, a product which is more potent and activates human kidney membranes with dose–response curves which are closer to the native product than is the Brewer sequence.

Furthermore bPTH(1–34) and hPTH(1–34)N which differ only in the amino acids in 3 positions, as compared to 6 for hPTH(1–34)B (fig.1), give very similar activation patterns.

Although obviously it cannot be inferred from our studies that the Niall sequence is the 'true' sequence, our results suggest that this sequence is more likely the active natural product. Other results concerning their relative immunological properties support this hypothesis [20]. Nevertheless arguments about the existence of isohormones or the conformational properties of hPTH (discussed [9,20]) cannot be eliminated. It seems, however, that the present available data justify using the Niall sequence rather than the Brewer sequence for evaluating physiological properties of hPTH.

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Table 1
Effect of hPTH(1–34)B on adenylate cyclase activation by hPTH(1–34)N and bPTH(1–34)

| | Adenylate cyclase act. (pmol cAMP/15 min/mg protein) | % |
|---|---|---|
| hPTH(1–34)N (10 μg/ml) | 9860 ± 740 | 100 |
| + hPTH(1–34)B (100 μg/ml) | 10 340 ± 960 | 95–115 |
| + hPTH(1–34)B (300 μg/ml) | 10 170 ± 1050 | 92–113 |
| bPTH(1–34) (10 μg/ml) | 10 520 ± 910 | 100 |
| + hPTH(1–34)B (100 μg/ml) | 11 050 ± 1020 | 90–110 |
| + hPTH(1–34)B (300 μg/ml) | 11 240 ± 1230 | 88–112 |

hPTH(1–34)N and bPTH(1–34) at submaximal concentrations were incubated in the presence of hPTH(1–34)B at 2 concentrations (submaximal and large excess)

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