

Parathyroid hormone-induced lipolysis in human adipose tissue

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Abstract Relative lipolytic activity of human parathyroid hormone-(1-34) (hPTH-(1-34)), hPTH-(3-34), desamino-Ser¹-hPTH-(1-34), and rat PTH-(1-34) was compared in human subcutaneous adipose tissues in vitro. Human PTH-(1-34), rat PTH-(1-34), and desamino-Ser¹-hPTH-(1-34) stimulated in vitro lipolysis significantly above basal level at the concentration of 10⁻⁶ M. Average increments of lipolytic rate were 2.39, 1.82, and 0.87 μ mol/g per 2 hr, respectively, being significantly different among the three groups. On the other hand, hPTH-(3-34)-induced lipolytic rate was 0.83 ± 0.18 μ mol/g per 2 hr, not significantly different from the basal level (0.71 ± 0.20 μ mol/g per 2 hr). The effect of hPTH-(3-34) on glycerol release stimulated by hPTH-(1-34), isoproterenol, or forskolin was subsequently investigated. Human PTH-(3-34) produced a dose-dependent inhibition of hPTH-(1-34)-stimulated lipolysis. In contrast, isoproterenol- and forskolin-induced lipolytic rates were not influenced by hPTH-(3-34). The effect of propranolol on hPTH-(1-34)- or isoproterenol-induced lipolysis was also studied. Propranolol dose-dependently inhibited isoproterenol-induced lipolysis but had no effect on lipolysis stimulated by hPTH-(1-34). These results suggest that the amino acids at positions 1 (serine) and 2 (valine) of PTH are critical for the stimulation of lipolysis in human adipose tissue. Human PTH-(1-34) causes lipolysis after binding to receptors distinct from β -adrenergic receptors of fat cells and possibly hPTH-(3-34) inhibits hPTH-(1-34)-stimulated lipolysis by competing at the level of PTH receptor.—Taniguchi, A., K. Kataoka, T. Kono, F. Oseko, H. Okuda, I. Nagata, and H. Imura. Parathyroid hormone-induced lipolysis in human adipose tissue. *J. Lipid Res.* 1987. 28: 490–494.

Supplementary key words PTH fragment • forskolin • isoproterenol • propranolol

Parathyroid hormone (PTH) is a single-chain polypeptide consisting of 84 amino acids and has many biological effects including induction of lipolysis (1, 2). The lipolytic action as well as the phosphaturic action can be reproduced by the first 34 residues at the amino terminus (1, 2). To our knowledge, however, it is not known whether PTH fragments shorter than the first 34 amino acids cause lipolysis of human adipose tissue. In this experiment, we in-

vestigated the effect of synthetic human PTH fragments, human PTH-(1-34) (hPTH-(1-34)), hPTH-(3-34), desamino-Ser¹-hPTH-(1-34), and rat PTH-(1-34) on glycerol release from human adipose tissue.

Another aim of the present study was to investigate the effect of hPTH-(3-34) on lipolysis induced by hPTH-(1-34), isoproterenol, and forskolin, since PTH-(3-34) was reported to inhibit rat renal cortex adenylate cyclase stimulated by PTH-(1-34) (3, 4) and since PTH-(1-34)-, isoproterenol-, or forskolin-induced lipolysis was postulated to be mediated by the adenylate cyclase system in fat cells (5–7).

MATERIALS AND METHODS

Patients under study were 18 males and 10 females aged 22 to 74 years who underwent abdominal surgery. They had no history or signs of diabetes mellitus, obesity, hypertension, and other endocrine diseases. Informed consent was obtained from each patient. Prior to the surgery, all patients were fasted and did not receive any fluid supplementation overnight. Anesthesia was performed with halothane and nitrous oxide. On the basis of previous reports (8, 9), it was assumed that variations in the duration and depth of anesthesia did not significantly affect the metabolism of fat cells in vitro. Subcutaneous adipose tissues were resected from the epigastric area. We used adipose tissue fragments instead of collagenase-treated fat cells because collagenase itself might alter the hormone responsiveness of the fat-cell membranes (10).

The patients were divided arbitrarily into two groups. One group consisted of 4 males and 4 females aged 22 to

Abbreviation: hPTH, human parathyroid hormone.

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73 years. The other comprised 14 males and 6 females aged 38 to 74 years. Adipose tissues of the former were used for the study of the relative lipolytic activity of hPTH-(1-34), rat PTH-(1-34), desamino-Ser¹-hPTH-(1-34), and hPTH-(3-34). Adipose tissues of the latter group were used for the examination of the effect of hPTH-(3-34) or propranolol on glycerol release stimulated by hPTH-(1-34), isoproterenol, and forskolin. In the present study, the sex of the individual had no influence on either basal or stimulated lipolysis (data not shown). Synthetic hPTH-(1-34), rat PTH-(1-34), desamino-Ser¹-hPTH-(1-34), and hPTH-(3-34) were kindly supplied by Toyo Jozo Co. Ltd., Tokyo, Japan.

Experiment 1

Adipose tissues weighing about 100 mg were preincubated for 10 min at 37°C in 0.75 ml of Krebs-Ringer phosphate buffer, pH 7.4, containing 3% bovine serum albumin and 5.5 mM glucose. Thereafter, hPTH-(1-34), rat PTH-(1-34), desamino-Ser¹-hPTH-(1-34), or hPTH-(3-34) dissolved in the same buffer was added to the incubation test tubes and incubated for 2 hr. The final concentrations of the peptides were 10⁻⁶M.

Experiment 2

The method of preincubation was the same as described in experiment 1 except for the addition of zero to 10⁻⁵ M hPTH-(3-34) or zero to 10⁻⁵ M propranolol. Thereafter,

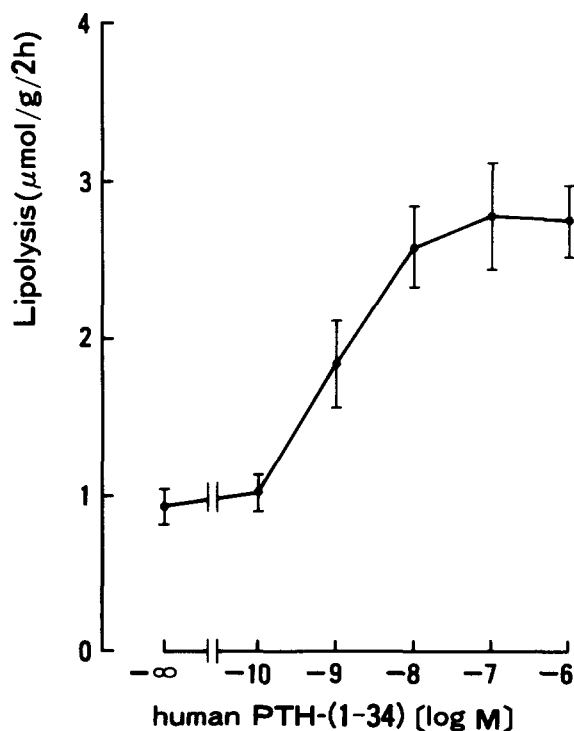


Fig. 1. Effects of human PTH-(1-34) on lipolysis in human adipose tissues. The ordinate shows the amount of glycerol released. Vertical bars represent mean \pm SEM of six experiments.

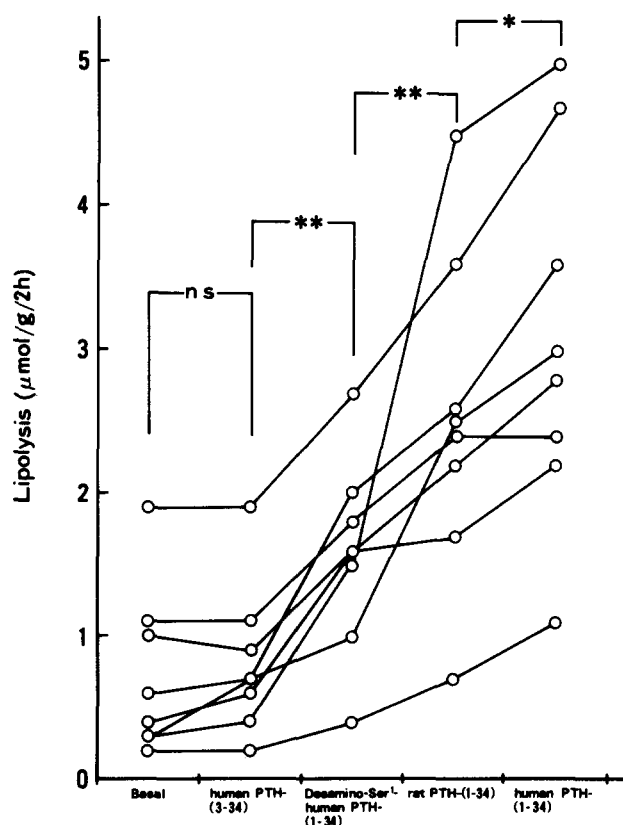


Fig. 2. Effects of human PTH-(3-34), desamino-Ser¹-human PTH-(1-34), rat PTH-(1-34), and human PTH-(3-34) on lipolysis in eight human adipose tissues. * $P < 0.02$; ** $P < 0.01$; n s, not significant.

10⁻⁸ M hPTH-(1-34), 9.4×10^{-7} M isoproterenol or 10⁻⁴M forskolin was added to the incubation mixtures and the test tubes were incubated for 2 hr.

Reactions were stopped by placing the samples in an ice bath and an aliquot of the medium was taken for the measurement of glycerol (11). Glycerol release was linear for at least 3 hr after 10 min preincubation time. Basal rates of lipolysis were defined as glycerol release in the absence of hormone.

Statistical analysis

Data are shown as mean \pm SEM. A nonparametric test (Wilcoxon's sign rank test) was used for statistical analysis, taking a value of $P < 0.05$ as significant.

RESULTS

The addition of hPTH-(1-34) significantly stimulated lipolysis in human subcutaneous adipose tissues at a concentration of 10⁻⁹ M ($P < 0.05$) and the maximum lipolytic rate was attained at a concentration of 10⁻⁸ to 10⁻⁶ M (Fig. 1). Isoproterenol caused lipolysis maximally at a concentration of 9.4×10^{-7} M in accord with our previous report (11). In contrast, the maximum lipolytic rate stimulated by forskolin was observed at a concentration of

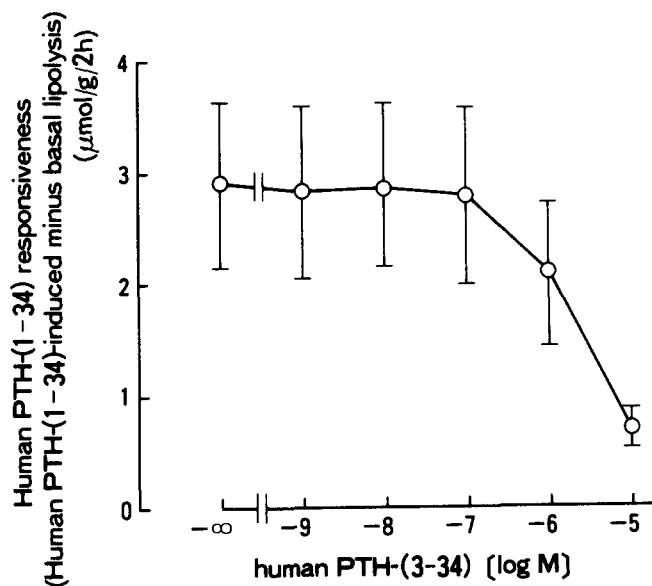


Fig. 3. Effects of human PTH-(3-34) on the responsiveness of human adipose tissues to 10^{-8} M human PTH-(1-34). Vertical bars represent mean \pm SEM of four experiments. The ordinate represents increment of lipolysis over basal levels.

10^{-4} M (data not shown). Hence, we used 10^{-8} M or 10^{-6} M hPTH-(1-34), 9.4×10^{-7} M isoproterenol, or 10^{-4} M forskolin in the following experiments.

Fig. 2 shows hPTH-(3-34)-, desamino-Ser¹-hPTH-(1-34)-, rat PTH-(1-34)-, hPTH-(1-34)-induced, and basal

lipolytic rates in eight human subcutaneous adipose tissues at the final concentrations of 10^{-6} M of the peptides. Lipolytic rates as expressed by glycerol released were 0.81 ± 0.18 , 1.58 ± 0.24 , 2.53 ± 0.41 , 3.10 ± 0.46 and 0.73 ± 0.21 $\mu\text{mol/g}$ per 2 hr, respectively. HPTH-(3-34) did not significantly stimulate lipolysis above basal level, although there was a tendency for slight lipolytic effect. On the other hand, desamino-Ser¹-hPTH-(1-34), rat PTH-(1-34), and hPTH-(1-34) stimulated lipolysis significantly. Average increments of lipolytic rate were 0.85, 1.80 and 2.37 $\mu\text{mol/g}$ per 2 hr, respectively.

Fig. 3 depicts the effect of various doses of hPTH-(3-34) (0 to 10^{-5} M) on glycerol release stimulated by 10^{-8} M hPTH-(1-34) in four human subcutaneous adipose tissues. HPTH-(1-34) responsiveness was defined as glycerol release in the presence of hPTH-(1-34) minus basal glycerol release. HPTH-(3-34) dose-dependently inhibited hPTH-(1-34) responsiveness and a half-maximum inhibition was observed at about 5×10^{-6} M added hPTH-(3-34).

Next, the effect of 10^{-6} M hPTH-(3-34) on lipolysis stimulated by hPTH-(1-34), isoproterenol, and forskolin was investigated (Fig. 4). The responsiveness to isoproterenol or forskolin was defined as glycerol release in the presence of isoproterenol or forskolin minus basal glycerol release. The responsiveness to hPTH-(1-34) was 2.91 ± 0.51 $\mu\text{mol/g}$ per 2 hr, which was significantly inhibited to 2.12 ± 0.49 $\mu\text{mol/g}$ per 2 hr by 10^{-6} M hPTH-(3-34) ($P < 0.01$). The responsiveness to isoproterenol in the

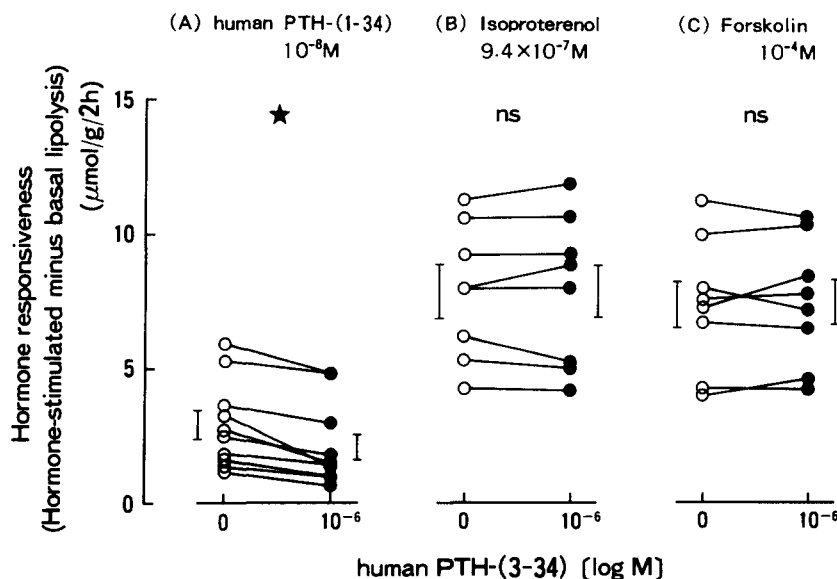


Fig. 4. Effects of 10^{-6} M human PTH-(3-34) on the responsiveness of human adipose tissues to 10^{-8} M human PTH-(1-34) (A), 9.4×10^{-7} M isoproterenol (B), and 10^{-4} M forskolin (C). The ordinate represents increment of lipolysis over basal levels. Open and closed circles represent mean of each individual in the absence and presence of 10^{-6} M PTH-(3-34), respectively. Lines connecting circles represent values in each subject. Vertical bars represent mean \pm SEM of each group. * $P < 0.01$, n s, not significant.

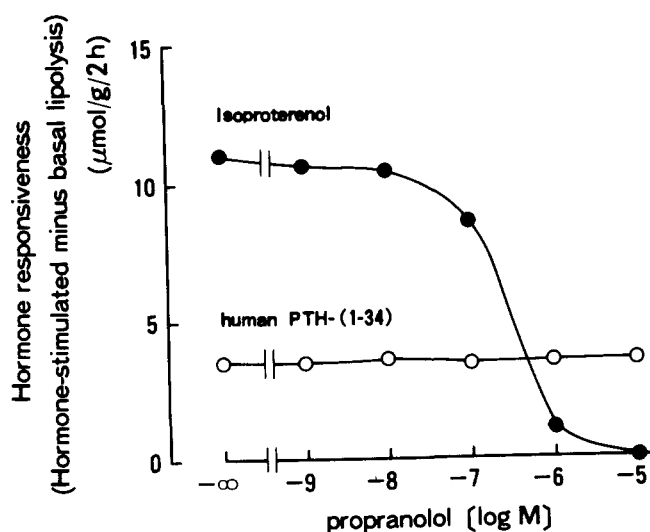


Fig. 5. Effects of propranolol on the responsiveness of human adipose tissues to 9.4×10^{-7} M isoproterenol (●) and 10^{-8} M human PTH-(1-34) (○). The ordinate represents increment of lipolysis over basal level. Each point is the mean of two experiments.

absence and presence of 10^{-6} M hPTH-(3-34) was 7.84 ± 1.01 and 7.90 ± 0.99 $\mu\text{mol/g}$ per 2 hr, respectively. The responsiveness to forskolin without and with added 10^{-6} M hPTH-(3-34) was 7.41 ± 0.89 and 7.55 ± 0.83 $\mu\text{mol/g}$ per 2 hr, respectively. The responsiveness to isoproterenol and forskolin was not influenced by 10^{-6} M hPTH-(3-34).

Finally, the effect of propranolol on isoproterenol- or hPTH-(1-34)-induced lipolysis was examined (Fig. 5). Propranolol dose-dependently inhibited the responsiveness to isoproterenol, and complete inhibition was achieved at 10^{-5} M added propranolol. In contrast, the responsiveness to hPTH-(1-34) was not altered by propranolol. Propranolol had no effect on basal lipolysis (data not shown).

DISCUSSION

The present study demonstrated that synthetic hPTH-(1-34) significantly stimulated lipolysis in human adipose tissues in vitro, whereas hPTH-(3-34) had no significant effect. This indicates that amino acids at positions 1 (serine) and 2 (valine) are critical for the stimulation of lipolysis in human adipose tissue. PTH is known to activate the adenylate cyclase system of the fat cell (5). Hence, it is possible that the presence of the first two amino acids is important for the stimulation of the adenylate cyclase system. This observation coincides with previous reports (3, 4) that the deletion of the two amino-terminal amino acids of PTH completely abolished the stimulatory effect on rat renal cortex adenylate cyclase system in vitro. It is not known which of the two amino

acids is more important in causing PTH-induced lipolysis in human adipose tissues. In our study, desamino-Ser¹-hPTH-(1-34) retained 50% of lipolytic activity of hPTH-(1-34); rat PTH-(1-34), whose amino acids are alanine (position 1) and valine (position 2), had 80% of lipolytic activity of hPTH-(1-34) when compared at the concentration of 10^{-6} M. From these results, it can be speculated that valine (position 2) is more important in causing PTH-induced lipolysis in human adipose tissue than serine (position 1), although further studies with hPTH-(2-34) or desamino-Val²-hPTH-(1-34) are required to settle this issue.

Propranolol inhibited isoproterenol-induced lipolysis but had no effect on hPTH-(1-34)-induced lipolysis, suggesting that hPTH-(1-34) causes lipolysis after binding to receptors distinct from β -adrenergic receptors. This observation coincides with a previous report (5) that PTH could activate the adenylate cyclase system of human fat cell ghosts via binding to an individual receptor separable from adrenergic receptor sites.

It is also well known that PTH, when the first two amino acids are removed, produces an inhibitory effect on the rat renal cortex adenylate cyclase system stimulated by hPTH-(1-34) (3, 4). Our present study demonstrated that the inhibitory effect of hPTH-(3-34) was observed also in lipolysis stimulated by hPTH-(1-34), and that a half-maximum inhibition was achieved at about 5×10^{-6} M added hPTH-(3-34), a concentration 500 times greater than that of hPTH-(1-34). This is very interesting because it has been reported that the concentration of PTH-(3-34) needed for 50% inhibition of PTH-(1-34) action is 300 times greater than that of PTH-(1-34) in kidney (12, 13).

The mechanism by which hPTH-(3-34) produces an inhibitory effect on hPTH-(1-34)-stimulated lipolysis is not known at present, but it is considered to be competitive inhibition at the PTH receptor, since isoproterenol- and forskolin-induced lipolytic rates were not influenced by hPTH-(3-34) and it is suggested that PTH causes lipolysis after binding to receptors distinct from β -adrenergic receptors. [14]

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