# Human Parathyroid Hormone (1-34) Transiently Increases the Excretion of Lysosomal Enzymes into Urine and the Size of Renal Lysosomes

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**It has been reported that the urinary excretion of N-acetyl-β-D-glucosaminidase (NAG), a lysosomal enzyme, transiently increases in human after treatment with human parathyroid hormone (hPTH)(1-34). We report here that hPTH(1-34) caused transient changes in the size and density of rat renal lysosomes following urinary excretion of NAG and other lysosomal enzymes tested. Percoll density gradient centrifugation revealed that hPTH(1-34) slightly but significantly increased the fraction of high density lysosomes (around 1.12 g/ml) 5-10 min after the treatment with hPTH(1-34), with a concomitant decrease in the fraction of intermediate density lysosomes (1.07-1.08 g/ml). On electron micrographs, some lysosomes in proximal tubules but not in distal tubules showed a change in morphology from circular to oval, and became enlarged and electron-dense 5-10 min after the treatment with hPTH(1-34). These responses to hPTH(1-34) were also reversible and transient. NAG excreted in urine after treatment with hPTH(1-34) had the molecular mass of a mature form in lysosomes and/or endosomes and was not a prepro- and/or pro-form of the enzyme. Thus, the changes in the density and size of renal lysosomes appear to be associated with the exocytosis of lysosomal enzymes by hPTH(1-34).**

**Key words: *N*-acetyl-β-D-glucosaminidase, cathepsins, lysosome, parathyroid hormone, urinary excretion.**

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The kidney is the major target of parathyroid hormone (PTH), and PTH increases the urinary excretion of calcium, phosphate, hydrogen ions and cAMP (*1*). In clinical studies, Mizunashi *et al.* found that infusion of human PTH (hPTH)-(1-34) transiently increases the urinary excretion of *N*-acetyl-β-D-glucosaminidase (NAG), a lysosomal enzyme, in normal subjects and idiopathic hypoparathyroidism patients, but not in pseudohypoparathyroidism type I patients (*2, 3*). We recently found similar effects of hPTH(1-34) in normal rats, and revealed that hPTH(1-34) transiently increases the urinary excretion of not only NAG but also various lysosomal enzymes, such as β-glucuronidase and acid phosphatase (ACP), but not the excretion of tubular brush border enzymes. These results suggest that the urinary excretion of lysosomal enzymes by hPTH(1-34) is a scope of physiological response in renal tubules, and is not caused by nephrotoxicity of hPTH(1-34). These *in vivo* results are supported by the finding *in vitro* that PTH enhances the release of lysosomal enzymes from isolated intestinal targeting cells, but not from non-targeting lung cells (*4*). However, the mechanisms underlying the urinary excretion of lysosomal enzymes caused by hPTH(1-34) and the effects of hPTH(1-34) on intracellular organelles have not been elucidated.

Lysosomes in normal rat kidney cells, non-secretory cells, have the properties of exocytotic vesicles like granules in secretory cells (*5*). In activated mast cells, the granules swell, fuse with each other, protrude from the cell surface during the process of degranulation and then are released into the extracellular milieu (*6, 7*). From these data, we speculate that hPTH(1-34) induces changes in the density and/or morphology of intracellular organelles, especially lysosomes, before the excretion of lysosomal enzymes in urine. In this paper, we demonstrated for the first time changes in the density and morphology of lysosomes in proximal tubules of the rat kidney after treatment with hPTH(1-34).

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Abbreviations: ABC, avidin-biotinylated enzyme complex; ACP, acid phosphatase; MCA, 4-methyl-coumaryl-7-amide; MCP, *m*-cresolsulfon-phthaleinyl-; NAG, *N*-acetyl-β-D-glucosaminidase; OK, opossum kidney; PO, horseradish peroxidase; PTH, parathyroid hormone; T-PBS, Tween-20 containing phosphate-buffered saline; Z, carbobenzoxy-.

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## MATERIALS AND METHODS

*Materials*—The active fragment of hPTH, hPTH(1-34) [bioactivity of 33,000 units/mg in an adenylate cyclase assay involving rat renal cortical membranes (*8*)], and the C-terminal peptide of the mouse NAG $\alpha$-subunit ($NH_2$-Ala-Gln-Pro-Ile-Ser-Val-Gly-Tyr-Cys-Glu-Gln-Glu-Phe-Glu-Gln-Thr-COOH) were synthesized by means of solid-phase procedures by Asahi Chemical Industry (Shizuoka). Affinity-purified biotin-labeled $F(ab')_2$ goat anti-rabbit IgG Fc fragment and avidin-biotinylated enzyme complex-conjugated horseradish peroxidase (ABC-PO) were purchased from Jackson Immunoresearch Laboratories (West Grove, PA) and Vector Laboratories (Burlingame, CA), respectively. Percoll and density marker beads were purchased from Pharmacia (Uppsala, Sweden). Carbobenzoxy (Z)-Arg-Arg-4-methyl-coumaryl-7-amide (MCA) and Z-Phe-Arg-MCA were purchased from Peptide Institute (Osaka). 4-Nitrotetrazolium violet was purchased from Nacalai Tesque (Kyoto). Most other chemicals were obtained from Wako Pure Chemical Industries (Osaka).

*Animals*—Eight-week-old male Wistar rats were purchased from the Shizuoka Laboratory Animal Center (SLC, Shizuoka). The animals were housed under a 12 h light-dark cycle at a room temperature of $24\pm1$°C and $50\pm10$% humidity, and fed a standard chow (CE-2, CLEA Japan, Tokyo) with tap water *ad libitum*. Experiments were performed at the age of 10 weeks.

*Measurements of Enzyme Activities and Urine Parameters*—Creatinine was measured using CRE-EN KAINOS® reagent (Kainos Laboratories, Tokyo). NAG activity was measured by a spectrophotometric method (*9*) with *m*-cresolsulfonphthaleinyl *N*-acetyl-$\beta$-D-glucosamine (MCP-NAG) as a substrate (NAG test Shionogi®; Shionogi Pharmaceuticals, Osaka). ACP was measured by the method of Kind and King (*10*). Cathepsins were measured by the method of Barrett and Kirschke (*11*) with Z-Phe-Arg-MCA and Z-Arg-Arg-MCA as substrates for cathepsin L and cathepsin B, respectively. Succinate reductase was measured as reported (*12*) using 4-nitrotetrazolium violet as a substrate. Alkaline phosphatase (ALP), leucine aminopeptidase (LAP), and $\gamma$-glutamyl transpeptidase ($\gamma$-GTP) were measured by use of an autoanalyzer (Hitachi 7070, Hitachi, Tokyo). One unit of enzyme activity was defined as the amount required to catalyze 1 $\mu$mol of substrate per minute at 37°C. To standardize the difference in urine volume, the urinary excretion of enzymes was expressed as units per gram creatinine (U/g Cr). Protein concentrations were measured with the bicinchoninic acid protein assay reagent (Pierce Chemical, Rockford, IL) according to Smith *et al.* (*13*) with bovine serum albumin (BSA) as a standard.

*In Vivo Perfusion of Rats at Awake*—*In vivo* perfusion of rats was performed by the method of Yamamoto *et al.* (*14*) with a minor modification. Briefly, rats were anesthetized with an intraperitoneal injection of sodium pentobarbital (Nembutal®, Dainabot, Chicago, IL) at the dose of 40 mg/kg body weight, and the left femoral vein and urinary bladder were cannulated for the purposes of infusion and urine collection, respectively. The rats were transfered to Bollman cages (KN-326 type-3; Natsume, Tokyo), and a sterilized nutrient solution (20 mM NaCl, 5 mM $MgCl_2$, 2.5 mM KCl, 1.25 mM $CaCl_2$, and 4% glucose) was infused at the constant rate of 3 ml/h. After infusion for 16 h, urine samples were collected every 30 min for 3 h. Human PTH(1-34) in 200 $\mu$l of the nutrient solution containing 0.1% BSA per 250 g body weight was intravenously infused at a dose of 20 U/kg for 3 min following the second urine collection. The urine samples collected were stored at 4°C, and the enzyme activities of NAG, ACP, cathepsin L, and cathepsin B and the amounts of creatinine were measured within 2 days.

*Analysis of the Density of Lysosomes in Rat Kidney*—A mitochondrial-lysosomal fraction was prepared by the method of Ohshita and Kido (*15*) with a minor modification. Briefly, rats were fasted for 16 h prior to the experiment, then injected with hPTH(1-34) *via* a tail vein. After 5, 10, 20, or 30 min, the abdomen was opened under pentobarbital anesthesia, and the kidneys were excised and rinsed several times with ice-cold 0.25 M sucrose. The cortices were separated from the medullae, and each pair of cortices was suspended in 10 ml of 0.25 M sucrose, then mildly disrupted with a Potter-type Teflon homogenizer. The homogenate was centrifuged twice at $540\times g$ for 10 min at 4°C to remove blood cells, and the supernatant (post-nuclear supernatant) was centrifuged at $18,000\times g$ for 20 min at 4°C. The precipitated mitochondria-lysosome fraction was carefully suspended in 24 ml of 40% iso-osmotic Percoll and centrifuged at $93,000\times g$ for 60 min at 4°C. After centrifugation, fractions of 1 ml were collected from the bottom of the tube for measurement of the activities of NAG, ACP, cathepsin L, cathepsin B, and succinate reductase. The density of each fraction was calibrated using density marker beads.

*Electron Microscopy*—Kidneys from rats fasted for 16 h were excised 5, 10, 20, and 30 min after the injection of hPTH(1-34) under pentobarbital anesthesia. Renal cortices were immediately cut into small pieces and prefixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2, at 4°C. One hour later, they were rinsed several times with 0.2 M phosphate buffer, pH 7.2, then fixed with 1% osmium tetroxide in 0.2 M phosphate buffer, pH 7.2, for 1 h at room temperature. After fixation, the tissues were dehydrated through an ascending ethanol series and embedded in EPON 812. Ultrathin sections were cut with an ultramicrotome (Reichert-Jung, Nussloch, Germany) and stained with uranyl acetate and lead citrate. Electron micrographs were obtained using a JEM-1200EXII microscope (Japan Electronics, Tokyo).

For determination of the size of lysosomes in proximal tubules, electron micrographs were taken at a magnification of 3,000× and developed to a final magnification of 6,000×. Five to seven fields in a micrograph of tubules were selected at random, and the lengths and widths of all lysosomes in the fields were measured. Small granules of less than 2 mm in diameter in the developed micrographs were excluded, since it was difficult to determine from their morphology whether they were lysosomes or not.

*Preparation of Anti-Mouse NAG α-Subunit Antibodies*—In this study, the C-terminal 16 amino acid peptide (*16*) of the mouse NAG $\alpha$-subunit ($NH_2$-Ala-Gln-Pro-Ile-Ser-Val-Gly-Tyr-Cys-Glu-Gln-Glu-Phe-Glu-Gln-Thr-COOH, purity greater than 90%) was synthesized, because the amino acid sequence of rat NAG was not available at the time of experiment. Antiserum against the peptide was prepared by the method of Kominami and Katunuma (*17*).

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Antigen affinity-purified IgG was prepared with a column of peptide-coupled Affigel 15 (Pierce, Rockford, IL) according to the manufacturer's instructions.

*Electrophoresis and Western Immunoblotting*—Aliquots of urine in 10% trichloroacetic acid were centrifuged at 10,000 × *g* for 10 min at 4°C. The precipitates were washed two times with cold acetone at −20°C, dried, and subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) by the method of Laemmli (*18*). SDS-PAGE mid-range standards (Daiichi Pure Chemicals, Tokyo) were used as molecular weight markers. For Western immunoblotting analysis, proteins were transferred electrophoretically to a nitrocellulose membrane (Nitropure®; Funakoshi, Tokyo). The membrane was soaked in 5% skim milk in 0.05% Tween-20 containing phosphate-buffered saline, pH 7.4 (T-PBS), for 2 h to block nonspecific binding, then reacted with 10 μg/ml of anti-NAG antibodies in 3% skim milk in T-PBS for 15 h at 4°C. After washing with T-PBS, it was further reacted with biotinylated anti-rabbit IgG Fc specific antibodies for 2 h, then with ABC-PO in T-PBS for 50 min at room temperature. The bound antibodies were detected with ECL Western blotting detection reagents (Amersham, Buckinghamshire, UK) according to the manufacturer's instructions. The intensities of the bands were measured with image analysis software, NIH Image version 1.47.

*Statistical Analysis*—All results are expressed as means ± SD. Differences between before and after drug treatment were analyzed by a paired *t*-test. Differences between two groups were analyzed by an unpaired *t*-test or Aspin-Welch's *t*-test. Differences among multiple groups were analyzed by a Dunnett test. A *p* value of less than 0.05 was considered significant.

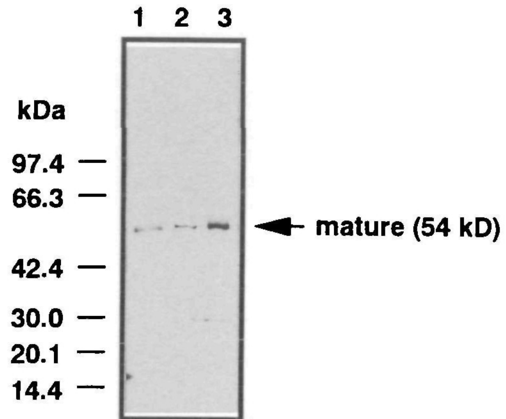


Fig. 3. **Western immunoblotting of NAG in urine.** Rat renal lysosomes (0.36 μg) (lane 1), urine samples (440 μg of protein) before (lane 2), and 30 min after (lane 3) infusion of hPTH(1-34) were subjected to SDS-PAGE followed by Western immunoblotting.

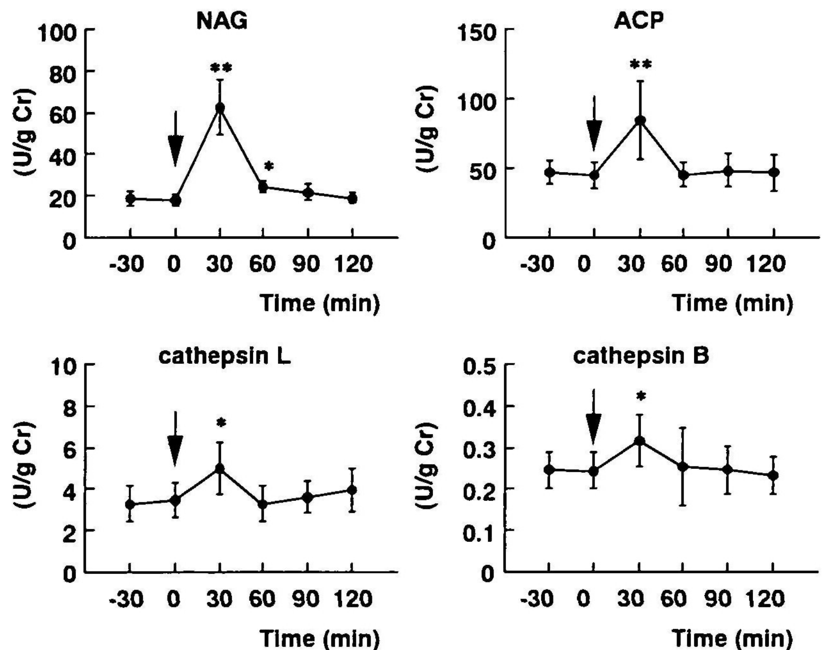


Fig. 1. **Effect of hPTH(1-34) on urinary excretion of lysosomal enzymes in rats.** Urine was collected every 30 min before and after infusion of hPTH(1-34), which is indicated by an arrow. The results are presented as means ± SD ($n=8$). *,** $p<0.05$, 0.01 *vs.* before infusion of hPTH(1-34), respectively.

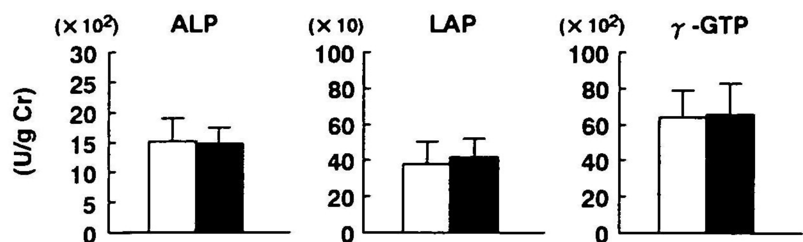


Fig. 2. **Effect of hPTH(1-34) on urinary excretion of the enzymes in brush border membranes.** The results are presented as mean ± SD ($n=8$). Open columns: before infusion of hPTH(1-34); closed columns: 30 min after infusion of hPTH(1-34).



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## RESULTS

*Effect of hPTH(1-34) on Urinary Excretion of Lysosomal Enzymes*—We studied the effect of hPTH(1-34) on urinary excretion of the abundant lysosomal enzymes, such as NAG, ACP, cathepsin L, and cathepsin B, in normal rat kidney. As shown in Fig. 1, limited amounts of all lysosomal enzymes tested were constitutively detected in urine, and hPTH(1-34) increased the urinary excretion of these lysosomal enzymes. The relative increase in the levels of NAG, ACP, cathepsin L, and cathepsin B by hPTH(1-34) was 3.0-, 1.8-, 1.3-, and 1.4-fold, respectively. Induction of urinary excretion of all these lysosomal enzymes was observed within the first 30 min after infusion of hPTH(1-34), and the values returned to the basal levels by 60 min (Fig. 1). On the other hand, urinary excretion of non-lysosomal enzymes, namely, alkaline phosphatase, leucine

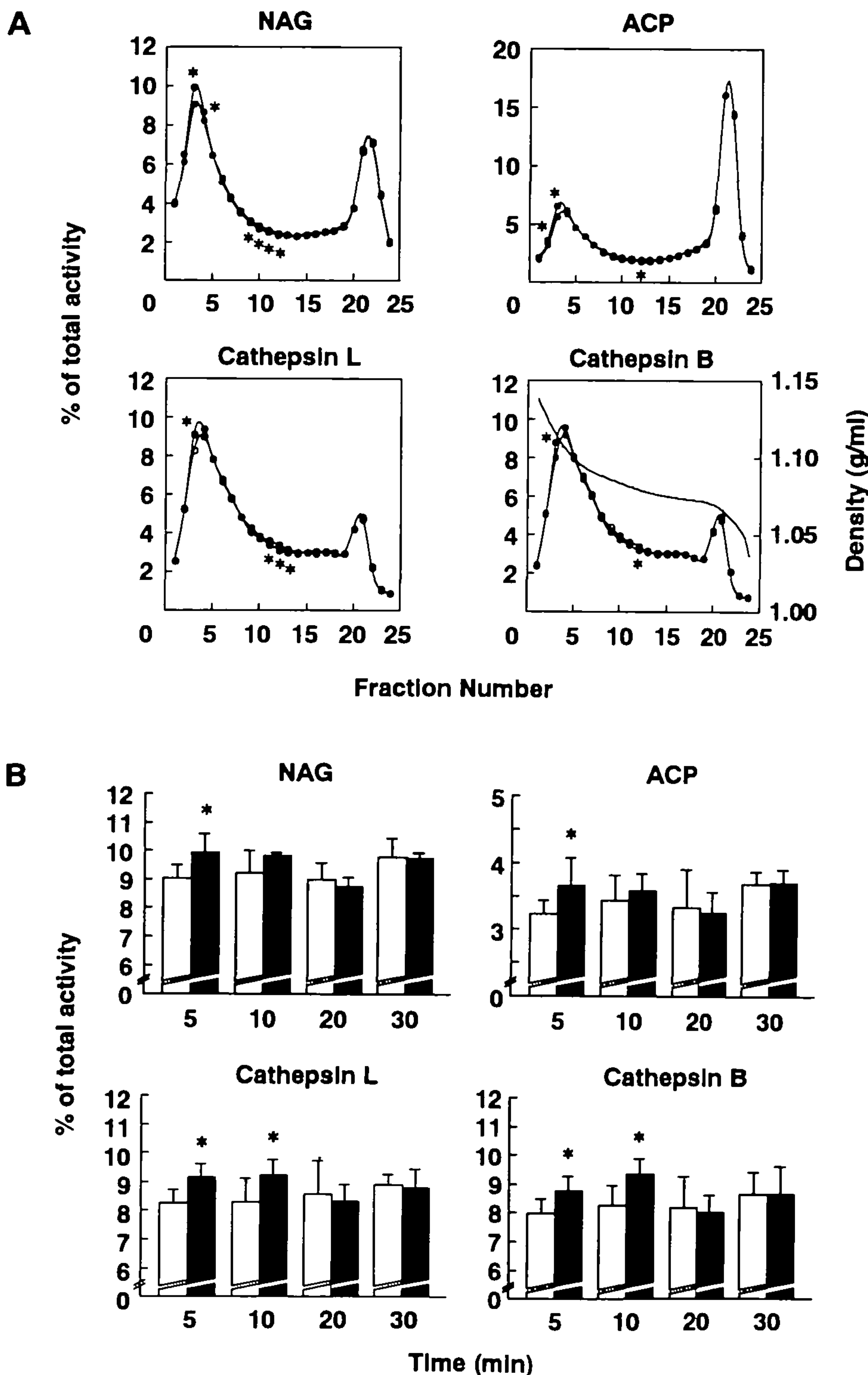


Fig. 4. **Effect of hPTH(1-34) on the density distribution of renal lysosomes.** A: Density distribution of renal lysosomes was determined by Percoll density gradient centrifugation as described under "MATERIALS AND METHODS." Results are means for six vehicle-treated (open circles) and six hPTH(1-34)-treated (closed circles) rats, 5 min after injection. B: Time course of distribution of high density lysosomes (NAG and cathepsins: fraction No 3; ACP: fraction No 2). Open columns: vehicle treatment group; closed columns: hPTH(1-34) treatment group. *$p<0.05$ *vs.* vehicle treatment group.

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aminopeptidase, and γ-glutamyl transpeptidase in brush border membranes, did not change (Fig. 2). Human PTH(1-34) at the dose used in this experiment did not affect the blood creatinine level or the amount of urinary creatinine excreted at any time during the experimental period (data not shown).

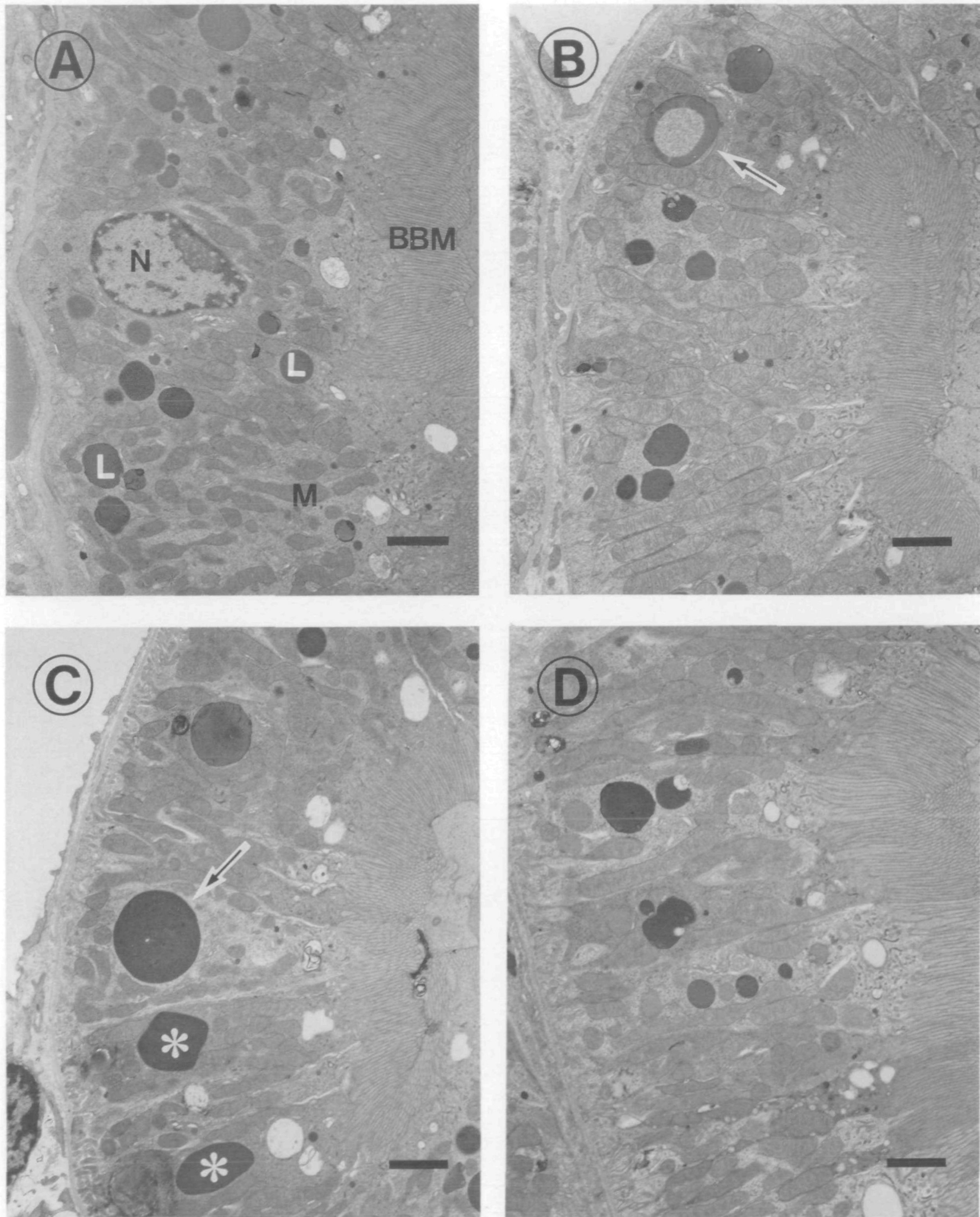


Fig. 5. **Morphology of proximal tubular cells.** Electron micrographs of proximal tubules 5 min after injection of vehicle (A) and 5 (B), 10 (C), and 20 min (D) after injection of hPTH(1-34). Large, nearly circular lysosomes with high and low densities (arrows), and oval lysosomes (asterisks) were observed 5-10 min after treatment with hPTH(1-34). BBM: brush border membrane; L: lysosome; M: mitochondrion; N: nucleus. Bars, 2 μm.

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*NAG Is Excreted in Urine in a Mature Form*—The precursors of lysosomal proteins are processed during trafficking through the endoplasmic reticulum and Golgi apparatus and finally converted to the mature forms in acidic organelles, *i.e.*, endosomes/lysosomes (*19*). To determine which organelle or traffic process is affected by hPTH(1-34), resulting in the urinary excretion of lysosomal enzymes, we determined the molecular mass of NAG in urine by means of SDS-PAGE and Western immunoblotting. The molecular masses of the protein band of NAG in urine before and after hPTH(1-34) infusion were both 54 kDa (Fig. 3), which is identical to that of the mature form of NAG in rat renal lysosomes, mouse macrophages (*20*), and human fibroblasts (*21*). The intensity of the NAG band was higher after hPTH(1-34) infusion than before infusion. These results suggest that excretion into urine of the mature form of NAG and, presumably, that of the mature forms of other enzymes in the endosomes/lysosomes is stimulated by hPTH(1-34).

*Slight but Significant Changes in the Density of Renal Lysosomes*—It is unclear what cellular events underlie the increased urinary excretion of lysosomal enzymes in response to hPTH(1-34). Thus, we first examined the effect of hPTH(1-34) on the density of renal lysosomes by Percoll density gradient centrifugation. The density distribution of lysosomes was determined as those of the lysosomal marker enzymes, NAG, ACP, cathepsin L, and cathepsin B. As shown in Fig. 4A, a broad density distribution of renal lysosomes (1.04-1.14 g/ml), with two peaks at densities around 1.12 and 1.06 g/ml, was observed, and each enzyme was distributed in lysosomes with various densities. NAG and cathepsins were more frequent in the higher density fractions of lysosomes, while the distribution of ACP was greater in the lower density fractions. These data are consistent with those reported previously (*15*). The difference in the distribution of these lysosomal enzymes might reflect the heterogeneity of lysosomes. In the vehicle-treated group, the distribution of lysosomal density was unchanged during the experimental periods. When hPTH(1-34) was administered intravenously, the fraction of heavy lysosomes at the density of around 1.12 g/ml increased slightly but significantly 5-10 min after the administration, with a concomitant and slight decrease in the fractions of lysosomes of intermediate density (1.07-1.08 g/ml) ($n=6$) (Fig. 4A). The change in the density distribution of lysosomes caused by hPTH(1-34) was transient and was found to be similar on analysis of the four different lysosomal marker enzymes. This change completely disappeared 20 min after the administration of hPTH(1-34) (Fig. 4B). Human PTH(1-34), however, did not affect the densities of mitochondria at any experimental time, as determined from the distribution of the activity of a mitochondrial marker enzyme, succinate reductase (data not shown).

*Changes in the Size of Tubular Lysosomes*—Since the change in lysosomal density caused by hPTH(1-34) may be associated with a change in their morphology, we next examined morphology of the lysosomes and other intracellular organelles of proximal and distal tubules in the kidneys of rats 5, 10, and 20 min after the administration of hPTH(1-34). Light microscopic analyses of the glomerular and tubular structures of rat kidney revealed no morphological changes in these tissues between 5 min and 2 h

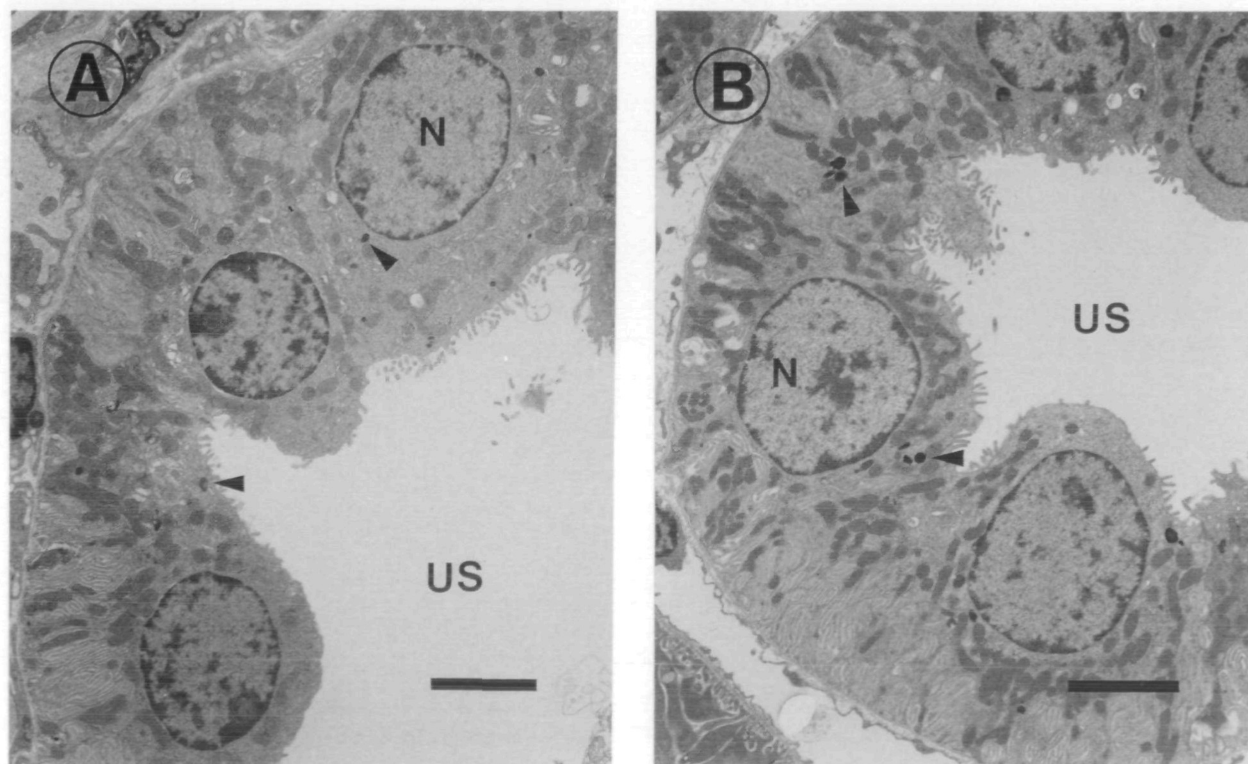


Fig. 6. **Morphology of distal tubular cells.** Electron micrographs of distal tubules 10 min after treatment with vehicle (A) and hPTH(1-34) (B). The architecture of tubules and cell organelles (arrowheads: lysosomes) did not show any alterations in the period of 5-30 min after treatment with hPTH(1-34). N: nucleus; US: urinary space. Bars, 2 $\mu$m.



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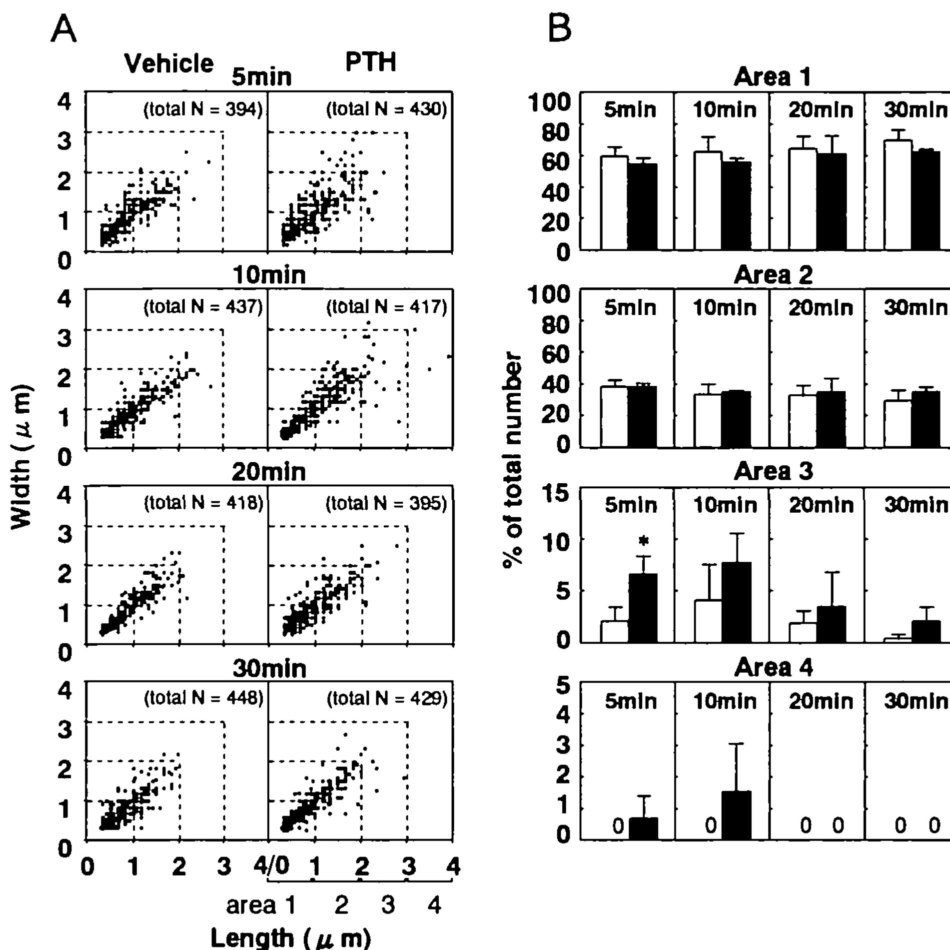


Fig. 7. **Changes in the size of lysosomes of proximal tubules by hPTH(1-34).** A: Change in the distribution of lysosomal size. The lengths and widths of all lysosomes in 5-7 fields in the proximal tubules of three rats were measured, and the data were classified into four groups (areas 1 to 4). B: Percentage of lysosomes distributed in each area. The results are presented as means±SD ($n=3$). Open bars: vehicle treatment group; solid bars: hPTH(1-34) treatment group, area 1: size<1 μm; area 2: 1≤size<2 μm; area 3: 2≤size<3 μm; area 4: 3≤size<4 μm, *$p<0.05$ *vs.* vehicle treatment group.

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after the administration of hPTH(1-34), indicating that this peptide hormone had no significant cytotoxic effect on rat kidney (data not shown). As shown in Fig. 5, some of the lysosomes of proximal tubules were transiently enlarged after hPTH(1-34) administration, and most of the enlarged lysosomes were electron-dense. These changes were remarkable 5-10 min after administration of the peptide hormone. However, no morphological changes caused by hPTH(1-34) were evident in the other organelles of the proximal tubules, such as mitochondria, plasma membranes, and brush border membranes. In distal tubules, lysosomes were fewer and much smaller than those in proximal tubules, as shown in Fig. 6. In this nephrone segment, no morphological changes in cellular architecture and organelles caused by hPTH(1-34) were evident.

The sizes of lysosomes in the proximal tubules were measured and the data were classified into four areas (areas 1 to 4) (Fig. 7A). The percentage of lysosomes distributed in each area is shown in Fig. 7B. Lysosomes in the proximal tubules were mostly distributed in areas 1 and 2 (96-99.6%; size: less than 2 μm). In the vehicle-treated group, there was no change in the lysosomal size distribution at any experimental time, and the lengths and widths of lysosomes were similar, indicating that lysosomes were nearly round (Fig. 7A). In the rats treated with hPTH(1-34), however, the lysosomes in proximal tubules were enlarged, and the percentages of lysosomes distributed in areas 3 and 4 (size: more than 2 μm) were significantly increased. This change was remarkable at 5 and 10 min. Along with this change, a slight decrease in the percentage of lysosomes in area 1 (size: less than 1 μm) was observed (Fig. 7B). Some of the enlarged lysosomes were oval (Fig. 7A). These morphological changes in lysosomes were transient and became normalized 20 min after the administration of hPTH(1-34).

## DISCUSSION

The present study revealed that hPTH(1-34) transiently increased the urinary excretion of not only NAG but also ACP, cathepsin L, and cathepsin B, which are major lysosomal enzymes in kidney. Since no increase in NAG in serum was detected in rats after infusion of hPTH(1-34) for 30 min, the enzyme excreted in urine originated from the kidney. This strongly suggests that other lysosomal enzymes may also originate from the kidney, but this remains to be confirmed. However, the excretion of lysosomal enzymes by hPTH(1-34) was transient and of limited amount, with each enzyme excreted accounting for less than 0.1% of the corresponding total lysosomal enzyme in rat kidney. The time course of slight but significant changes in both the density and the size of renal lysosomes induced by hPTH(1-34) were compatible with that of the limited excretion of these lysosomal enzymes in urine. The effect of

hPTH(1-34) on rat kidney was specific for lysosomes and was not observed for mitochondria, because the density gradient profile and the morphology of mitochondria were not affected by the treatment with hPTH(1-34).

Lysosomes are dynamic systems, and their density and size change under various cellular conditions and with lysosome tropic drugs (*22-25*). A biphasic pattern of the density gradient distribution of renal lysosomes was previously reported (*15*), and hPTH(1-34) slightly but significantly shifted the density of lysosomes from intermediate density (1.07-1.08 g/ml) to heavier density at around 1.12 g/ml. This change disappeared within 20 min. A similar small shift in the density of lysosomes has been reported in rat liver perfused with a medium deficient in amino acids (*24*). The time course of the increase in the density of lysosomes coincided with that of the increase in urinary excretion of lysosomal enzymes. Furthermore, hPTH(1-34) at the doses of 1 U, 20 U, and 100 U per kg body weight increased the fraction of the high density lysosomes (fraction No 3) in a dose-dependent manner as analyzed by the distribution of NAG (data not shown). These results suggest that the urinary excretion of lysosomal enzymes induced by hPTH(1-34) is accompanied by a change in the lysosomal density, although the mechanism underlying these phenomena is currently unknown.

PTH/PTHrP receptors are distributed in the proximal and distal tubules (*26*), but significant change in the lysosomal morphology was observed only in the proximal tubules after hPTH(1-34) treatment. In the proximal tubules, enlarged (size more than 2 $\mu$m) and electron-dense lysosomes were observed after hPTH(1-34) treatment. These findings may correspond to the increase in the density of lysosomes. Concomitantly, a slight decrease in the proportion of lysosomes with intermediate densities may also correspond to the slight decrease in the proportion of lysosomes of less than 1 $\mu$m in diameter.

Lysosomal enzymes are synthesized on ribosomes as precursors, processed during trafficking through the endoplasmic reticulum and the Golgi apparatus, and finally converted to mature forms in acidic organelles, *i.e.*, endosomes/lysosomes (*19*). To determine the intracellular origin of the lysosomal enzymes excreted into urine after treatment with hPTH(1-34), we analyzed the molecular mass of NAG in urine. A single immunoreactive NAG band was always observed during the experiments, and its intensity increased following hPTH(1-34) treatment. The molecular mass of NAG in urine was 54 kDa, indicating that the secreted NAG is the mature form (*20, 21*). Since late endosomes contain mature lysosomal acid hydrases, which are the same enzymes as those in lysosomes (*27*), mature enzymes excreted in urine by hPTH(1-34) may originate from the lysosomes and/or endosomes. Consistent with this notion, Rodríguez *et al.* demonstrated that the mature form of cathepsin D was secreted from lysosomes and/or endosomes upon elevation of intracellular $Ca^{2+}$ in normal rat kidney cells (*7*).

Although the possibility has not been completely ruled out that the excretion of lysosomal enzymes into urine is a result of cytotoxicity of hPTH(1-34), hPTH(1-34) did not disrupt the architecture of organelles, including lysosomes, in the proximal and distal tubules. Light microscopically, no significant morphological change was seen in the kidney after treatment with hPTH(1-34). Furthermore, hPTH(1-34) did not stimulate the urinary excretion of alkaline phosphatase, leucin aminopeptidase, or $\gamma$-glutamyl transpeptidase, sensitive makers of renal tubular damage (*28-30*). These results suggest that the increase in the density and size of lysosomes and the increase in the excretion of lysosomal enzymes into urine after treatment with hPTH-(1-34) are probably physiological responses to hPTH(1-34) in renal tubules.

As suggested by Mizunashi *et al.* (*2, 3*), the measurement of lysosomal enzymes in response to hPTH(1-34) administration may help to determine the renal function and to diagnose certain types of renal diseases. Further studies on the cellular mechanisms underlying the excretion of lysosomal enzymes and the changes in the density and the size of renal lysosomes in response to hPTH(1-34) are now under investigation.

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