Parathyroid Hormono-hydrolyzing Enzymes in Human Kidney

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Synopsis

Human kidney tissue obtained at autopsy was homogenized in 0.15 M KCl and centrifuged at 600 or 100,000 g to test the supernatants for the activity of hydrolyzing 125I-labelled bovine parathyroid hormone through incubation at 37°C for 1 hour and measurement of trichloroacetic acid-soluble 125I released. PTH-hydrolyzing activity was apparently soluble and mainly located in the supernatant fraction of centrifugation at 100,000 with the optimal pH of 4.5, unlike the rat kidney microsomal parathyroid hormone-hydrolyzing enzyme with optimal pH of 8.5. The activity per unit tissue weight slightly increased with advance in age but the total activity in both kidneys decreased with advance in age.

Parathyroid hormone (PTH) is mainly inactivated by the kidney through hydrolysis by a microsomal enzyme in rat (Fujita et al., 1969, Fujita et al., 1970; Martin et al., 1969; Vajda et al., 1969). Despite the important role of PTH in the metabolism of calcium and phosphorus, no information is yet available on the corresponding enzymes in the kidneys of other species. The present study was therefore undertaken to study human kidney with regard to the PTH-inactivating enzymes (PTHase).

Materials and Methods

Kidneys were obtained from human cadavers at the Tokyo Metropolitan Medical Examiner’s Office 5–10 hr after death and kept frozen until the time of assay. After removing the hilar and capsular connective and adipose tissue, renal parenchyma including cortex and medulla was prepared into 10% homogenate with 0.15 M KCl in a Waring blender. Centrifugation was carried out at 600 g for 3 min to remove nuclei and destroyed tissue fragments, and the supernatant was again centrifuged at 100,000 g for 1 hr to separate some of the particulate fraction. Activity of hydrolyzing 125I-labelled parathyroid hormone (125I-PTH) was measured as reported previously (Fujita et al., 1969), using 0.2 ml of the supernatant of centrifugation at 600 g and 100,000 g, 0.1 ml of 125I-PTH solution, 0.025 ml of 10 mg/ml aqueous solution of partially purified bovine parathyroid hormone (TCA-PTH, Wilson) with biological activity of 240 USP units/mg, 0.2 ml of either 0.2 M Na-citrate buffer, pH 4.5, or 0.2 M Na-borate buffer, pH 8.5, and distilled water to make up 1 ml. For the construction of pH-optimal curves, Na-citrate and Na-borate buffers covering the pH range of 1.2–10.0 were used. The mixture was placed in a 15 ml test tube and incubated with shaking for 60 min in a water bath at 38°C. After adding 2 ml of 10% trichloroacetic acid (TCA) to stop the reaction, followed by 0.05 ml of 10% bovine serum albumin, the mixture was centrifuged at 3,000 r.p.m. and 1 ml of the supernatant was used for counting 125I radioactivity (A) in an automatic well-type scintillation counter (Aloka). One tube containing the same amount of 125I-PTH in the same volume without TCA was used to count the total radioactivity (T), while TCA-soluble 125I-PTH already present before incubation (B) was measured through adding TCA to the reaction mixture before incubation. TCA-soluble 125I released from 125I-PTH was expressed as \( \frac{A-B}{T-B} \times 100\% \). Since the TCA-PTH in the reaction mixture had the activity of 60 USP units, the activity of the sample to hydrolyze parathyroid hormone was expressed as USP units inactivated: \( \frac{A-B}{T-B} \times 60 \) units. 125I-PTH, prepared from highly purified bovine PTH (CMC-PTH, Wilson)
with biological activity of 3000 USP units/mg by the method of Hunter and Greenwood (1962) had the specific activity of 230 mCi/mg.

**Results**

Human renal PTH ase activity showed a major peak at pH 4.5, as shown in Figure 1. Unlike rat kidney, no peak is evident around pH 8.5.

PTH ase activity per wet tissue weight of the kidney was much lower in man than in rats especially in the 600 g supernatant. (Fig. 2). In rat kidney, 600 g supernatant had much higher PTH ase activity than 100,000 g supernatant, especially at pH 8.5, apparently due to the presence of PTH ase with optimal pH at 8.5 in the microsomes, which are present in the 600 g supernatant but not in the 100,000 g supernatant. In human kidney homogenate, on the other hand, the activity was quite similar between 600 g and 100,000 g supernatants evidently speaking against localization of PTH in microsomes or other particulate fractions precipitates at 100,000 g. In Figure 3, the similarity of PTH ase activity between 600 g and 100,000 g is again demonstrated through plotting the activity of 600 g supernatant on the ordinase and 100,000 g supernatant on the abscissa. Most of the points are located close to the line dividing the quadrant into two equal portions. More closed circles appear below the line suggesting a slightly higher activity in 100,000 g supernatant than in 600 g supernatant at pH 4.5, while more open circles appear above the line, indicating somewhat higher activity in 600 g supernatant than in 100,000 g supernatant at pH 8.5. PTH ase activity per unit protein weight scarcely changed with advance.
in age, although a slight but significant positive correlation may be calculated (Fig. 4). Similar result was obtained for PTHase activity per unit wet tissue weight. However, total PTH hydrolyzing activity for both kidneys significantly declined with advance in age (Fig. 5).

![Graph](image)

**Fig. 4.** PTHase activity of human kidney per unit protein weight, supernatant of 600 g centrifugation at pH 4.5.

![Graph](image)

**Fig. 5.** PTHase activity of human kidney per total kidney tissue, supernatant of centrifugation at 600 g, pH 4.5. Calculation of linear regression is based on cases without gross renal abnormalities. Six cases of uremia due to chronic renal insufficiency are shown separately.

**Discussion**

Parathyroid hormone shares the fate to be mainly inactivated by the kidney with many other peptide hormones. Enzymatic mechanism of such inactivation was studied especially with regard to the rat kidney microsomal enzymes rather extensively (Maruyama et al., 1970). While the optimal pH of this microsomal enzyme was 8.5, another enzyme with optimal pH at 4.5 was shown to be present in the kidney and also spleen of rats (Fujita et al., 1969). In human kidney, according to the results of the present study, no such microsomal enzyme with optimal pH at 8.5 is apparently present in significant amount, while the enzyme with optimal pH at 4.5, mainly localized in the supernatant fraction, is apparently present in an amount comparable to that of rat. Although no marked change was seen in the concentration of this enzyme activity per unit tissue weight and per unit protein weight, total activity for both kidneys significantly declined with advance in age. However, such change was so mild that the physiological significance is rather questionable, although it is tempting to speculate that such decrease in parathyroid hormone-inactivating activity might reflect the gradual decrease of the function of renal cells with advance in age.

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**References**


