Measurement of Thyroid Stimulating Hormone (TSH) in Human Urine

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Abstract

Using a highly sensitive and specific immunoradiometric assay kit for human TSH, we measured TSH concentrations in unprocessed urines in normal subjects, in patients with primary hypothyroidism, and patients with renal disease. In five of ten normal subjects TSH was detectable in urine samples (<20 – $69 \mu\text{U/day}$). In five patients with hypothyroidism, the urinary TSH excretion was increased. In seven out of ten patients with nephrotic syndrome, eight out of nine patients with chronic renal failure and two patients with tubular dysfunction, the urinary TSH excretion was increased. The urinary TSH excretion correlated significantly with both urinary protein excretion and urinary β_2 -microglobulin excretion. These results suggest that the renal handling of TSH involves both glomerular filtration and tubular re-absorption, and that urinary TSH excretion is increased when serum TSH is increased and either glomerular or tubular function is impaired.

It is reported that the kidney is the major site of metabolism of TSH (Utiger, 1986). However, TSH has been reported to be undetectable in unconcentrated urine (Kuku *et al.*, 1974). Furthermore, the immunoreactive TSH measured in urinary concentrates was reported to be an assay artefact (Van Herle *et al.*, 1978). Recently, a highly sensitive and specific immunoradiometric assay kit for human TSH, which

allows detection of levels down to $0.03 \mu\text{U}$ TSH/ml, has become commercially available (Yoshida *et al.*, 1986 a and b). Using this kit, we measured urinary TSH excretion in normal subjects and in patients with primary hypothyroidism and renal diseases, and compared this with the serum TSH concentration and urinary protein and β_2 -microglobulin excretions. We also measured urinary excretion of the alpha subunit of the glycoprotein hormones and the TSH-beta subunit.

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Materials and Methods

Ten euthyroid patients were studied as controls. They were 7 males and 3 females, aged 19 to 39 years. None of them had signs of thyroid disease or had received any medication known to influence thyroid hormone concentration. Their serum thyroxine (T_4), 3, 5, 3'-triiodothyronine (T_3) and TSH concentrations were all within normal limits. Their serum creatinine was normal and none had proteinuria evaluated with Multistix (less than 15 mg/dl). Five patients with primary hypothyroidism, 14 patients with chronic glomerulonephritis (urinary protein excretion ≤ 3.5 g/day, creatinine clearance (Ccr) ≥ 20 ml/min), 10 patients with nephrotic syndrome (urinary protein excretion > 3.5 g/day), 9 patients with chronic renal failure ($Ccr < 20$ ml/min) and two patients with tubular dysfunction (Wilson's disease and hypokalemic nephropathy with chronic diarrhoea) were also examined. One patient with hypothyroidism, 8 with chronic glomerulonephritis, 5 with nephrotic syndrome and 3 with chronic renal failure were treated with 10 to 60 mg of prednisolone daily at the time of urine collection. The urine was collected for 24 h.

TSH concentrations in serum and urine were measured with a sensitive immunoradiometric assay kit (TSH Riabead II, Dainabott Co., Japan) (Yoshida *et al.*, 1986 a and b). In brief, 200 μ l of either standard solutions or patients samples were incubated for 2 h at room temperature with beads which had been coated with monoclonal antibody that binds only to a human TSH beta-subunit determinant. After the beads were washed with distilled water, 200 μ l of 125 I-anti human TSH monoclonal antibody was added and incubated for 2 h at room temperature. The amount of tracer bound specifically to the coated beads was measured with a gamma counter. The detection limit calculated from the results of the zero standard plus 2SD was 0.03 μ U/ml. The coefficient of variation was 2.4–3.9% for intra-assay and 9.0–9.5% for inter-assay. Luteinizing hormone (LH) and follicle-stimulating hormone (FSH) did not interfere with this assay unless concentrations of LH and FSH exceeded 250 and 500 mIU/ml, respectively. Urinary TSH levels in normal men are in the same range as in post-menopausal women. Furthermore, in three pregnant women with hy-

perthyroidism, whose serum glycoprotein alpha subunit concentrations were all increased (more than 50 ng/ml), serum TSH concentrations were all less than the lower limit of sensitivity of 0.03 μ U/ml. The beta subunit of TSH did not interfere with this assay unless its concentration exceeded 50 ng/ml. We recovered 90% and 96% of added TSH, respectively, when 0.1 and 0.4 μ U TSH were mixed with 200 μ l urine. When 200, 100, 50 and 25 μ l of urine with an increased TSH concentration were assayed in this system, the TSH values obtained were 7.03, 3.41, 1.71 and 0.83 μ U/ml, respectively. Human serum albumin, sodium chloride, urea, calcium chloride and magnesium sulfate were added at various concentrations to 1 ml of urine from a patient with chronic renal failure, and 200 μ l samples were assayed. Human serum albumin over the range 1–3% did not affect the assay. Similarly, sodium chloride, urea, calcium chloride and magnesium sulphate, which were reported to affect the TSH RIA (Kuku *et al.*, 1974; Van Herle *et al.*, 1978), did not modify the assay at concentrations less than 1 M. Changes in the pH of urines from 5.0 to 8.5, adjusted with HCl or NaOH, had no effect on the assay.

A Urine sample (500 μ l) obtained from a patient with chronic renal failure with a very high concentration of TSH (10.5 μ U/ml; 7.2 mU/day) was fractionated by gel chromatography on a 1.5 $\times$ 40 cm column of Sephadex G-100. Elutions were carried out at 4°C with 0.05 M Tris-HCl buffer, pH 7.4, and collected as 2-ml fractions. The flow rate was 0.4 ml/min. 125 I-TSH, 125 I-alpha and 125 I-TSH-beta subunits were also chromatographed as internal markers.

The urinary β_2 -microglobulin concentration was measured with a RIA kit (Pharmacia β_2 -micro RIA). Urinary concentrations of the alpha and beta subunits of human TSH were measured by radioimmunoassays (Kourides *et al.*, 1975), using reagents providing by NIDDKD and NIH. The subunits were iodinated with carrier free 125 I by the chloramin T method (Kourides *et al.*, 1975). 125 I-human TSH was obtained from Daiichi Radioisotope Co., Japan.

The correlation between two different variables was calculated by linear regression analysis.

Results

The elution profiles of ¹²⁵I-human TSH and the urine with increased TSH on gel filtration analysis are shown in Fig. 1. This shows that immunoreactive TSH is eluted as a single symmetrical peak and ¹²⁵I-TSH was eluted with an identical peak. The peak for albumin in urine eluted earlier,

and those for ¹²⁵I-alpha and ¹²⁵I-TSH-beta subunits eluted later than the TSH peak, respectively.

TSH was detectable in urine samples in 50% of the 10 euthyroid controls (0-69 μU/day) and the mean value was 29±31 (Mean±SD) μU/day. In all patients with primary hypothyroidism, urinary TSH excretion was increased and the mean value was 305±175 μU/day. Urinary TSH ex-

Fig. 1. Gel-filtration profiles of immunoassayable TSH in urine (●—●) and ¹²⁵I-TSH (×—×). A urine sample (500 μl) with an increased TSH concentration was fractionated by gel chromatography on a 1.5×40 cm column of Sephadex G-100. Elutions were carried out at 4°C with 0.05 M Tris-HCl buffer, pH 7.4, and collected as 2-ml fractions. The flow rate was 0.4 ml/min.

	N	0.02	0.1	1	10	mean serum TSH concentration (μU/ml)
Euthyroid Control	10	○ ○ ○ ○ ○ ○ ○ ○ ○ ○				1.3
Hypothyroidism	5		● ○ ○ ○ ○	○		204
Chr. Glomerulo-Nephritis	14	● ● ● ● ● ● ● ● ● ● ● ● ● ●	○	●		1.3
Nephrotic Syndrome	10	● ● ● ● ● ● ● ● ● ●		○ ○ ○ ● ● ○ ○ ○ ○		3.4
Chr. Renal Failure	9		○ ● ● ● ● ○ ○ ○ ○ ○ ○	○ ○ ○ ○ ○	●	3.3
Tubular Dysfunction	2			○		○ 4.4

Fig. 2. Urinary TSH excretion in euthyroid controls, in patients with primary hypothyroidism, chronic glomerulonephritis, nephrotic syndrome, chronic renal failure and tubular dysfunction. * with nephrotic syndrome; ● treated with prednisolone.

cretion was increased in one patient with chronic glomerulonephritis, 7 with nephrotic syndrome, 8 with chronic renal failure and 2 with tubular dysfunction (Fig. 2). However, urinary TSH was not detected in 10 patients with chronic glomerulonephritis, and 3 with nephrotic syndrome, most of whom were on prednisolone, and their

mean serum TSH concentration was 1.3 μ U/ml, which was identical to that in euthyroid controls (Fig. 2). The mean serum TSH concentration in each group is shown in Fig. 2. In euthyroid controls, the mean TSH clearance was 0.023 ml/min, which was about 0.02% of Ccr. Urinary TSH excretion correlated significantly with both

Fig. 3. Correlation of urinary TSH excretion with urinary protein excretion. $n=49$; $r=0.59$, $P<0.001$; $\times$ Normal Control, Δ Hypothyroidism, $\circ$ Chronic glomerulonephritis, $\square$ Chronic renal failure, $\odot$ Nephrotic syndrome, $\oplus$ Tubular dysfunction, $\bullet$ $\blacksquare$ treated with prednisolone.

Fig. 4. Correlation of urinary TSH excretion with urinary β_2 -microglobulin excretion. $n=44$; $r=0.46$, $P<0.001$. See also Fig. 3.

Fig. 5. Correlation of urinary TSH excretion with creatinine clearance. $n=46$; $r=-0.30$, $P<0.05$. See also Fig. 3.

		1	10	100 $\mu\text{g/day}$
Euthyroid	8	o o	o o o	o o
Hypothyroid	2		o	●
Chr. Glomerulo-nephritis	3	o	o o	
Nephrotic Syndrome	7	o o	o o o	o o
Chr. Renal Failure	8		o o o *	o o *
Tubular Dysfunction	2		o	●

Fig. 6. Urinary alpha subunit excretions in euthyroid controls, in patients with primary hypothyroidism, chronic glomerulonephritis, nephrotic syndrome, chronic renal failure and tubular dysfunction. * with nephrotic syndrome; ● post menopausal women.

urinary protein excretion (Fig. 3) and urinary β_2 -microglobulin excretion (Fig. 4). There was also a significantly negative correlation between the urinary excretion of TSH and Ccr (Fig. 5). However, no correlation was found between the urinary TSH excretion and serum TSH concentration (data not shown).

In euthyroid controls, the mean excretion of alpha subunit in urine samples was $3.0 \pm 1.7 \mu\text{g/day}$, ranging from 1.0 to $5.1 \mu\text{g/day}$.

In some patients with nephrotic syndrome and chronic renal failure, the urinary excretion of alpha subunit was increased. In the postmenopausal women, urinary alpha subunit excretion was somewhat higher (Fig. 6).

Urinary excretion of TSH-beta subunit was not detectable ($<0.5 \text{ ng/ml}$) in most of the 30 urine samples tested. In only two patients with chronic renal failure was the urinary concentration of TSH-beta sub-

unit detectable (1.3 and 1.4 ng/ml, respectively).

Discussion

It is well established that the kidney plays a major role in the removal of polypeptide hormones (e. g. insulin, glucagon, growth hormone, parathyroid hormone etc.) from the systemic circulation. The major route for the removal of peptides from the renal circulation is glomerular filtration. Filtered peptides are efficiently reabsorbed in the proximal tubules and relatively little appears in the urine (Maack *et al.*, 1979; Rabkin and Kitaji, 1983).

Investigation of TSH in the rat has shown that the kidney is the major site of metabolism of TSH (Bakke and Lawrence, 1962; Kuku *et al.*, 1979). Human TSH clearance is reduced in patients with renal failure, indicating that the kidney participates in TSH excretion, but little TSH is found in urine (Utiger 1986). Kuku *et al.* (1974) measured TSH in concentrated urine by RIA and found that it was increased in patients with primary hypothyroidism and decreased in patients with hyperthyroidism. On the other hand, Van Herle *et al.* (1978) reported that immunoreactive TSH measured in urinary concentrates was an assay artefact. Urinary TSH excretion had not been studied in patients with renal diseases.

In the present series, TSH was measured directly in human urine by a sensitive and specific immunoradiometric assay. The TSH measured in urine had immunological and gel chromatographic characteristic similar to those of human TSH. In hyperthyroid pregnant women, the serum alpha subunit concentration was greatly increased, but the serum TSH was not detectable. TSH-beta subunit did not interfere with this assay unless its concentration exceeded 50 ng/ml and the urinary TSH-beta subunit concentration was less than 1.5 ng/ml. These re-

sults indicate that TSH subunits did not react with our assay.

In normal subjects the mean excretion of TSH in urine was 29 μ U/day which is about one fifth that reported by Kuku *et al.* (1974). The urinary TSH excretion was increased in most patients with primary hypothyroidism, nephrotic syndrome, chronic renal failure and tubular dysfunction. Mean TSH clearance in euthyroid controls was about 0.02% of Ccr, indicating that filtered TSH is efficiently reabsorbed in the tubules. These results indicate that the renal handling of TSH is completely different from that of thyroid hormones (Yoshida *et al.*, 1980; Faber *et al.*, 1987). The urinary TSH excretion correlated significantly with both urinary protein and β_2 -microglobulin excretions. There was also a significant negative correlation between the urinary excretion of TSH and Ccr. Therefore, the urinary TSH excretion seems to be increased when serum TSH is increased and also when either glomerular or tubular function is impaired. These results suggest that the renal handling of TSH involves both glomerular filtration and tubular reabsorption.

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