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# Renal Clearance of the Thyrotropin-Releasing Hormone-Like Peptide Pyroglutamyl-Glutamyl-Prolineamide in Humans

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**ABSTRACT**

TRH-like peptides have been identified that differ from TRH (*p*Glu-His-ProNH$_2$) in the middle aminoacid. We have estimated TRH-like immunoreactivity (TRH-LI) in human serum and urine by RIA with TRH-specific antiserum 8880 or with antiserum 4319, which binds most peptides with the structure *p*Glu-X-ProNH$_2$. TRH was undetectable in serum (<25 pg/mL), but TRH-LI was detected with antiserum 4319 in serum of 27 normal subjects, 21 control patients, and 12 patients with carcinoid tumors (range 17–45, 5–79, and 18–16,600 pg/mL, respectively). Because serum was kept for at least 2 h at room temperature, which causes degradation of TRH, *p*Glu-Phe-ProNH$_2$, and *p*Glu-Tyr-ProNH$_2$, serum TRH-LI is not caused by these peptides. On high-performance liquid chromatography, serum TRH-LI coeluted with *p*Glu-Glu-ProNH$_2$ (<EEP-NH$_2$), a peptide produced in, among others, the prostate. Urine of normals and control patients also contained TRH-LI (range 1.14–4.97 and 0.24–5.51 ng/mL, respectively), with similar levels in males and females. TRH represented only 2% of urinary TRH-LI, and anion-exchange chromatography and high-performance liquid chromatography revealed that most TRH-LI in urine was <EEP-NH$_2$. In patients with carcinoid tumors, increased urinary TRH-LI levels were noted (range 1.35–962.4 ng/mL). Urinary TRH-LI correlated positively with urinary creatinine, and the urinary clearance rate of TRH-LI was similar to the glomerular filtration rate. In addition, serum TRH-LI was increased in 17 hemodialysis patients (43–373 pg/mL). This suggests that serum <EEP-NH$_2$ is cleared by glomerular filtration with little tubular resorption. The possible role of the prostate as a source of urinary TRH-LI was evaluated in 11 men with prostate cancer, showing a 25% decrease in urinary TRH-LI excretion after prostatectomy (0.19 ± 0.02 *vs.* 0.15 ± 0.01 ng/μmol creatinine, mean ± SEM). However, TRH-LI was similar in spontaneously voided urine and in urine obtained through a nephrostomy cannula from 16 patients with unilateral urinary tract obstruction (0.15 ± 0.01 *vs.* 0.14 ± 0.01 ng/μmol creatinine). These data indicate that: 1) TRH-LI in human serum represents largely <EEP-NH$_2$, which is cleared by renal excretion; 2) part of urinary <EEP-NH$_2$ is derived from prostatic secretion into the blood and not directly into urine; and 3) urinary <EEP-NH$_2$ can be used as marker for carcinoid tumors. (*J Clin Endocrinol Metab* **82:** 3068–3073, 1997)

EARLY STUDIES concerning the presence of extrahypothalamic TRH (<EHP-NH$_2$)[1] have indicated that immunoreactive TRH is present in peripheral tissues, blood, and urine (for reviews, see Refs. 1 and 2). Subsequent studies revealed the presence of TRH-like immunoreactivity (TRH-LI) distinct from authentic TRH in, among others, pituitary, male reproductive system, and semen (3–10) and indicated that TRH-LI in blood and urine is probably not caused by TRH (11, 12). Recently, naturally occurring tripeptides have been found that share the N- and C-terminal aminoacids with TRH but differ in the middle aminoacid (for reviews, see Refs. 1, 13) and cross-react, to varying extents, with antisera raised against TRH (6, 14–16). In human prostate and seminal fluid <EQP-NH$_2$ and <EFP-NH$_2$ have been identified (17–19), whereas <EYP-NH$_2$ has been found in alfalfa (20). Another TRH-like peptide is <EEP-NH$_2$, which has been detected in pituitary, prostate, and seminal fluid of various mammalian species (including man, rat, and rabbit) (7, 8, 21–25). Although knowledge of the biological function of this peptide is limited, <EEP-NH$_2$ has been found to increase the capacitation and fertilization capacity of mouse and human sperm (26, 27) and is therefore also called: fertilization promoting peptide (13). Moreover, <EEP-NH$_2$ increases the motility of sperm from certain infertile men (19) and inhibits the acrosome reaction of mouse sperm (28). In rats, <EEP-NH$_2$ has effects on pituitary GH secretion (23, 29), adrenal corticosterone secretion (30), and behavior (31). Though high levels of <EEP-NH$_2$ can antagonize effects of TRH (23, 32), the biological activity of <EEP-NH$_2$ is probably not exerted via the TRH receptor (13, 32, 33).

Recently we reported on the presence of <EEP-NH$_2$ in a human neuroblastoma cell line (34) and in serum of patients with metastatic carcinoid tumors (16). In a preliminary study, we observed that serum and urine of normal subjects also contained TRH-LI. Because blood lacks pyroglutamylaminopeptidase I, a peptidase with broad specificity (25, 35), but contains pyroglutamylaminopeptidase II, an enzyme with a narrow substrate specificity that degrades TRH but not <EEP-NH$_2$ (25, 36), we hypothesized that TRH-LI in serum and urine was <EEP-NH$_2$. In the present paper, we provide evidence that indeed most TRH-LI in serum and urine represents <EEP-NH$_2$. In addition, we studied the possibility

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[1] The one-letter aminoacid code is used, where D = Asp, E = Glu, <E = *p*Glu, F = Phe, G = Gly, H = His, *P* = Pro, Q = Gln, and Y = Tyr.

that urinary TRH-LI is derived from prostatic secretion and may be used as a marker for carcinoid tumors.

## Subjects and Methods

### *Subjects*

The studies were done according to rules of the hospital medical ethics committee. Normal healthy subjects [17 women aged 25–46 (median 28) yr, 10 men aged 27–57 (median 39) yr], patients with metastatic carcinoid tumors [3 women aged 58–65 (median 62) yr, 9 men aged 30–70 (median 64) yr], control patients [14 women aged 20–76 (median 50) yr, 7 men aged 19–71 (median 54) yr], anuric patients undergoing regular hemodialysis [7 women aged 27–73 (median 44) yr, 10 men aged 43–80 (median 47) yr], patients with short-lasting unilateral urinary tract obstruction temporarily treated by unilateral nephrostomy [5 women aged 41–76 (median 47) yr, 11 men aged 22–70 (median 47) yr], and patients with prostate cancer [11 men aged 52–65 (median 61) yr] participated in this study.

No drugs were used by the healthy normal subjects, except for contraceptives (9 of the 17 females), and none of them had a history of renal disease. Ten of the 12 patients with carcinoid tumors were treated with SRIH analog octreotide. The group of control (*i.e.* noncarcinoid) patients consisted of 5 patients with disorders of lipid metabolism (most of them were on lipid-lowering drugs), 5 patients with Cushing's syndrome (2 patients with an adrenocortical tumor and 3 patients with untreated Cushing's disease), 3 patients with primary hyperparathyroidism, 2 patients with gonadotropin-secreting or clinically nonfunctioning pituitary tumors, 2 patients with neurinomas/neurofibromas, 2 subjects who underwent a routine check-up, 1 patient with colonic cancer, and 1 patient with untreated active acromegaly. None of these patients was known to have renal disease or been treated with medications affecting renal function. When appropriate, hypopituitarism in patients with pituitary tumors was treated by replacement therapy. None of the patients with primary hyperparathyroidism or pituitary tumors was known with the multiple endocrine neoplasia-I syndrome.

### *Collection of serum and urine*

Basal, nonfasting blood samples were drawn from a peripheral vein. Blood was taken from the anuric patients before hemodialysis. After centrifugation, serum was kept for at least 2 h at room temperature to ensure enzymatic TRH degradation. The normal subjects delivered a morning urine sample, whereas 24-h urine was collected from control and carcinoid patients. From these persons, urine was collected on the same day as blood was sampled. In patients with obstructive uropathy, 24-h urine was collected from the obstructed kidney through a nephrostomy cannula and by spontaneous micturition. From patients with prostate cancer, 24-h urine was collected the day before prostatectomy and again 3–7 (median 5) days later. Serum and urine were kept at −20 C before the measurements.

### *Serum degradation of TRH-LI*

Serum degradation of <EHP-$NH_2$ (TRH), <EDP-$NH_2$, <EEP-$NH_2$, <EQP-$NH_2$, <EFP-$NH_2$, and <EYP-$NH_2$ was tested by adding 10 ng peptide to 5 mL normal human serum kept at room temperature. At several time intervals, 0.2-mL aliquots were removed and mixed with 1 mL methanol to stop further peptide degradation. After centrifugation, supernatants were removed, dried under a stream of nitrogen at room temperature, dissolved in 1 mL phosphate buffer (pH 7.4), and stored at −20 C until analysis of TRH-LI. After removal of aliquots for RIA measurements, the rest of the serum was extracted with methanol to determine the nature of the residual TRH-LI by isocratic reverse-phase high-performance liquid chromatography (HPLC).

### *Chromatography*

*Urine.* To 1 vol urine were added 2 vol ethanol. The mixture was kept at 4 C for 1 h, and after centrifugation, the supernatant was isolated and dried under a stream of nitrogen. The residue was reconstituted in 2 mL 0.05 M Tris-HCl (pH 7.6) containing 0.02% (wt/vol) sodium azide, and 7500 cpm [$^{125}$I]<EHPG were added as calibration marker. The nature of the TRH-LI in the extracts was analyzed by QAE-Sephadex A-25 (Pharmacia, Uppsala, Sweden), anion-exchange chromatography, and isocratic reverse-phase HPLC as previously reported (16, 34). TRH-LI in the fractions was assayed by RIA using antiserum 4319. Before each session, the HPLC column was calibrated with 2000 cpm [$^3$H]<EHP-$NH_2$ and 2000 cpm [$^3$H]<EEP-$NH_2$. Blank runs were performed between sample analyses to prevent contamination. Elution positions of synthetic TRH-like peptides were determined in separate sessions for both anion-exchange chromatography and HPLC. Recovery of TRH-LI after the chromatographic procedures was at least 85%.

*Serum.* To 5 mL serum was added 7.5 mL 1% (wt/vol) trifluoracetic acid, and the mixture was applied to a Sep-Pak C18 cartridge (Waters, Milford, MA). After washing with 1% trifluoracetic acid and water, TRH-LI was eluted with methanol. The solvent was evaporated, the residue was taken up in HPLC buffer, filtered through a Centricon-10 concentrator (Amicon, Capelle a/d IJssel, The Netherlands), and analyzed by HPLC as described above.

### *Assays*

Levels of TRH-LI were determined by RIA using antiserum 4319 or 8880 (14, 16). Although both antisera were raised against the same antigen, antiserum 8880 specifically recognizes TRH and hardly cross-reacts with <EEP-$NH_2$, whereas antiserum 4319 detects most peptides with the general structure <EXP-$NH_2$ (14–16). Antisera 4319 and 8880 were used at a final dilution of 1:20,000 and 1:50,000, respectively, in the RIAs employing [$^{125}$I]TRH as tracer, unlabeled TRH as standard, and 0.1–0.2 mL serum or 0.1 mL diluted urine extract as sample. Detection limits for assays with antisera 4319 and 8880 are 5–10 and 15–25 pg TRH/mL, respectively, and intra- and interassay variation varied between 8 and 14%. Regardless of the antiserum used, TRH-LI levels are presented as TRH equivalents. Because urea may interfere with the measurement of TRH-LI (11), the effect of urea was tested in the RIA; even high urea levels (165 mmol/L) did not displace [$^{125}$I]TRH from antisera 4319 and 8880. Creatinine and prostate specific antigen (PSA) were measured by routine laboratory methods.

### *Chemicals*

<EHP-$NH_2$ and <EEP-$NH_2$ were purchased from Bissendorf (Hannover, Germany); <EFP-$NH_2$, <EQP-$NH_2$ and <EHPG from Peninsula (Belmont, CA); and <EDP-$NH_2$ and <EYP-$NH_2$ from UCB (Brussels, Belgium). [$^3$H]TRH (43 Ci/mmol) was obtained from Amersham (Aylesbury, UK), and [$^3$H]<EEP-$NH_2$ (30 Ci/mmol) was a gift of Dr. S. M. Cockle (Reading, UK). [$^{125}$I]TRH and [$^{125}$I]<EHPG were prepared and purified by HPLC as previously described (37). All other chemicals were of analytical grade.

### *Statistical analyses*

The results are presented as means ± SEM or range and median. Statistical tests included Mann-Whitney *U* test, Wilcoxon matched-pairs signed-ranks test, and linear regression analysis. $P \leq 0.05$ was considered significant.

## Results

### *Serum and urinary creatinine levels*

Serum creatinine was within normal limits (60–110 μmol/L) in most subjects, with the exception of one carcinoid patient (140 μmol/L); all hemodialysis patients (range 320-1543 μmol/L); and 2 patients with unilateral urinary tract obstruction (134 and 182 μmol/L). Creatinine was lower in nephrostomy urine (4950 ± 520 μmol/L) than in spontaneously voided urine (6910 ± 1010 μmol/L).

### *TRH-LI in serum and urine of normals, control patients, and patients with metastatic carcinoids*

TRH-LI was undetectable by RIA with TRH-specific antiserum 8880 in sera of normal subjects, control patients, and

patients with metastatic carcinoid tumors (<25 pg/mL), but TRH-LI was detected in these sera using nonspecific antiserum 4319 (Table 1). With values between 18 and 16600 pg/mL, mean serum TRH-LI in carcinoid patients was significantly higher than in normal subjects and control patients (Fig. 1, Table 1). Substantial amounts of TRH-LI were detected by RIA with antiserum 4319 in morning urine of normal subjects (Table 1), whereas only low levels were found by RIA with the TRH-specific antiserum 8880 (women 29 ± 3, men 39 ± 6 pg/mL). Serial dilutions of urine produced a dose-response curve parallel to those of <EEP-$NH_2$ and TRH in the RIA with antiserum 4319 (Fig. 2), and <EEP-$NH_2$ added to urine was recovered quantitatively (data not shown). Although TRH-LI levels in 24-h urine of control patients were, in general, lower than values in morning urine of normal subjects, this difference disappeared when urinary TRH-LI was corrected for urinary creatinine content (Table 1). Urinary TRH-LI was not different between males and females (Table 1). Levels of TRH-LI in 24-h urine of patients with metastatic carcinoid tumors varied between 1.35 and 962.4 ng/mL, which is significantly higher than values in control male patients (Fig. 1, Table 1).

In normal subjects and control patients, no significant correlation was found between serum and urine levels of TRH-LI (Fig. 1; r = 0.27, $P = 0.07$), even after correction of the latter for the urinary creatinine content (r = 0.07). Urinary TRH-LI, however, correlated significantly with urinary creatinine (r = 0.76, $P < 0.01$). In patients with metastatic carcinoid tumors, serum TRH-LI correlated significantly with urinary TRH-LI (Fig. 1; r = 0.95, $P < 0.01$).

Based on the data concerning urine production and levels of TRH-LI in serum and urine of the 21 control patients, the urinary clearance rate of serum TRH-LI was calculated from the formula: $[\text{TRH-LI}]_{\text{urine}} \times$ urinary production rate $\div [\text{TRH-LI}]_{\text{serum}}$, yielding a value of 117 ± 21 mL/min.

*Serum degradation of TRH-LI*

Disappearance of TRH-like peptides was tested in human serum kept at room temperature (Fig. 3). No degradation of <EDP-$NH_2$, <EQP-$NH_2$, and <EEP-$NH_2$ was observed, and HPLC analysis showed that these peptides remained intact (data not shown). On the other hand, <EHP-$NH_2$ (TRH), <EFP-$NH_2$, and <EYP-$NH_2$ were degraded rapidly with half-lives of 19, 31, and 16 min, respectively.

*Chromatography of TRH-LI in serum and urine*

The nature of TRH-LI in serum of normal subjects was studied by HPLC, and most TRH-LI was found to coelute with <EEP-$NH_2$ (data not shown). Ethanol-extracted urine from normal subjects was examined by anion-exchange chromatography and HPLC, and typical results are shown in Fig. 4. Most urinary TRH-LI was retained on the QAE-Sephadex A-25 anion-exchange column and, thus, was clearly separated from authentic TRH, as well as basic and neutral TRH-LI, which are not retained on this column. Further analysis by HPLC revealed that urinary TRH-LI coeluted with <EEP-$NH_2$.

*TRH-LI in serum of anuric patients*

Serum TRH-LI measured by RIA with antiserum 4319 in 17 anuric patients was significantly higher (127 ± 18 pg/mL, $P < 0.01$) than that in control patients (18 ± 4 pg/mL). No difference in serum TRH-LI was observed between female and male hemodialysis patients (110 ± 20 and 138 ± 29 pg/mL, respectively). HPLC analysis of sera from 2 of these patients indicated that TRH-LI coeluted quantitatively with <EEP-$NH_2$ (data not shown). Serum TRH-LI in these patients correlated significantly with serum creatinine levels (r = 0.52, $P < 0.025$).

*TRH-LI in urine of patients with unilateral urinary tract obstruction*

TRH-LI was estimated by RIA using antiserum 4319 in urine from patients with unilateral urinary tract obstruction. TRH-LI levels in urine sampled from the nephrostomy cannula were lower than in spontaneously voided urine, but this difference disappeared when TRH-LI values were corrected for the urinary creatinine content (Table 2). No significant difference existed between female and male patients regarding TRH-LI in nephrostomy cannula urine (0.13 ± 0.03 and 0.15 ± 0.01 ng/μmol creatinine, respectively) or spontaneously voided urine (0.15 ± 0.01 and 0.14 ± 0.03 ng/μmol creatinine, respectively). Urinary TRH-LI in these patients

**TABLE 1.** Serum TRH-LI levels, and urinary TRH-LI and creatinine levels (means ± *SEM*) in normal subjects, control patients, and patients with metastatic carcinoid tumors

| | Serum TRH-LI (pg/ml) | Urine TRH-LI (ng/mL) | Urine Creatinine (μmol/mL) | Urine TRH-LI/creatinine (ng/μmol) |
|---|---|---|---|---|
| Normal subjects | | | | |
| Women (17) | 26 ± 2 | 2.28 ± 0.17 | 13.2 ± 1.4 | 0.18 ± 0.01 |
| Men (10) | 26 ± 1 | 2.42 ± 0.38 | 13.8 ± 2.2 | 0.20 ± 0.02 |
| Control patients | | | | |
| Women (14) | 16 ± 3 | 1.67 ± 0.09[a] | 10.4 ± 2.0[a] | 0.17 ± 0.02 |
| Men (7) | 22 ± 10 | 1.64 ± 0.25 | 9.2 ± 1.7 | 0.19 ± 0.03 |
| Carcinoid patients | | | | |
| Women (3) | 117 ± 39[b] | 7.33 ± 4.10 | 5.4 ± 0.9[a] | 1.50 ± 0.83 |
| Men (9) | 2345 ± 1828[ab] | 174.8 ± 114.4[ab] | 8.7 ± 1.6[a] | 22.95 ± 16.38[ab] |

Morning urine was collected from the normal subjects, whereas 24-h urine was collected from the patients. TRH-LI was estimated by RIA with the nonspecific antiserum 4319. Number of individuals is *between parentheses.*

[a] $P < 0.01$ compared with normal subjects.

[b] $P < 0.01$ compared with control patients.

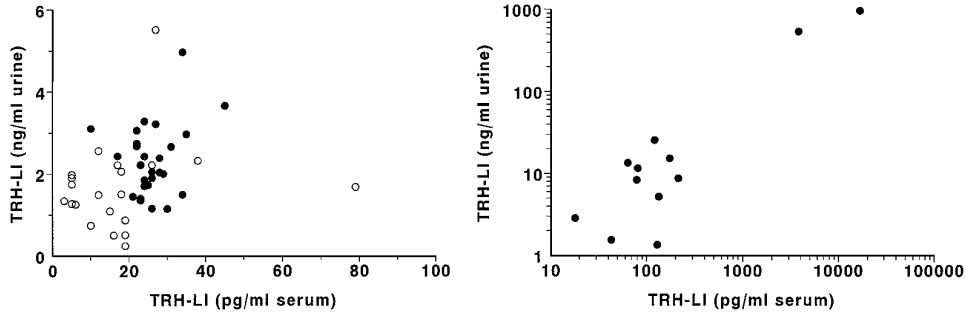


FIG. 1. Relation between serum and urine levels of TRH-LI in 27 healthy normal subjects (*closed circles, left panel*), 21 control patients (*open circles, left panel*), and 12 patients with metastatic carcinoid tumors (*right panel*). TRH-LI was determined with the nonspecific antiserum 4319. No authentic TRH could be detected in these serum samples with the TRH-specific antiserum 8880.

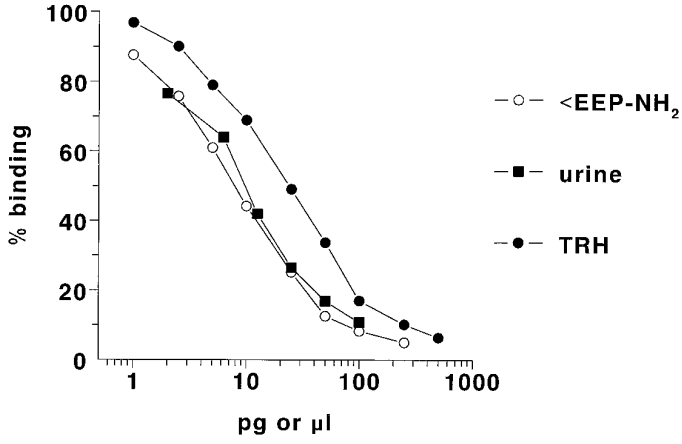


FIG. 2. Inhibition of [$^{125}$I]TRH binding to antiserum 4319 by increasing concentrations of TRH or <EEP-$NH_2$, or increasing volumes of urine.

correlated significantly with urinary creatinine ($r = 0.93$, $P < 0.01$).

*Serum PSA and urinary TRH-LI in men before and after prostatectomy*

Prostatectomy reduced serum PSA from 9.7 ± 2.2 to 0.8 ± 0.3 µg/L ($P < 0.01$, normal <10 µg/L). Levels of TRH-LI in urine, as estimated by RIA using antiserum 4319, were higher before than after prostatectomy (1.7 ± 0.3 vs 1.0 ± 0.1 ng/mL; $P < 0.02$), even after correction for urinary creatinine content (0.19 ± 0.02 and 0.15 ± 0.01 ng/µmol creatinine, respectively; $P < 0.02$).

## Discussion

We have investigated the presence and nature of TRH-LI in human serum and urine. Our two antisera, of which antiserum 8880 is TRH-specific (14–16), enabled us to distinguish between authentic TRH and TRH-like peptides. No authentic TRH could be detected in serum by RIA with TRH-specific antiserum 8880, but TRH-LI was detected with the nonspecific antiserum 4319. It should be stressed, however, that serum was kept for at least 2 h at room temperature to ensure TRH degradation. In blood collected directly into methanol or ethanol to block enzymatic degradation of TRH, undetectable or barely detectable concentrations of authentic TRH have been reported (11, 38). Because <EFP-$NH_2$ and <EYP-$NH_2$ also are rapidly degraded in serum, these peptides also are unlikely to contribute to serum TRH-LI. On the other hand, <EDP-$NH_2$, <EQP-$NH_2$, and <EEP-$NH_2$ are not degraded and thus may contribute to serum TRH-LI. HPLC

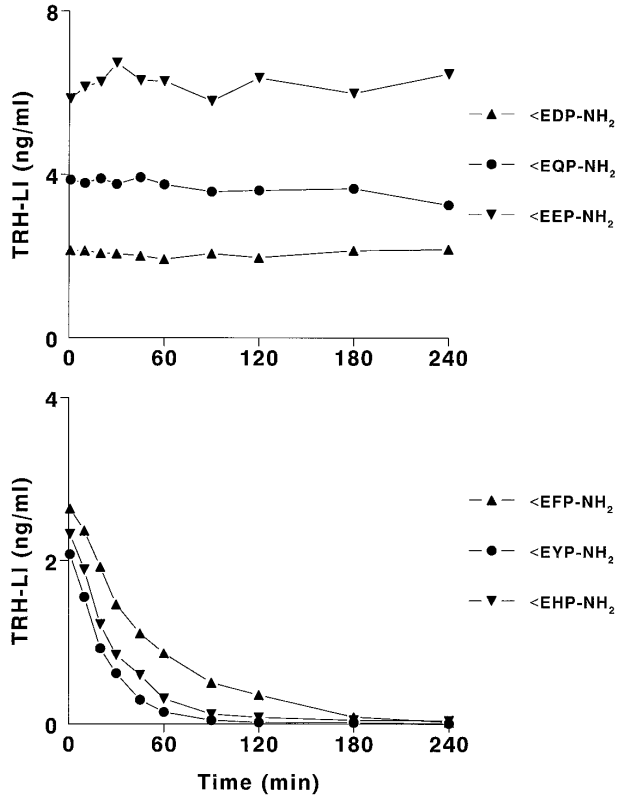


FIG. 3. Degradation of TRH and TRH-like peptides in human serum kept at room temperature. Ten nanograms of peptide was added to 5 mL serum and aliquots of 0.2 mL were removed at various time intervals and mixed with 1 mL methanol. The extracts were assayed for TRH-LI using the nonspecific antiserum 4319.

analysis revealed that most TRH-LI in serum of normal subjects represents <EEP-$NH_2$. Recently, <EEP-$NH_2$ also has been identified in serum of elderly men (39) and in patients with metastatic carcinoid tumors (16).

In urine of healthy subjects, significant amounts of TRH-LI were measured by RIA using antiserum 4319. Only ~2% of urinary TRH-LI was accounted for by authentic TRH, which is in agreement with Suhonen-Malm (38), who reported that urinary TRH varied between 2 and 12 pg/mL. Further analysis revealed that most urinary TRH-LI was retained on the anion-exchange column and coeluted with synthetic <EEP-$NH_2$ on HPLC. Thus, substantial amounts of <EEP-$NH_2$ are present in human urine.

The presence of <EEP-$NH_2$ in serum and urine suggests that <EEP-$NH_2$ is cleared from blood by renal excretion. This

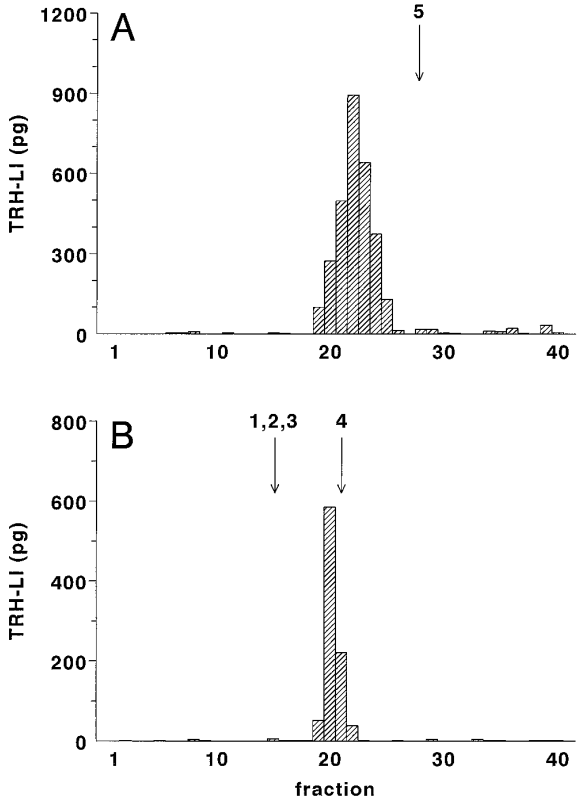


FIG. 4. Typical analysis of TRH-LI in ethanol-extracted urine from a healthy subject by QAE-A25 Sephadex anion-exchange chromatography (A) and isocratic reverse phase HPLC (B). *Arrows* indicate elution positions of <EHP-$NH_2$ (TRH; 1), <EDP-$NH_2$ (2), <EQP-$NH_2$ (3), <EEP-$NH_2$ (4), and [$^{125}$I]<EHPG (5). TRH-LI in the fractions was determined by RIA with the nonspecific antiserum 4319.

**TABLE 2.** Levels of TRH-LI and creatinine (means ± SEM) in spontaneously voided urine and in urine collected from a nephrostomy cannula in 17 patients with unilateral urine tract obstruction

| | TRH-LI (ng/mL) | Creatinine (μmol/mL) | TRH-LI/creatinine (ng/μmol) |
|---|---|---|---|
| Spontaneous | 1.09 ± 0.23 | 6.9 ± 1.0 | 0.15 ± 0.01 |
| Nephrostomy cannula | 0.73 ± 0.11[a] | 5.0 ± 0.5[a] | 0.14 ± 0.01 |

[a] $P < 0.01$ compared with spontaneously voided urine.

is supported by our observations that serum TRH-LI is increased in anuric patients on hemodialysis and that a good correlation exists between TRH-LI and creatinine in urine. For control (*i.e.* noncarcinoid) patients, the urinary clearance rate of TRH-LI amounted to 117 ± 21 mL/min, which is similar to the normal glomerular filtration rate (~125 mL/min). Thus, <EEP-$NH_2$ seems to be cleared from blood by glomular filtration with little tubular reabsorption. This is in contrast to most peptides, which are reabsorbed or undergo significant renal metabolism (40, 41). We have recently found also that after iv injection of <EEP-$NH_2$ in rats, the peptide is recovered quantitatively in the urine (42). Thus, it seems that urinary <EEP-$NH_2$ can be used as an indicator of the total amount of <EEP-$NH_2$ released into the blood.

The similarity of serum and urinary TRH-LI in men and women suggests minor contributions of prostatic <EEP-$NH_2$ secretion. This conclusion is corroborated by the effect of prostatectomy in patients with prostate cancer. Although urinary TRH-LI was reduced 5 days after prostatectomy, this reduction amounted to only 25%. However, it should be mentioned that TRH-LI is virtually absent from prostate cancer tissue (43). Therefore, the effect of prostatectomy in patients with prostate cancer may not be representative of the contribution of the prostate to serum and urinary TRH-LI levels in healthy males. A major contribution of prostatic <EEP-$NH_2$ secretion directly into the urinary tract is also unlikely because patients with unilateral nephrostomy had similar TRH-LI levels per μmol creatinine in urine collected from the renal pelvis, which is not contaminated by prostatic secretory products, and in spontaneously voided urine. Together, our results suggest that a major source of <EEP-$NH_2$, other than the prostate, is present in humans.

As for an alternative source of <EEP-$NH_2$, this peptide is present in the pituitary of various species, including rat, rabbit, pig, and domestic fowl (see Ref. 13 for review). However, analysis of TRH-LI in serum obtained by bilateral inferior petrosal sinus sampling in humans suggests that hypophyseal <EEP-$NH_2$ is a negligible source of serum TRH-LI[2]. In analogy with the presence of <EYP-$NH_2$ in alfalfa (20), serum and urinary <EEP-$NH_2$ may be derived from food. However, urinary TRH-LI in normal subjects is not affected by fasting[3], suggesting a minor dietary intake of <EEP-$NH_2$. Also in rats, evidence has been obtained that <EEP-$NH_2$ in serum and urine is not derived from food (42). Thus, further research is needed to identify the origin of <EEP-$NH_2$ in serum and urine.

No significant correlation was found between serum and urinary TRH-LI in healthy subjects and in control patients. Variable serum TRH-LI levels have been detected previously by us (16) in control subjects (range 9–193 pg/mL, n = 175), and recent studies in our laboratory suggest an episodic variation in serum TRH-LI in healthy women[2]. The latter phenomenon may explain the lack of correlation between serum and urinary TRH-LI in normal subjects and in control patients.

The finding that <EEP-$NH_2$ is excreted in urine has possible clinical implications. In healthy subjects, serum TRH-LI is low, but increased levels have been found in 36 of 72 patients with carcinoid tumors (16, present study). In absolute terms, however, serum levels remain rather low in many of these patients. TRH-LI levels in urine are ~100× higher than in serum, and urinary TRH-LI correlates with serum TRH-LI in carcinoid patients. Therefore, urinary TRH-LI may be a more accurate and sensitive parameter for evaluation of patients with carcinoid tumors than serum TRH-LI. In the present study, serum TRH-LI was higher than the upper normal limit (75 pg/mL; Ref. 16) in 8 of the 12 patients with metastatic carcinoid tumors, whereas urinary TRH-LI was higher than the upper normal limit (0.31 ng/μmol creatinine) in 10 of the 12 patients. Urinary 5-hydroxyindoleacetic acid excretion was elevated in 10 of the subjects, and the 2 patients who did not have increased urinary 5-hydroxyindoleacetic

[2] W. J. de Greef, W. W. de Herder, C. B. Lambalk, W. Klootwijk, E. Sleddens-Linkels and T. J. Visser. The TRH-like peptide pyroglutamyl-glutamyl-prolineamide in human serum is not secreted from the pituitary gland. (Manuscript submitted for publication).

[3] W. Klootwijk, E. J. Rolleman, G. Henneman, T. J. Visser and W. J. de Greef, unpublished observations.

acid excretion showed elevated urinary TRH-LI levels. Therefore, urinary TRH-LI may be used to identify gastrointestinal carcinoids, a group of tumors with variable biochemical, morphological, and biological characteristics (44).

In conclusion, we have demonstrated that most TRH-LI in human blood is caused by <EEP-$NH_2$, which is cleared from blood by renal excretion. The prostate is not a major source of urinary TRH-LI. Finally, urinary <EEP-$NH_2$ may be used as marker for carcinoid tumors.

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