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


ABSTRACT

TRH-like peptides have been identified that differ from TRH (pGlu-His-ProNH2) 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 pGlu-X-ProNH2. 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, pGlu-Phe-ProNH2, and pGlu-Tyr-ProNH2, serum TRH-LI is not caused by these peptides. On high-performance liquid chromatography, serum TRH-LI coeluted with pGlu-Glu-ProNH2 (<EEP-NH2), a peptide produced in, among others, the prostate. Urine of normals and control patients also contained TRH-LI (range 1.14–4.97 and 0.24–5.11 ng/mL, respectively), with similar levels in males and females. TRH represented only 2% of urinary TRH-LI, and anion-exchange chromatography revealed that most TRH-LI in urine was <EEP-NH2. 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-NH2 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-NH2, which is cleared by renal excretion; 2) part of urinary <EEP-NH2 is derived from prostatic secretion into the blood and not directly into urine; and 3) urinary <EEP-NH2 can be used as marker for carcinoid tumors. (J Clin Endocrinol Metab 82: 3068–3073, 1997)
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 nephropathy [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 SRH 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 adrenal cortical 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 urine of 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 <EEP-NH₂ (TRH), <EDP-NH₃, <EEP-NH₂, <EQP-NH₂, <EYP-NH₂, and <EYP-NH₂ 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 [¹²⁵I]-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 antisera 4319. Before each session, the HPLC column was calibrated with 2000 cpm [³H]EHP-NH₂ and 2000 cpm [³H]-EEP-NH₂. 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%.

**Assays**

Levels of TRH-LI were determined by RIA using antisera 4319 or 8880 (14, 16). Although both antisera were raised against the same antigen, antisera 8880 specifically recognizes TRH and hardly cross-reacts with <EEP-NH₂, whereas antisera 4319 detects most peptides with the general structure –EEP-NH₂ (14–16). Antisera 4319 and 8880 were used at a final dilution of 1:2000 and 1:5000, respectively, in the RIAs employing [¹²⁵I]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 [¹²⁵I]TRH from antisera 4319 and 8880. Creatinine and prostate specific antigen (PSA) were measured by routine laboratory methods.

**Chemicals**

<EEP-NH₂ and <EEP-NH₃ were purchased from Bissendorf (Hannover, Germany); <EDP-NH₂, <EQP-NH₂, and <EHPG from Peninsula (Belmont, CA); and <EDP-NH₂ and <EYP-NH₂ from UCB (Brussels, Belgium). [¹¹]TRH (43 Ci/mmol) was obtained from Amersham (Aylesbury, UK), and [³H]-EEP-NH₂ (30 Ci/mmol) was a gift from Dr. S. M. Cockle (Reading, UK). [¹²⁵I]TRH and [³H]-EEP-NH₂ were purchased from Bissendorf (Hannover, Germany). 

**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 ≤ 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 in serum and urine of normals, control patients, and patients with metastatic carcinoids

TRH-LI was undetectable by RIA with TRH-specific antisera 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 antisemum 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 antisemum 4319 in morning urine of normal subjects (Table 1), whereas only low levels were found by RIA with the TRH-specific antisemum 8880 (women 29 ± 3, men 39 ± 6 pg/mL). Serial dilutions of urine produced a dose-response curve parallel to those of <EEP-NH₃ and TRH in the RIA with antisemum 4319 (Fig. 2), and <EEP-NH₃ 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: [TRH-LI] urinary × urinary production rate / [TRH-LI] 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 <EEP-NH₂, <EEP-NH₂, and <EEP-NH₂ was observed, and HPLC analysis showed that these peptides remained intact (data not shown). On the other hand, <EHP-NH₂ (TRH), <EEP-NH₂, and <EYP-NH₂ 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-NH₂ (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-NH₂.

**TRH-LI in serum of anuric patients**

Serum TRH-LI measured by RIA with antisemum 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-NH₂ (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 antisemum 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 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).

### 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

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

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 antisemum 4319. Number of individuals is between parentheses.

*P* < 0.01 compared with normal subjects.

*b* *P* < 0.01 compared with control patients.
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 <EEP-NH₂ and <EYP-NH₂ also are rapidly degraded in serum, these peptides also are unlikely to contribute to serum TRH-LI. On the other hand, <EDP-NH₂, <EOP-NH₂, and <EEP-NH₂ are not degraded and thus may contribute to serum TRH-LI. HPLC analysis revealed that most TRH-LI in serum of normal subjects represents <EEP-NH₂. Recently, <EEP-NH₂ 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-NH₂ on HPLC. Thus, substantial amounts of <EEP-NH₂ are present in human urine.

The presence of <EEP-NH₂ in serum and urine suggests that <EEP-NH₂ is cleared from blood by renal excretion.
that urinary is recovered quantitatively in the urine (42). Thus, it seems women suggests minor contributions of prostatic elution positions of radiography (A) and isocratic reverse phase HPLC (B). Arrows indicate elution positions of <EEP-NH₂ (TRH; 1), <EDP-NH₂ (2), <EQP-NH₂ (3), <EEP-NH₂ (4), and [¹²⁵I]<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

<table>
<thead>
<tr>
<th></th>
<th>TRH-LI (ng/mL)</th>
<th>Creatinine (µmol/mL)</th>
<th>TRH-LI/creatinine (ng/µmol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spontaneous</td>
<td>1.09 ± 0.23</td>
<td>6.9 ± 1.0</td>
<td>0.15 ± 0.01</td>
</tr>
<tr>
<td>Nephrostomy</td>
<td>0.73 ± 0.11</td>
<td>5.0 ± 0.5</td>
<td>0.14 ± 0.01</td>
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<tr>
<td><em>P &lt; 0.01</em></td>
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</table>

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-NH₂ seems to be cleared from blood by glomerular 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-NH₂ in rats, the peptide is recovered quantitatively in the urine (42). Thus, it seems that urinary <EEP-NH₂ can be used as an indicator of the total amount of <EEP-NH₂ released into the blood.

The similarity of serum and urinary TRH-LI in men and women suggests minor contributions of prostatic <EEP-NH₂ 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-NH₂ 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-NH₂ other than the prostate, is present in humans.

As for an alternative source of <EEP-NH₂, 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-NH₂ is a negligible source of serum TRH-LI². In analogy with the presence of <EYP-NH₂ in alfalfa (20), serum and urinary <EEP-NH₂ may be derived from food. However, urinary TRH-LI in normal subjects is not affected by fasting ¹, suggesting a minor dietary intake of <EEP-NH₂. Also in rats, evidence has been obtained that <EEP-NH₂ in serum and urine is not derived from food (42). Thus, further research is needed to identify the origin of <EEP-NH₂ 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. 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-NH₂ 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 acid excretion were women.


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-NH₂ which is cleared from blood by renal excretion. The prostate is not a major source of urinary TRH-LI. Finally, urinary <EEP-NH₂ may be used as marker for carcinoid tumors.

Acknowledgments

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References