Turnover of thyrotropin-releasing hormone in patients with chronic renal failure and chronic alcoholic liver disease

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Abstract. Homogenates of liver and kidney tissues are efficient in degrading TRH, but the liver contains only membrane-bound pyroglutamyl aminopeptidase, active in degrading TRH at the extracellular side of cell membranes. In the present study the effect of liver and kidney failure on the degradation of infused TRH was investigated in man. In 7 uremic patients (group I) and 7 patients with chronic alcoholic liver disease (group II) plasma clearance rate, half-time of disappearance (t1/2) and half time of disappearance of TRH in serum in vitro (t1/2p) was determined. The plasma clearance rate, t1/2 and t1/2p were, respectively, 19.8±6.2 ml · kg−1 · min−1, 6.6±1.5 min and 16.4±6.2 min in group I versus 28.2±4.8 ml · kg−1 · min−1, 9.3±2.6 min and 25.3±15 min (mean ± so) in group II. The volume of distribution of TRH was 19.9±% of the body weight in group I and 36.5±% in group II. The calculated half-time in the extravascular tissue compartment (t1/2v) was 5.4±1.4 min in group I and 9.2±2.7 min in group II patients (mean ± so). TRH metabolism in the uremic patients was almost identical to that previously reported in normal subjects. In the patients with chronic liver disease plasma clearance rate was significantly greater than in normal subjects, indicating an increased TRH-degrading enzyme activity in the tissue compartment. However, owing to the very large expansion of this compartment, the t1/2 and t1/2p were significantly prolonged. Hence, half-time determination of TRH is no reliable indicator of overall TRH degradation in patients with liver disease.

Hypothalamic TRH acts as a hormone on the anterior pituitary, whereas the extrahypothalamic TRH distributed throughout the central nervous system probably participates in neurotransmission (1). The TRH located in peripheral organs such as the gut, pancreas, thyroid, and reproductive system (2-5) may have endocrine, neurotransmitter as well as paracrine functions (6-10). Of crucial importance for these cellular communications is an adequate clearance of TRH from its sites of action by tissue and serum degradation. Especially homogenates of animal liver and kidney tissues were reported to have a high TRH degrading activity (11,12). However, there is lack of information on the ability of these organs to inactivate TRH in vivo in animals as well as in man. In the present investigation data on serum and tissue degradation of TRH were obtained in patients with impaired liver or kidney function.

Patients and Methods

Seven uremic patients and 7 patients with alcoholic liver disease participated in the study.

The uremic patients comprised 3 men and 4 women (group I). Mean age was 52 years (range 20-57). Five of the patients were treated with hemodialysis 5 h 3 times a week. Their renal disease were chronic glomerulonephritis (N=2, medical treatment phenobarbital 100 mg/day and nitrazepam 5 mg/day, respectively), chronic pyelonephritis (bilaterally nephrectomized, N=1, medical treatment theophyllamine 600 mg/day), chronic nephropathy (not classified, N=1, medical treatment acetylsalicylic acid 75 mg/day and persantine 100 mg/day), and nephrocalcinosis (owing to hyperoxaluria, N=1, medical treatment nifedipine 60 mg/day and acetylsalicylic acid 100 mg/day). The two other patients had polycystic renal disease (no medical treatment) and renal amyloidosis secondary to rheumatoid arthritis (medical treatment furosemide 160 mg/day, propranolol 240 mg/day and hydra-
lazine 100 mg/day). Mean serum creatinine was 794 μmol/l (range 566-1041, reference range 55-110), mean serum urea 26.0 mmol/l (range 21.5-31.8, reference range 3.0-7.5) and mean total serum CO₂ was 19 mmol/l (range 14-23, reference range 22-30).

Patients with alcoholic liver disease included 6 men and 1 woman (group II). Mean age was 43 years (range 29-59). The diagnosis of alcoholic liver disease was suspected because of chronic alcohol abuse, stigmata of chronic liver disease and biochemical findings. Liver biopsy showed cirrhosis in 4 and severe steatosis in one patient. Biopsy was not performed in 2 patients. Four of the patients had esophageal varices and 4 patients had different degrees of ascites at the time of investigation. Three patients were treated with spironolactone (100-200 mg/day) and furosemide (80-120 mg/day). One of these patients received thiamide 900 mg/day. One patient received daily bendroflumethiazide 7.5 mg combined with potassium chloride 1719 mg. Two patients received no medicine. Mean serum albumin was 30.1 g/l (range 20.1-47.0, reference range 36-50), mean alkaline phosphatase 261 U/l (range 166-454, reference range 70-272), serum bilirubin was 45 μmol/l (range 8-145, reference <21), and serum gamma-globulin 17.4 (range 8.1-23, reference range 7-14 g/l).

Medicine was not given the last 24 h prior to the investigation.

Informed consent was obtained from all the participants. The study was in accordance with the Helsinki II Declaration and approved by the local Medical Ethical Committee.

**Materials**

Synthetic TRH was from Sigma Chemical Co., St. Louis, MO, and Roche, Basel, Switzerland. SP-Sephadex C-25 was from Pharmacia Fine Chemicals, Uppsala, Sweden. Tritiated TRH (specific activity 106.3 Ci/mmol) was from New England Nuclear, Boston, MA. [3H]TRH was purified by SP-Sephadex C-25 chromatography shortly before use. The immunoreactivity of the purified [3H]TRH was 0.66 pmol/10⁶ cpm when measured in the TRH radioimmunoassay. Diethylether and anhydrous methanol were obtained from Merck, Darmstadt, FRG.

**TRH infusion**

TRH, 10 nmol/min was constantly infused into an antecubital vein for 60 min. Blood samples of 6 ml were taken through an indwelling catheter 3 min before, during the infusion, and at 3, 6, 9, 12, 15, 18, 21 and 24 min after its end. Enzymatic degradation of TRH in blood samples was prevented by cooling as described previously (13). The samples were kept on ice, immediately mixed with 250 IU heparin and centrifuged for 2 min at 2°C. Plasma (2 ml) was mixed with traces of [3H]TRH for recovery studies and extracted with methanol. After evaporation, the methanol extract was chromatographed on a SP-sephadex C-25 column. TRH was measured in the eluate by radioimmunoassay.

**Serum degradation of TRH**

Shortly before each infusion 40 ml of blood was withdrawn. At time, 0, 0.8 ml serum at 37°C was added to each of 16 glass tubes at 37°C. Half of the tubes contained 25 μl 0.04 mol/l phosphate buffer, pH 7.2 (control tubes); the other half contained 8 pmol TRH in 25 μl of the buffer to obtain a concentration similar to that seen after infusion of 10 nmol TRH/min. Methanol (4 ml) was added to two control tubes and to TRH-containing tubes at 0, 20, 40, and 60 min of incubation at 37°C. After centrifugation, the supernatants were evaporated and redissolved in 0.2 mol/l phosphate buffer, pH 7.2, for the RIA of TRH. The TRH anti-serum was highly specific for alterations in the N- and C-terminal part of the molecule, but poorly recognized minor alterations in the histidyl residue.

**Calculations**

The methods for calculation of the half-life of disappearance of TRH in the extravascular tissue compartment were described in detail recently (14). The pharmacokinetic principle is determination of plasma clearance rate and fractional rate of degradation of TRH in vivo by means of a constant TRH infusion and simultaneous determination of the fractional degradation rate of TRH in serum in vitro.

In all calculations, the pre-infusion level of TRH-like immunoreactivity in plasma and serum was subtracted. This does not represent genuine TRH and is not affected by TRH degrading enzymes (13). The average pre-infusion TRH-like immunoreactivity was 3.1 and 4.7% of steady state plasma TRH in uremic patients and in patients with chronic liver disease, respectively. Plasma clearance rate (PCR) was calculated as the ratio of the rate of infusion of TRH to its plasma concentration at steady state (mean of 40, 50 and 60 min) (15). Total volume of distribution (V) was (PCR . t½)/0.693 (16). The fractional rate of degradation of TRH in vivo (Kd) and in serum in vitro (Kd) was the slope of the disappearance curves in vivo and in vitro calculated by least squares regression analysis. In patients with a hematocrit within the reference range (6 of the patients in group II), the plasma volume (Vp) was determined from age, sex and body weight by use of a nomogram (17). In patients with a hematocrit below the reference range (all patients in group I and one patient in group II), the blood volume (Vb) was determined and Vp was calculated from the formula: Vp = Vb - (hematocrit . 0.9) (17). The volume of distribution of TRH in the extravascular tissue compartment (Vv) was Vv = V - Vp. The fractional degradation of TRH in this compartment (Kv) was Kv = K - (Vp/Vv) . (K - Kp - Kd).
(13), where $K_u$ is the fractional rate of excretion of TRH from $V_p$ in urine. For calculation of $K_u$ in the patients with liver disease, a renal excretion of 6.7% of infused TRH is used, which is the mean value in 12 normal subjects (13). In the uremic patients $K_u$ was supposed to be zero.

Statistical evaluation
The Student’s non-paired $t$-test of logarithmically transformed values was used for statistical evaluation, with a 5% limit of statistical significance. Data are given as mean±1so or as mean and range.

Results
The plasma TRH concentrations at 40 and 50 min of infusion were 103.8±13.0 and 101.8±17.5% (mean±sd) of the concentration at 60 min in group I, and 97.2±10.3 and 106.9±11.7% in group II. Thus, TRH concentrations were in steady state in both group I (mean 6.27 nmol/l) and group II (mean 9.45 nmol/l) at the discontinuation of TRH infusion at 60 min.

The plasma clearance rate, volume of distribution, and the half-life of TRH in vivo, in serum, and in tissues are depicted in Table 1 for uremic patients and patients with chronic alcoholic liver disease. For comparison, the results of a previous study on pharmacokinetics of TRH in normal subjects are also given (14).

The mean values for $t_{1/2}$, PCR and V of TRH in the uremic patients were not different from those found in normal subjects.

Fig. 1 shows the relationship between plots of individual values of $t_{1/2}^p$ (x) and $t_{1/2}^i$ (y) in the uremic patients. The correlation graph was $y=0.19x + 2.27$, $r=0.84$, $p=0.02$. The plots lie near the correlation graph of $t_{1/2}^p$ to $t_{1/2}^i$ of TRH in normal subjects: $y=0.15x + 3.80$, $r=0.95$, $p<0.001$, $N=14$. (In normal men the correlation was $y=0.17x + 3.46$, $r=0.91$, $p=0.004$ and in normal women $0.13x + 4.10$, $r=0.96$, $p<0.001$).

Fig. 2 shows the relationship between plots of $t_{1/2}^p$ and $t_{1/2}^i$, of TRH in the patients with chronic alcoholic liver disease (group II). The plots are widely scattered around the correlation graph of $t_{1/2}^p$ to $t_{1/2}^i$ in normal subjects.

In these patients the correlation between $t_{1/2}^p$ and $t_{1/2}^i$ was $y=0.06x + 7.55$, $r=0.35$. This correlation was not statistically significant ($p=0.44$). Nearly similar figures were obtained if only data from the 6 male patients with liver disease were used for the calculations (Fig. 2). The $t_{1/2}^p$ and $t_{1/2}^i$, in group II were significantly prolonged as compared with the $t_{1/2}^p$ and $t_{1/2}^i$, of TRH of normal subjects. The $t_{1/2}^p$ was significantly longer in the male subjects of group II (27.1±15.5 min, $N=6$) than in the normal male subjects (12.6±6.7 min, $N=7$).

Values for PCR and V of TRH in group II were significantly greater than those found in normal subjects. The two patients with the most severe ascites had the greatest volume of distribution of TRH (48.3 and 38.9% of body weight). However, there was no correlation between the parameters of TRH metabolism and clinical state, the medical

<table>
<thead>
<tr>
<th>N</th>
<th>PCR (m1 · kg−1 · min−1)</th>
<th>$t_{1/2}^i$ (min)</th>
<th>$t_{1/2}^p$ (min)</th>
<th>$t_{1/2}^i$ (min)</th>
<th>V (% body weight)</th>
</tr>
</thead>
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<tr>
<td>Normal women</td>
<td>7</td>
<td>22.2±4.0</td>
<td>6.0±1.4</td>
<td>21.1±10.2</td>
<td>6.9±1.5</td>
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<td>7</td>
<td>21.2±5.6</td>
<td>7.3±1.4</td>
<td>12.6±6.7</td>
<td>5.6±1.2</td>
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<tr>
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<td>14</td>
<td>21.7±4.7</td>
<td>6.6±1.5</td>
<td>16.8±9.4</td>
<td>6.2±1.4</td>
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<tr>
<td>Uremic patients (Group I)</td>
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<td>19.8±6.2</td>
<td>6.6±1.5</td>
<td>16.4±6.2</td>
<td>5.4±1.4</td>
</tr>
<tr>
<td>Patients with liver disease (Group II)</td>
<td>7</td>
<td>28.2±4.8</td>
<td>9.3±2.6</td>
<td>25.3±15.0</td>
<td>9.2±2.7</td>
</tr>
<tr>
<td>Group II (men only)</td>
<td>6</td>
<td>27.4±4.6</td>
<td>9.5±2.7</td>
<td>27.1±15.5</td>
<td>9.4±2.9</td>
</tr>
</tbody>
</table>

PCR: plasma clearance rate of TRH; $t_{1/2}, t_{1/2}^p$ and $t_{1/2}^i$: half-lives of disappearance of TRH in vivo, in serum (in vitro), and in tissues, respectively; V: volume of distribution of TRH. $p<0.01$ (observed versus normal men and women). $p<0.05$ (observed versus normal men). Statistical comparison with normal men.
treatment given until 24 hours prior to the investigation, biochemical indices of liver function or the degree of cholestasis.

Discussion
The permeability of the blood-brain barrier to TRH is poor (18). Thus, degradation of the infused TRH in the tissue compartment is mainly occurring in peripheral organs.

Inactivation of TRH in tissues is catalysed by three enzymes. One is the TRH specific membrane-bound pyroglutamyl aminopeptidase (type II) with peripheral location in liver and lungs. Owing to similar physical, chemical and biological properties this enzyme and the TRH-degrading serum enzyme are suggested to be derived from the same gene (19). The two other enzymes are cytosolic with ubiquitous distribution and are less specific. They are type I pyroglutamyl aminopeptidase and the post proline cleaving enzyme. For review of TRH-degrading enzymes see Wilk (20) and Bauer (21).

In a previous study we found that the rate of disappearance of infused TRH in serum and tissues was very constant in normal subjects and independent of sex, phase of menstrual cycle, and time of day. The half-life of disappearance of TRH in serum and tissues of normal subjects were strongly linearly correlated (14).

Homogenates of kidney tissue are especially efficient in degrading TRH, although the pyroglutamyl aminopeptidase (type II), recently reported to be located at the extracellular side of cell membranes is absent in the kidney (19,21). In the patients with chronic renal failure, the parameters of TRH metabolism were not significantly different.
from those found in normal subjects. Hence, a normal amount of functioning kidney cells seems not to be important for degradation of infused TRH.

Probably infused TRH is not degraded by cytosolic enzymes since TRH as other neurotransmitters seems not be taken up by cells (21). This is consistent with our finding of a volume of distribution of TRH, which is approximately 20% of the body weight in normal subjects and in patients with renal failure. Hence TRH is distributed exclusively or nearly exclusively in the extravascular compartment. Consequently renal excretion of TRH in normal subjects is caused only by glomerular filtration and not by tubular transport. The normal mean value for renal excretion of infused TRH, 6.7% (14), corresponds to a renal clearance of 103 ml/min. A decrease in rate of TRH disappearance owing to the absence of a renal TRH clearance of this order of magnitude would hardly be detectable.

The normal close relationship between the activities of the TRH-degrading serum enzyme (as expressed by $t_{1/2p}$) and the membrane-bound pyroglutamyl aminopeptidase (as expressed by $t_{1/2i}$) was not significantly altered in the uremic patients.

Our expectation before this study was that the plasma clearance rate of TRH would be low in the patients with chronic liver disease. This was based on the reduction in liver tissue, and on the expectation that reduced hepatic blood flow and increased amounts of intracellular fluid (22) might impair transport of TRH to the membrane bound pyroglutamyl aminopeptidase of the liver cells. Surprisingly the plasma clearance rate was found to be significantly higher (30%) in these patients than in normal subjects. This was not due to enhanced degradation of TRH in the intravascular compartment since direct measurements of $t_{1/2p}$ in vitro showed a reduction in TRH degradation in plasma. Consequently, the high plasma clearance rate could only be explained by an increase in total TRH-degrading enzyme activity in the extravascular compartment.

The $t_{1/2i}$ was significantly prolonged in the patients with chronic liver disease, indicating that the fractional rate of degradation of TRH in the extravascular compartment was reduced. However, owing to the very large expansion of the volume of distribution in these patients, the total degradation of TRH in the extravascular compartment was high. This gives rise to an increase in plasma clearance rate.

Owens et al. (23) studied growth hormone kinetics in patients with chronic liver disease and found prolonged $t_{1/2}$ and increased plasma clearance rate of growth hormone. They contended that $t_{1/2}$ of growth hormone was an unreliable index for the metabolism of growth hormone in these patients. Correspondingly, we conclude that $t_{1/2}$ does not reflect the metabolism of TRH in patients with chronic alcoholic liver disease.

The origin of the TRH-degrading serum enzyme is not known, but the prolonged $t_{1/2p}$ in the male patients with chronic liver disease may indicate that it is synthesized in the liver. Another mechanism behind the low TRH degradation in plasma could be loss of enzyme owing to leakage into the interstitial fluid. This could be the origin of the enhanced enzyme activity in the tissue compartment. However, the TRH-degrading serum enzyme, which is structurally very similar to the membrane-bound pyroglutamyl aminopeptidase is of high molecular weight (280,000). Notably our measurements of TRH degradation in vitro is a measure of the activity of circulating TRH-degrading enzyme(s) only. If small amounts of TRH in serum are degraded in vivo by enzymes exposed by the endothelial lining of the blood vessels (24), this will be included in the calculated tissue degradation of TRH.

It is at present unknown, which enzymes are responsible for the increased TRH-degrading activity in the extravascular, extracellular tissue compartment of patients with chronic liver disease. Also the putative role of enhancers or inhibitors of TRH degradation remains to be clarified.

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References


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