CLINICAL STUDY

Changes in metabolism of TRH in euthyroid sick syndrome

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Abstract

Objective: The aim of this study was to examine the metabolism of a simple dose, intravenously administered TRH bolus of 200 µg, in patients with euthyroid sick syndrome (ESS).

Patients and Methods: A TRH test was performed on ten ESS patients and ten controls upon admission (d1) and after recovery (d2). Blood samples were collected at 0, 10, 20 and 30 min after TRH injection. We analyzed the volume of distribution (V_d), the plasma clearance rate (PCR), the fractional clearance rate (FCR), the half-life ($t_{1/2}$) and the TSH response to the injection of TRH.

Results: All patients had lower tri-iodothyronine (T_3) levels compared with controls (0.9 ± 0.1 nmol/l vs 1.9 ± 0.1 nmol/l; $P < 0.0001$; mean $\pm$ s.d.; paired t -test). In addition, the V_d (16.7 ± 5.9 l vs 30.6 ± 0.6 l; $P < 0.0005$) and PCR (2.0 ± 0.80 l/min vs 3.3 ± 0.25 l/min; $P < 0.0005$) were found statistically lowered in patients than in controls, whereas FCR (0.119 ± 0.01 per min vs 0.110 ± 0.01 per min; $P < 0.025$) was found increased in patients as opposed to controls. The $t_{1/2}$ of exogenously administered TRH was increased in ESS compared with controls (7.2 ± 0.7 min vs 6.3 ± 0.6 min; $P < 0.005$). TSH response to TRH was found significantly repressed at 10, 20 and 30 min after TRH injection. On d2, these findings had reverted to normal and no changes regarding the kinetics of TRH and the response of TSH could be detected between patients and controls.

Conclusions: The results demonstrate an impairment of TRH metabolism in ESS. The findings may suggest altered enzymatic activity, responsible for TRH degradation in states of acute ESS. These changes might be involved in the pathogenesis of ESS and represent part of an adaptive mechanism to this syndrome.

European Journal of Endocrinology 141 337–341

Introduction

The euthyroid sick syndrome (ESS) is characterized by normal or even decreased thyroxine (T_4) levels, decreased tri-iodothyronine (T_3 , i.e. low T_3 syndrome), usually elevated reverse T_3 (rT_3) levels, normal basal thyrotropin (TSH) serum concentrations, blunted TSH response to thyrotropin-releasing hormone (TRH) testing and decreased nocturnal TSH surge (1–3). Free T_4 values, as determined by equilibrium dialysis, are frequently increased and may be secondary to reduced serum clearance rates of T_4 (4). The constellation of ESS is observed in a variety of clinical disorders, from carbohydrate deficiency, surgical intervention, liver or kidney failure, and in various situations encountered in intensive care unit patients (5–7). The etiopathogenesis of ESS is multi-factorial, based essentially on impaired T_4 binding to serum carrier proteins; it is caused by circulating inhibitors, such as indoxyl sulfate and hippuric acid (8). The decreased serum T_4 binding

leads to reduced T_4 uptake from blood to tissues, and consequently to lower T_4 clearance rates (9). Cytokines, especially tumor necrosis factor (TNF) and interleukin-1 (IL-1), modulating type I iodothyronine deiodinase (ID-I) activity, seem also to be involved in the pathogenesis of the ESS (10, 11). Consequently, decreased activity of ID-I causes a lower T_3 production rate and a decreased rT_3 clearance rate, which leads to the observed reduced levels of circulating T_3 and the increases in rT_3 levels.

The hypothalamic neuropeptide TRH is inactivated by the TRH-degrading ectoenzyme, a TRH-specific metalloproteinase, which is mainly regulated by thyroid hormones (12). A disturbed TRH feedback regulation by the thyroid hormones in ESS may conduct changes in TRH secretion and metabolism. Indeed, in experimental ESS the content of proTRH mRNA in the paraventricular nucleus (PVN) is inappropriately low, despite low circulating levels of thyroid hormones (13, 14). However, there are no reports regarding TRH metabolism and the activity of the enzymes responsible for

peripheral serum degradation of TRH in ESS in humans. It was the aim of the study to investigate the kinetics of TRH, and the TSH response to TRH, in patients with severe ESS.

Patients and methods

Ten patients with a median age 51 years (range 32–60 years), admitted into the intensive care unit because of myocardial infarction ($n = 6$), bronchopneumonia ($n = 3$), stroke ($n = 1$) and concomitant ESS, were included in the study. None of the patients had preexisting thyroid, psychiatric, metabolic or endocrine diseases, liver failure (prothrombin time $<20\%$) or renal failure, and none was under treatment with glucocorticoids, somatostatin, dopamine or dopamine antagonists, i.e. drugs known to affect TSH secretion. Ten normal, euthyroid persons with a median age 48 years (range 27–58 years), who were mainly clinic personnel, served as the control group. Thyroid drugs or other medicaments known to affect the hypothalamic–pituitary–thyroid axis were not being taken by any of the individuals.

Verbal consent was obtained from all patients or relatives before inclusion in the study.

The pharmacokinetics of TRH were studied using compartmental analysis, following single bolus i.v. administration of the compound. A standard TRH test (Antepan 200 μg , Henning-Berlin GmbH, Berlin, Germany) was performed on all subjects shortly after admission (d1). TRH was administered to the controls and eight patients between 0600 and 0900 h, whereas two patients received TRH administration between 1100 and 1300 h. Blood samples were collected at 0, 10, 20 and 30 min after TRH administration. T_4 and T_3 were measured at baseline, while TSH and TRH were measured at all above mentioned time intervals. The test was repeated approximately 4 weeks later (d2) upon the patients' recoveries, and just before being discharged.

Assays

Blood TRH was measured by a specific and very sensitive RIA. The samples were collected in cold methanol (90%). They were carefully mixed and double-evaporated at 37°C . The extract was dissolved with 1 ml of distilled water and lyophilized. The final extract was dissolved with 220 μl of phosphate-buffered potassium (pH 7.4) and was assayed (15). TRH recovery of graded quantities of synthetic TRH to blood samples was between 101 and 105%. Intraassay coefficient of variation (CV) between 20 and 80% binding was calculated at $4.2 \pm 1.0\%$ on four assays. The sensitivity was 1 pg (3 fmol) per tube. Serum TSH levels were measured immunometrically using acridinium ester labeled monoclonal TSH antibody and a second monoclonal TSH antibody, fixed to coated tubes (LUMI test-TSH, BRAHMS,

Berlin, Germany). The analytic (interassay) sensitivity was 0.04 mU/l. The normal range was between 0.3 and 3 mU/l. The intraassay CV was 4.7%. Serum T_4 and T_3 levels were measured by immunoluminometric assays (Lumi- T_4 and Lumi- T_3 , BRAHMS, Berlin, Germany). The intraassay CV values were 4.4% and 4.8% respectively, and the normal values were 66–155 nmol/l and 1.2–2.7 nmol/l respectively.

Pharmacokinetics

The two sets of ten TRH kinetic data sets of six points each were batched as controls versus ESS groups. Each batched group was then fitted sequentially to sums of 1 and 2 exponential using the expert system program DIMSUM (16, 17). The single exponential model $A\exp(-\lambda t)$ fitted best, according to the six statistical criteria built into DIMSUM. The plasma clearance rate, $\text{PCR} = 100\lambda/A$; distribution volume, $V_d = 100/A$, fractional clearance rate, $\text{FCR} = \lambda$; and half-life, $t_{1/2} = \ln 2/\lambda$ were computed, where A is expressed as % dose/volume and λ has t^{-1} units.

The model that fitted best for both groups was one of the simplest, consisting of one compartment each for TRH and TSH, with a time delay compartment in-between.

The combined TRH disappearance and TSH appearance data were also batched as above and fitted into several kinetic models of increasing complexity.

Statistical analysis

Data are expressed as mean $\pm$ s.d. The level of statistical significance was $P < 0.05$. A paired t -test was used for the comparison of hormonal results between ESS and controls. The relationships between variables were calculated by simple and multiple linear regression analysis.

Results

Table 1 presents some demographic data and biochemical parameters in ESS patients and controls.

Table 1 Data and various parameters of ESS patients and controls (mean $\pm$ s.d.).

	ESS ($n = 10$)	Controls ($n = 10$)	P values
Age (years)	51 $\pm$ 8	48 $\pm$ 9	n.s.
Weight (kg)	66.2 $\pm$ 9	64.3 $\pm$ 8	n.s.
T_4 (nmol/l)			
(n.r. 66–155)	85.7 $\pm$ 5.1	121.6 $\pm$ 7.6	n.s.
T_3 (nmol/l)			
(n.r. 1.2–2.7)	0.9 $\pm$ 0.1	1.9 $\pm$ 0.1	<0.001
TSH (mU/l)			
(n.r. 0.3–4.0)	0.7 $\pm$ 0.2	0.8 $\pm$ 0.2	n.s.
Δ -TSH (mU/l)	2.0 $\pm$ 0.3	6.5 $\pm$ 1.5	n.s.

n.r., normal range. P values obtained using paired t -test.

Figure 1 Degradation curve of TRH in blood of ten ESS patients compared with ten controls, following a single-dose (i.v. 200 µg) injection of TRH.

Total T_4 in ESS was numerically lower but statistically similar to that of the control population (ESS: 85.7 ± 5.1 , Control: 121.6 ± 7.6 nmol/l; $P > 0.05$). However, in ESS patients total T_3 levels were statistically significantly decreased compared with the controls (0.9 ± 0.1 nmol/l vs 1.9 ± 0.1 nmol/l; $P < 0.001$). Basal TSH (0.7 ± 0.2 mU/l) as well as Δ -TSH (peak-basal; 2.0 ± 0.3 mU/l) were found to be lower, but not statistically significant, compared with the controls (0.8 ± 0.2 mU/l; 6.5 ± 1.5 mU/l; n.s., respectively).

Figure 1 shows the degradation curve of TRH in ESS and in controls after intravenous TRH administration. The disappearance of TRH in blood is shown as an exponential decay curve. Each set of data was fitted individually for both single and biexponential functions. In all cases, single exponential fitted best.

Table 2 presents the calculated pharmacokinetic parameters for the single-dose study of TRH in ESS and in controls. Time of peak concentration (T_{max}) was, in all patients and controls, at 2 min after the

injection of TRH. The mean peak TRH concentrations (C_{max}) was higher in the ESS patients (54.370 ± 8.990 fmol/ml) compared with controls (13.400 ± 1.020 fmol/ml; $P < 0.0005$). V_d and PCR were smaller in ESS (16.7 ± 5.9 l; 2 ± 0.8 l/min respectively)

Table 2 Pharmacokinetic parameters of intravenously administered TRH in ESS patients and controls (mean $\pm$ s.d.).

	ESS	Controls	P values
T_{max} (min)	2	2	
C_{max} (fmol/ml)	54.370 ± 8.990	13.400 ± 1.020	<0.0005
V_d (l)	16.7 ± 5.9	30.6 ± 0.6	<0.0005
PCR (l/min)	2.0 ± 0.80	3.3 ± 0.25	<0.0005
FCR (min^{-1})	0.119 ± 0.01	0.110 ± 0.01	<0.025
$t_{1/2}$ (min)	7.2 ± 0.7	6.3 ± 0.6	<0.005

T_{max} , time to C_{max} ; C_{max} , maximum plasma concentration; V_d , volume of distribution; PCR, plasma clearance rate; FCR, fractional clearance rate; $t_{1/2}$ = half-life. P values obtained using paired *t*-test.

Figure 2 TRH-test in ten ESS patients at admission (d1), after recovery (d2), and in ten controls (means $\pm$ s.d.). The blunted TSH response on d1 was reverted on d2. * $P < 0.002$ compared with d2 and to controls (paired t -test).

compared with controls (30.6 ± 0.6 l/min; 3.3 ± 0.25 l/min; $P < 0.0005$ respectively). In contrast, FCR was found increased in ESS compared with controls (0.119 ± 0.01 vs 0.110 ± 0.01 ; $P < 0.025$). The $t_{1/2}$ was computed longer in ESS (7.2 ± 0.7 min vs 6.3 ± 0.6 min; $P < 0.005$ compared with normal subjects).

Figure 2 presents the TRH test in ESS and in controls. In ESS, TSH response to TRH administration was found repressed by 63.8% at 10 min (1.27 ± 0.4 mU/l vs 1.99 ± 0.4 mU/l; $P < 0.002$), by 52.2% at 20 min (1.97 ± 0.3 mU/l vs 3.77 ± 0.7 mU/l; $P < 0.002$), and by 36.7% at 30 min (2.69 ± 0.3 mU/l vs 7.32 ± 1.6 mU/l; $P < 0.002$) compared with controls. The test was repeated after recovery of the patients, and this time no statistically significant changes were observed compared with controls (TRH, d2: PCR = 3.1 ± 0.26 l/min).

Discussion

The results demonstrate an impairment of TRH metabolism in ESS. Different factors might be involved. A fundamental determinant is constituted by altered activity of the enzymes which break down TRH. It is known that T_3 and/or T_4 and estradiol are the regulators of the tissue-specific activity of TRH-degrading ectoenzyme (12, 18). ESS has been associated with low thyroid

hormone levels coupled with 'inappropriately' normal TSH serum concentration. Thus, acute ESS might influence the biological function of the TRH-degrading ectoenzyme and, consequently, the degradation of TRH in these patients. As basal TSH levels are normal, it is difficult to assert that a deficiency in hypothalamic TRH secretion in our patients exists. Therefore, we deduce that a disturbed TRH metabolism, as we found in terms of V_d , PCR, FCR and $t_{1/2}$, may affect the biological efficiency of TRH. In fact, TRH V_d was reduced by 54.5% and PCR by 60.6% in ESS compared with controls; in contrast, FCR was found increased. These findings might be explained by a reduced activity of the enzymes catalyzing TRH, which may lead to a delay of degradation, as was shown by the prolongation of $t_{1/2}$. This could be co-responsible for the maintenance of 'normal' low TSH levels, in spite of the presence of low thyroid hormone serum concentrations. However, the mechanism is not very clear. One could hypothesize a partial TRH resistance induced by the illness, as has been reported in a study using TRH infusions, but it seems rather improbable, as our patients were in acute phases of disease (19). Cytokines such as TNF and IL-1 which may indirectly reduce the content of TRH and of pro-TRH in RNA in the PVN can also play a substantial role (20, 21). Consequently, the insufficiency of the PVN to respond to ESS, by increasing the TRH biosynthesis, may assist as a concurrent mechanism or as a contributor to ESS by maintaining 'inappropriately' normal TSH levels and by lowering the set-point for thyroid hormones on the thyrotroph (22). Additionally, low T_3 concentrations at hypothalamic level in ESS may alter the hypothalamic thyroid feedback control (23). Our results, upon performance of the TRH test in ESS, indirectly support that the pituitary response to TRH administration is restored after T_3 levels are apparently fully recovered. Moreover, in the classical feedback regulatory system, low T_3 levels in ESS may affect the transduction of TRH signals at the thyrotroph by regulating the number of corresponding receptors (24). The TSH response to TRH was found slightly overshoot at discharge compared with controls. This phenomenon has been previously reported (14, 25). The mechanism is not clear. One possible explanation could be a transient elevation of TSH as a result of the normalization of T_3 serum concentration (26). Another mechanism could be an increased sensitivity of TSH receptors in patients recovering from ESS.

Finally, the concept of the disturbed neuroendocrine response in ESS possibly being partly of hypothalamic origin, was supported in a recent study that used *in situ* hybridization. Research showed a positive correlation of total TRH mRNA in the PVN and serum concentrations of TSH and T_3 in severely critically ill patients just before death (27).

The disturbed TRH degradation in ESS may also lead to impaired binding of TRH to the different classes of its receptors. It has been reported that different ligands,

including opioid peptides and substance P, can compete for these receptors (28). Thus, in ESS, an increase of ligands, which antagonize the binding of TRH to its receptors, may reduce the action of TRH at a molecular level.

Clearly, there are numerous factors to take into account regarding ESS when defining precise conditions in any given tissue, such as blood flow rates, levels of receptor expression, the relative affinities and disassociation–association rates of TRH and its receptors. In addition to the modulation of these factors, which regulate the endocrine function, other local conditions are also possible, such as compartmental metabolic modifications, which may represent a subtle mechanism for altering the local action of a hormone. We believe that our results provide new aspects regarding a multi-variable syndrome such as ESS.

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Received 19 April 1999

Accepted 24 June 1999