Plasma neurotensin levels in humans: relation to hormone levels in diseases involving the hypothalamo–pituitary–thyroid axis

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This study was aimed to investigate, in humans, the possible relationship between plasma neurotensin (NT) levels and the activity of the hypothalamo–pituitary–thyroid axis. Neurotensin was measured by radioimmunoassay in 14 healthy adult volunteers and in 41 patients among whom 10 were considered as controls and 31 had thyroid dysfunction according to free thyroxine and thyrotropin plasma values. Basal NT levels were not significantly different in healthy adults and in control patients: 9.7 ± 1.1 fmol/ml (mean ± SEM) vs 13.3 ± 2.9 fmol/ml, respectively. In patients with central hypothyroidism the NT level was significantly lower (5.7 ± 1.2 vs healthy volunteers and controls; p < 0.05) and in patients with peripheral hypothyroidism and hyperthyroidism the NT level was significantly higher (35.9 ± 12.8 and 29.9 ± 9.5 fmol/ml, respectively, vs healthy adults (p < 0.01) and vs controls (p < 0.05)). After thyrotropin-releasing hormone (TRH) injection (250 µg iv) in nine subjects (two control patients, five patients with hypothyroidism and two patients with hyperthyroidism), NT levels decreased independently of the endocrine status from mean values of 13.4 ± 8.4 at basal level to 7.3 ± 0.8 fmol/ml 30 min after injection (p < 0.01 on paired percentage decrease values). These data suggest that plasma NT levels in humans depend upon the pituitary–thyroid status and indicate that TRH could exert a negative regulation on circulating NT levels.

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Neurotensin (NT) is a biologically active peptide originally isolated from bovine hypothalamus (1) and later from bovine and human intestine (2, 3). Neurotensin levels are also measurable in rat (4) and human plasma (5, 6), and a possible peripheral source of NT from lymphocytes has been shown (7). In the brain, NT has been localized in various structures and acts as a neurotransmitter. In rats, it has been shown that NT plays a role in the regulation of dopamine systems (8–10) as well as in anterior pituitary hormone secretion (11–15). In contrast, in humans, Blackburn et al. (16) did not observe any significant changes in plasma pituitary hormone concentrations after infusion of NT (at a mean dose of 2.3 pmol/kg in a restricted number of subjects). However, a variation of circulating NT levels after growth hormone injection was observed in children with growth delay, raising the possibility that circulating levels of NT may be involved in the regulation of pituitary secretions (6).

In the present work, we have tested the possibility of a relationship between NT plasma levels and plasma free thyroxine (FT₄) or thyrotropin (TSH) concentrations. We also examined the effects of TRH administration on plasma NT levels. The hypothesis was that circulating NT levels may have a physiological implication in the control of pituitary–thyroid function.

Methods

Subjects

Fifty five subjects were included in this study:

(i) Fourteen healthy volunteers (five females (F) and nine males (M)) aged 23–57 years (33.5 ± 2.7, mean ± SEM) were recruited from the Clinical Investigation Center (CIC, Hôpital Saint-Antoine, Paris, France). None of the subjects had clinical or laboratory evidence of any disease or were taking any medication.

(ii) Forty-one patients, ranging from 1 month to 35 years old, were investigated for thyroid disease and were classified into four groups (1–4) according to FT₄ and TSH levels at the time of sampling. Patients from group 1 (5F, 5M, 13.6 ± 2.7 years old) were considered as “controls”, with FT₄ levels ranging from 12 to 20 pg/ml. Group 2 (5F, 3M, 23 ± 3.4 years old) consisted of patients with hypothyroidism of central origin with an FT₄ value below 9 pg/ml and low TSH values (< 1 µU/ml). Patients from group 3 (7F, 3M, 10.2 ± 3.2 years old) had hypothyroidism of peripheral origin with low values of FT₄ (< 9 pg/ml) and high TSH values (> 10 µU/ml). Group 4 (10F, 3M, 8.4 ± 2.7 years old) included patients with peripheral hyperthyroidism with high FT₄ values above 22 pg/ml and TSH below 0.5 µU/ml.
All subjects (or parents) were informed of the nature and purpose of the study before blood sampling. Their voluntary consent was obtained. This study was approved by the local ethics committee.

Protocols

Venous blood samples (5 ml) were collected from healthy volunteers at the time of another protocol performed at the CIC and from patients at the time of a biological examination, after an overnight fast, between 09.00 and 10.00 h in sterile tubes containing EDTA (100 μl of 100 mmol/l EDTA, Sigma, Saint-Quentin Falavier, France) and aprotinin (250 μl = 250 μU, Bayer Pharma, Germany).

The investigation protocol included a TSH stimulation test with one iv injection of 250 μg of TRH in nine subjects (two control patients, five patients with hypothyroidism and two with hyperthyroidism). Blood samples were collected at 0 and 15, 30, 45 and 60 min after TRH injection.

Plasma samples were obtained by centrifugation for 20 min at 4°C and stored at −20°C. The samples were examined within 6 months of storage.

Hormonal evaluation

Determination of NT concentration in plasma. The NT concentration was measured using a radioimmunoassay (RIA) after plasma extraction using C18 columns (Waters Sep-Pak Cartridge, Millipore, Saint-Quentin-en-Yvelines, France) and propanol elution as described previously (6). By using this protocol, the plasma was concentrated fourfold. The iodination of NT (Neosystem, Strasbourg, France) was performed using the lactoperoxidase method (17). The purified peptide was obtained by filtration on SP-Sephadex G25 (Pharmacia, LKB, Uppsala, Sweden) in 1 mmol/l TRIS·HCl (pH 8.6). Neurotensin antibody (purified IgG) was developed in this laboratory by immunizing rabbits with NT coupled to tetanic anatoxin. This antibody cross-reacts with NT and NT(1–11) but not with Neuremolin N or NT(8–13). The specific IgGs were incubated at 1:15,000 final concentration with tracer and standards or plasma samples in 60 mmol/l phosphate buffer, 10 mmol/l EDTA and 0.3% BSA (BioSepra, France), pH 7.4. Standards (1–1000 fmol/tube) and plasma (150 μl) were assayed in triplicate (total volume 1 ml). Incubation was performed for 72 h at 4°C with constant shaking. Separation of antibodies bound to the tracer was carried out by precipitation with 16% polyethylene glycol and 0.08% bovine globulin in RIA buffer. After removal of the supernatants, the radioactivity was measured by scintillation counting in the pellets. The sensitivity of the assay was 1 fmol/ml. The coefficient of variation was lower than 6% intra-assay and lower than 10% interassay. The precision of the plasma assay was 8.48 ± 1.67% (mean ± SEM percentage variation on six samples).

Determination of FT4 concentration. The FT4 determination was carried out by RIA using a Kodak Clinical Diagnostic IM 5051 kit (Amersham, UK). Normal values are 15.7 ± 3.3 pg/ml (mean ± SD).

Determination of TSH concentration. The TSH concentration was measured with an IRMA COPL 07 kit (CIS Bio International, RIA-Gnost, h-TSH, Gif sur Yvette, France). Normal values are 1.6 ± 0.8 μU/ml (mean ± SD).

Statistics

All data are expressed as means ± SEM. Differences between the groups were analyzed by Student’s t-test after log transformation of the data and/or by Wilcoxon’s rank sum test. Differences in the plasma NT and TSH concentrations before and after TRH injection were analyzed by Student’s paired t-test on percentage variation values. The correlation between NT and TSH values was established with Spearman’s rank correlation coefficient. The level of significance was chosen as p < 0.05.

Results

In 14 healthy adults subjects, the plasma NT concentration was 9.7 ± 1.1 fmol/ml (mean ± SEM). In group 1...
(control patients: FT₄ = 15.49 ± 0.58 pg/ml, TSH = 0.42 ± 0.49 µU/ml) the NT level was 13.3 ± 2.9 fmol/ml. No significant difference was found between the basal level of NT in healthy volunteers and control patients (group 1). In patients with central hypothyroidism (group 2: FT₄ = 8.09 ± 0.70 pg/ml, TSH = 0.63 ± 0.21 µU/ml) the NT levels were significantly lower, at 5.7 ± 1.2 fmol/ml (p < 0.05 vs healthy subjects and controls). Patients with peripheral hypothyroidism (group 3: FT₄ = 3.84 ± 1.39 pg/ml, TSH = 58.32 ± 22.76 µU/ml) had significantly higher NT concentrations of 35.9 ± 12.6 fmol/l (p < 0.05 vs control patients and p < 0.01 vs healthy subjects). In subjects with hyperthyroidism (group 4: FT₄ = 24.76 ± 1.45 pg/ml, TSH = 0.13 ± 0.02 µU/ml) the NT values were also elevated, at 29.9 ± 9.5 fmol/ml (p < 0.05 vs control patients and p < 0.01 vs healthy subjects) (Fig. 1).

Following iv administration of TRH, plasma NT levels decreased in eight out of the nine subjects studied. The mean NT values decreased significantly from 13.4 ± 8.4 before TRH injection to 7.3 ± 0.8 fmol/ml 30 min after injection (p < 0.01 on paired percentage decrease values) (Table 1 and Fig. 2). Considering all the individual values together, the correlation between NT and TSH values after TRH administration is negative and significant (r = -0.717, p < 0.03).

Finally, no correlation was found between the NT level and the age of subjects in any of the groups.

Discussion

The aim of our study was to evaluate the possible relationship between NT and TSH/FT₄ plasma levels in humans.

Table 1. Thyrotropin (TSH) and neurotensin (NT) plasma levels before (○) and 30 min after TRH injection (250 µg iv).a

<table>
<thead>
<tr>
<th>Patients no.</th>
<th>TSH (µU/ml)</th>
<th>NT (fmol/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>30 min</td>
</tr>
<tr>
<td>1</td>
<td>1.10</td>
<td>5.50</td>
</tr>
<tr>
<td>2</td>
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</tr>
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<td>5</td>
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</tr>
<tr>
<td>6</td>
<td>0.98</td>
<td>14.00</td>
</tr>
<tr>
<td>7</td>
<td>12.10</td>
<td>90.00</td>
</tr>
<tr>
<td>8</td>
<td>0.08</td>
<td>0.17</td>
</tr>
<tr>
<td>9</td>
<td>0.09</td>
<td>0.04</td>
</tr>
<tr>
<td>Mean ± SEM</td>
<td>0.69 ± 0.36</td>
<td>3.86 ± 3.38</td>
</tr>
</tbody>
</table>

a The significance of the results was calculated on a percentage variation of NT and TSH concentrations before and after TRH injection: p < 0.01 vs respective basal values.

b Nos. 1 and 2: control patients; nos. 3–7: hypopituitarism; nos. 8 and 9: hyperpituitarism.

c Data have been log transformed before statistical analysis.

Fig. 2. Individual responses of plasma neurotensin (NT) levels (●) after TRH injection (250 µg iv) in two patients with different TSH (△) responses. (a) Patient with postsurgical hypothyroidism and normal response of TSH to TRH injection. (b) Patient with hyperthyroidism and very low levels of TSH, non-responsive to TRH injection.

There are several arguments in favor of a role of NT in the control of hypotalamo-pituitary functions. Neurotensin has been found at high concentration in the hypothalamus, where it is located in perikarya and processes (8). High-affinity binding sites have also been demonstrated in hypothalamic tissues (15, 18). Finally, the anterior pituitary contains NT expressing cells as well as high-affinity binding sites for the peptide (14, 19).
Changes in the secretion of several pituitary hormones (LH, PRL, GH, TSH) have been reported following iv or intra-cerebroventricular (icv) injection of NT in rats (14). Immunoneutralization with specific NT antibody prevents an increase of PRL release or enhancement of LH release following icv or intrahypothalamic injection of NT. These results indicate that NT can act on PRL and LH regulation and this action could be of physiological significance (14). However, in experimental models, contradictory results have been reported on the in vitro direct effect of NT on the regulation of TSH. Vijayan et al. (12) have shown that TSH secretion was stimulated in hemipituitaries incubated with various doses of NT, while Askew et al. (20) reported an inhibitory action of NT on TSH secretion in cultured pituitary cells. Similar discrepancies have been observed in vivo because intraventricular injection of NT either increased or decreased plasma TSH concentration and blunted the TSH rise observed following systemic TRH administration (11, 12). In contrast, iv injection of NT produced a rise in plasma TSH (12). Some of these differences have been attributed to the route of administration and the different doses used. It is also possible that the different responses are due to feedback mechanisms. Indeed, it was suggested that T3 exerted a negative feedback on its own secretion via inhibition of TSH release through NT action (21, 22). In addition, some pituitary cells showed a co-localization of NT and TSH (19) and both hypothalamic and pituitary NT appeared to be regulated by thyroid hormones (22).

Finally, thyroideectomy induces an important decrease in the synthesis and pituitary content of NT (23, 24). Together, these findings support a physiological role for NT in the control of TSH secretion.

The presence of NT in rat and bovine plasma was demonstrated in 1976 by Carraway and Leeman (25). Several studies pointed out the problems that are encountered in performing RIA measurements on unextracted human plasma (4, 5, 26). For these reasons, we have performed an easy and reproducible technique using extracted plasma (2 ml) on a Sep-Pak column eluted with propanol (6). Even if Blackburn et al. (16) were unable to demonstrate any effect of NT injection on TSH levels in humans with a restricted number of subjects (N = 5), the present findings show a possible relation between plasma NT levels and pituitary–thyroid status. This suggests that TRH itself may decrease plasma NT levels, independently of its effect on TSH, because a decrease in plasma NT was observed even in the absence of TSH increase (two cases). Although the plasma NT level changes in various endocrine states of the thyroid axis, it does not seem to be strictly related to the plasma level of FT4 or TSH. The NT level was elevated under both high or low concentrations of FT4 and TSH (such as in the case of peripheral hypothyroidism and hyperthyroidism). However, these findings may be related to the different sites of action of NT directly on thyrotrpe cells in the pituitary, and on TRH release in the hypothalamus (27).

In conclusion, the present data obtained from human subjects are in agreement with previous experimental studies performed in rats. They indicate that plasma NT may play a physiological role in the regulation of hormone secretion in diseases with disregulation of the hypothalamo–pituitary–thyroid axis and suggest a negative effect of TRH on the circulating NT concentration.

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