Glucocorticoids decrease thyrotropin-releasing hormone messenger ribonucleic acid expression in the paraventricular nucleus of the human hypothalamus

Alkemade, A.; Ummehopa, U.A.; Wiersinga, W.M.; Swaab, D.F.; Fliers, E.

DOI
10.1210/jc.2004-1430

Publication date
2005

Published in
Journal of clinical endocrinology and metabolism

Citation for published version (APA):
Glucocorticoids Decrease Thyrotropin-Releasing Hormone Messenger Ribonucleic Acid Expression in the Paraventricular Nucleus of the Human Hypothalamus

A. Alkemade, U. A. Unmehopa, W. M. Wiersinga, D. F. Swaab, and E. Fliers

Netherlands Institute for Brain Research (A.A., U.A.U., D.F.S., E.F.), 1105 AZ Amsterdam, The Netherlands; and Department of Endocrinology and Metabolism (A.A., W.M.W., E.F.), Academic Medical Center, University of Amsterdam, 1100 DE Amsterdam, The Netherlands

The way glucocorticoids affect TRH mRNA expression in the paraventricular nucleus of the hypothalamus is still unclear. In view of its relevance for Cushing's syndrome and depression, we measured TRH mRNA expression in human hypothalami obtained at autopsy by means of quantitative TRH mRNA in situ hybridization. In corticosteroid-treated subjects (n = 10), TRH mRNA hybridization signal was decreased as compared with matched control subjects (n = 10) (Mann-Whitney U test, P = 0.02). By inference, hypercortisolism as present in patients with Cushing's syndrome or major depression may contribute to lower serum TSH or symptoms of depression by lowering hypothalamic TRH expression. (J Clin Endocrinol Metab 90: 323–327, 2005)

In Clinical Conditions such as Cushing's syndrome, nonthyroidal illness (NTI), and major depression, an endogenous hypercortisolism is often present. In these conditions changes in the hypothalamus-pituitary-thyroid (HPT) axis also occur (1–3). A possible role for glucocorticoids as attenuating modulators of the HPT axis has been proposed for patients with Cushing's disease (4) and major depression (5). In these patients complex associations occur between TSH and ACTH responses to specific stimulation with TRH and CRH (5). TRH neurons in the paraventricular nucleus (PVN) are involved in the neuroendocrine regulation of the HPT axis (6), and a clear correlation with thyroid hormone serum levels indicated that TRH in the PVN can be considered a major determinant of thyroid hormone status in patients with NTI (7). The mechanism by which glucocorticoids influence TRH gene expression in the PVN is still unclear, although a glucocorticoid receptor has been identified in TRH neurons in the PVN, and a glucocorticoid response element is present on the TRH gene (8). In vitro experiments have shown that dexamethasone can either stimulate or inhibit TRH expression in cultured hypothalamic neurons, dependent on the dose in the medium (9, 10). Dexamethasone treatment decreases hypothalamic CRH mRNA and TRH mRNA in the rat PVN, whereas opposite effects are observed after adrenalectomy (11). In humans glucocorticoids decrease TSH secretion (12), whereas TSH is increased during metyrapone-induced hypocortisolism (13). No data were, however, present on the effects of glucocorticoids on the HPT axis at the level of the hypothalamus in humans. In the present study, we investigated, therefore, the effect of corticosteroids on TRH mRNA in the PVN of the human hypothalamus. TRH mRNA was measured in corticosteroid-treated patients by means of in situ hybridization in combination with quantification by computer-assisted image analysis. For a matched control group, we studied subjects without corticosteroid treatment and without a primary psychiatric or neurological disease.

Subjects and Methods

Subjects

We studied the hypothalamus of 10 subjects that were treated with corticosteroids until death and 10 matched controls. Doses of corticosteroids varied among patients but exceeded the normal daily production rate of 20 mg hydrocortisone in at least eight of 10 cases (14). Daily doses and cortisol equivalents are presented in Table 1 (15). Brain material was obtained from The Netherlands Brain Bank at The Netherlands Institute for Brain Research in accordance with the formal permission for a brain autopsy and the use of human brain material and clinical information for research purposes. All the brains were systematically investigated by a neuropathologist. Exclusion criteria for corticosteroid-treated patients were: 1) use of antiepileptics, dopamine, or opiates within 4 wk before death or use of amiodarone; 2) known thyroidal disease; 3) mechanical ventilation; and 4) psychiatric or neurological disease. Patients and control subjects were matched for sex and, as closely as possible, severity and duration of fatal illness in an attempt to match for possible interference by NTI (7, 16). Exclusion criteria for control subjects were identical with the addition of corticosteroid treatment within 4 wk before death. Clinicopathological data are presented in Table 1.

Histology

Hypothalami were fixed in 10% phosphate-buffered formalin at room temperature for 3–14 wk. Tissues were dehydrated in a graded ethanol series, cleared in xylene, and embedded in paraffin. Coronal serial sections (6 μm) were made from the level of the lamina terminalis to the mammillary bodies. Depending on availability, either the left or right hemihypothalamus was used. Every 100th section was collected on a chromealum gelatin-coated slide with 0.5% BSA (Sigma, Zwijndrecht, The Netherlands) in distilled water followed by Nissl staining (0.5%...
<table>
<thead>
<tr>
<th>Subject no.</th>
<th>Sex</th>
<th>Age (yr)</th>
<th>PMD</th>
<th>Fix</th>
<th>Side</th>
<th>Corticosteroids; daily dose; cortisol equivalents, duration, and indication for treatment</th>
<th>Cause of death; clinical diagnoses; duration of fatal illness</th>
<th>TRH mRNA values (a.u.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>99125</td>
<td>F</td>
<td>40</td>
<td>10</td>
<td>98</td>
<td>L</td>
<td>Methylprednisolone; 1000 mg; 5000 mg, 3 d before death for exacerbation systemic lupus erythematosus, in addition to chronic prednisone</td>
<td>Multi-organ failure; renal insufficiency, systemic lupus erythematosus, sepsis, corticosteroid induced diabetes; 3 wk</td>
<td>11.63</td>
</tr>
<tr>
<td>97156</td>
<td>F</td>
<td>77</td>
<td>2</td>
<td>47</td>
<td>R</td>
<td>None</td>
<td>Septic shock, icterus; metastasized pancreas carcinoma, sepsis; 3 wk</td>
<td>Hypovolemic shock; pharynx carcinoma, upper intestinal tract bleeding, unsuccessful resuscitation; 2 d</td>
</tr>
<tr>
<td>98096</td>
<td>F</td>
<td>65</td>
<td>14</td>
<td>91</td>
<td>L</td>
<td>Dexamethasone; 12 mg; 320 mg, since 17 d before death for pancytopenia</td>
<td>Shock; probably cardiac failure, myelodysplastic syndrome, acute myeloid leukemia, type II diabetes; 3 d</td>
<td>13.49</td>
</tr>
<tr>
<td>98024</td>
<td>F</td>
<td>49</td>
<td>16</td>
<td>31</td>
<td>L</td>
<td>None</td>
<td>Respiratory insufficiency; mycosis fungoides, chronic obstructive pulmonary disease, atrial fibrillation, ischemic heart disease, pneumonia; 1 d</td>
<td>Respiratory insufficiency; pneumonia, metastasized bronchus carcinoma, cachexia; 3 d</td>
</tr>
<tr>
<td>98139</td>
<td>F</td>
<td>78</td>
<td>6</td>
<td>32</td>
<td>R</td>
<td>Prednisone; 40 mg; 160 mg, 1 d before death for exacerbation COPD, in addition to chronic prednisone</td>
<td>Sepsis; respiratory insufficiency, Wegener's granulomatosis, renal insufficiency, urothelium carcinoma, hyperparathyroidism; 1 wk</td>
<td>12.52</td>
</tr>
<tr>
<td>99162</td>
<td>M</td>
<td>38</td>
<td>11</td>
<td>37</td>
<td>L</td>
<td>Prednisone; 20 mg; 80 mg, chronic prednisone treatment for vasculitis in the framework of Wegener's granulomatosis</td>
<td>Multi-organ failure; HIV, hepatosplenomegaly, Hodgkin lymphoma, cachexia; 1 wk</td>
<td>19.97</td>
</tr>
<tr>
<td>97075</td>
<td>M</td>
<td>33</td>
<td>18</td>
<td>32</td>
<td>L</td>
<td>Dexamethasone; 12 mg; 320 mg, 7 d before death for high intracranial pressure</td>
<td>Brain edema; multitrauma by traffic accident, subarachnoidal bleeding, subdural hematoma; 6 d</td>
<td>3.95</td>
</tr>
<tr>
<td>97082</td>
<td>M</td>
<td>36</td>
<td>29</td>
<td>42</td>
<td>R</td>
<td>None</td>
<td>Intracerebral hemorrhage; metastasized choriocarcinoma of the testis, coma and hemi-paresis, due to intracerebral hemorrhage; 6 d</td>
<td>Subarachnoidal bleeding; chronic myeloid leukemia, thrombocytopenia, splenomegaly; 1 d</td>
</tr>
<tr>
<td>98133</td>
<td>M</td>
<td>64</td>
<td>8</td>
<td>30</td>
<td>L</td>
<td>Prednisone; 80 mg; 320 mg, chronic prednisone as palliative pain therapy in preterminal phase</td>
<td>Hemorrhage in the brain stem; generalized atherosclerosis, with moderate chronic renal failure, diverticulosis coli, hemorrhage in the brain stem; 1 d</td>
<td>10.32</td>
</tr>
<tr>
<td>98072</td>
<td>M</td>
<td>79</td>
<td>17</td>
<td>31</td>
<td>L</td>
<td>None</td>
<td>Respiratory insufficiency; unsuccessful resuscitation, chronic obstructive pulmonary disease, malignant tumor of the right lung; 1 d</td>
<td>Electromechanical dissociation during heart catheterization; ischemic heart disease, recent myocardial infarction; 2 d</td>
</tr>
<tr>
<td>98130</td>
<td>M</td>
<td>83</td>
<td>8</td>
<td>nd</td>
<td>L</td>
<td>Prednisolone; dose unknown; 11 d before death for exacerbation COPD</td>
<td>Pneumonia; AIDS, candidiasis, herpes, lymphoma, cytomegalovirus, acute renal insufficiency; 1 month</td>
<td>6.75</td>
</tr>
<tr>
<td>94039</td>
<td>M</td>
<td>78</td>
<td>nd</td>
<td>88</td>
<td>L</td>
<td>None</td>
<td>Multi-organ failure; M. Kahler, metastasized prostate carcinoma, urosepsis, renal insufficiency; 6 wk</td>
<td>Respiration arrest; chronic renal insufficiency, kidney transplantation; 1 d</td>
</tr>
<tr>
<td>90010</td>
<td>M</td>
<td>24</td>
<td>17</td>
<td>29</td>
<td>L</td>
<td>Corticosteroids; dose unknown; 13 d before death for pancytopenia</td>
<td>Probable cardiac arrest; renal insufficiency, kidney transplantation; 1 d</td>
<td>5.30</td>
</tr>
<tr>
<td>94109</td>
<td>M</td>
<td>82</td>
<td>5</td>
<td>32</td>
<td>L</td>
<td>None</td>
<td>Probable cardiac arrest; unsuccessful resuscitation, Bechterew's disease, atrial fibrillation, renal insufficiency; 1 d</td>
<td>Respiratory insufficiency; chronic obstructive pulmonary disease, pneumonia, left-sided pneumothorax; 2 wk</td>
</tr>
<tr>
<td>96419</td>
<td>M</td>
<td>29</td>
<td>7</td>
<td>nd</td>
<td>L</td>
<td>Prednisone; at least 10 mg; at least 40 mg, 6 d before death as immunosuppressive agent after kidney transplantation</td>
<td>Probable acute myocardial infarction; decompensatio cordis, severe left-sided cardiac failure based on mitral valve insufficiency and coronary sclerosis; 4 wk</td>
<td>13.13</td>
</tr>
</tbody>
</table>

F, Female; m, male; Fix, fixation period in days; L, left; R, right; nd, not determined; PMD, postmortem delay before fixation in hours; Side, side of the hypothalamus that was studied; COPD, chronic obstructive pulmonary disease.
thionine in distilled water) for the establishment of the anatomical boundaries of the PVN. Additional immunocytochemical staining for vasopressin was performed for more precise delineation of the rostral and caudal border of the PVN as described previously (17, 18).

In situ hybridization for TRH mRNA

In situ hybridization was performed through the entire PVN using a systematic random sampling procedure. Every 100th section of the area in which the PVN was located was mounted on RNase-free 2% aminoalkyl-silane-coated slides. The hybridization procedure has been described previously (19).

In short, sections were dried in a stove set at 37 °C for at least 2 d. Sections were deparaffinized in xylene, brought through graded ethanol, treated with 0.2 M HCl, and washed in PBS. Subsequently sections were deproteinized with proteinase K (10 μg/ml, 37 °C, 30 min). The protease treatment was stopped in glycine buffer and slides were washed in PBS. Probes were diluted in hybridization buffer. Seventy microliters of hybridization buffer containing 8 × 10^7 dpm of ^35S-labeled TRH probe complementary to bp 330–549 of the human prepro-TRH cDNA (10) was applied to each section. Sections were coveredslipped and hybridized overnight at 66 °C. Coverslips were removed in 2 × standard saline citrate (SSC) at 37 °C, and sections were washed sequentially for 45 min at 60 °C in 1× SSC, 0.1× SSC, 0.01× SSC, and finally three times for 1 h in 0.001× SSC. Sections were dehydrated in 300 mM ammonium acetate (pH 5.5)/ethanol 100% at volume ratios of 1:1, 3:7, 1:9, and 0:1, respectively, and dried in a stream of cool air for 5 min. Sections were apposed directly to autoradiography film (Amersham, Buckinghamshire, UK) and exposed for 2 d. The time of exposure was determined experimentally. Films were developed for 2.5 min in D-19 developer (Kodak) and fixed in Maxfix (Kodak) for 10 min. After rinsing in running tap water, the films were dried.

Sections were hybridized in two sessions within 1 wk using one batch of labeled probe. Matched couples of control subjects and subjects treated with corticosteroids were hybridized in the same session. We used RNase-treated sections (0.2 mg/ml RNase in PBS for 1 h at 37 °C before protease treatment) as a negative control in both sessions.

Quantitative analysis of TRH mRNA in situ hybridization

For quantification of the TRH mRNA in situ hybridization signal, we used radioactive standards. The methods of densitometry and quantification have been published elsewhere (14). In short, gray values of the film autoradiograms were analyzed by computer-assisted densitometry using an Interaktives Bild-Analysen system image analysis system (Kontron Electronik, Munich, Germany) and software developed at our institute. The relationship between the gray values and the amount of radioactive label present in the PVN. This was used as a relative measure for the amount of TRH mRNA in the PVN and expressed in arbitrary units (a.u.).

Statistical analysis

Differences between groups (corticosteroid treated vs. control) were tested with a Mann-Whitney U test (0.05 level of significance). A multivariate regression analysis (stepwise, dependent variable total TRH mRNA hybridization signal; 0.05 level of significance) of the factors postmortem delay, fixation duration, side of the hypothalamus, and age was performed.

Results

All subjects showed a clear hybridization signal in the region of the PVN using the TRH mRNA antisense probe. The RNase-treated sections yielded a completely negative hybridization signal on the film autoradiograms, supporting specificity of the procedure. The intensity of the signal showed a strong interindividual variation (Fig. 1). The TRH mRNA hybridization signal was significantly decreased in subjects who were treated with corticosteroids (median 6.21 a.u.), compared with control subjects (median 10.49 a.u., \( P = 0.02 \)) (Fig. 2).

A multivariate regression analysis of the factors postmortem delay, fixation duration, side of the hypothalamus, and age showed no influence of these factors on TRH mRNA hybridization signal.

Discussion

The specificity of the TRH cRNA probe was supported in an earlier study by displacement studies with unlabeled probe, by the absence of hybridization signal using a labeled sense probe and the absence of interfering homologies (19). In agreement with earlier studies (7), we observed a strong interindividually variation, which can be explained in part by NTI. Borson-Chazot et al. (20) reported lateralization of TRH concentrations in the human hypothalamus, with a left prominence for TRH. However, lateralization was not observed by a later study in suicide victims (21). We studied...
mostly the left side of the hypothalamus (n = 15) but also the right side (n = 5) when the left side of the PVN was not available for our research. A possible interference of lateralization was tested with a linear regression analysis but showed no effect.

TRH mRNA expression is influenced by nutritional status. In rats a decrease in TRH mRNA is observed during starvation (22). In the present study, two cachectic patients were included. Because these two patients were in the control group, the decrease in TRH mRNA expression in the corticosteroid-treated group may in fact have been underestimated in the present study.

Because discrete changes in thyroid hormone levels, including slightly lower serum free $T_3$, have been described in healthy elderly people, age can be considered a possible confounder for TRH mRNA in the hypothalamus (23). However, no effect of age has been observed in our previous studies on TRH expression in the human PVN (7, 19, 24), whereas NTI can be considered a major determinant of TRH expression. We therefore matched subjects in the present study for severity and duration of illness in an attempt to match for NTI rather than age. We did analyze a possible effect of age using a multivariate regression analysis but again found no effect on TRH mRNA in this study.

Cortisol equivalents in this study were based on peripheral action of glucocorticoids (15), whereas it is unknown what concentrations are reached in the brain. Rat studies have indicated that brain uptake of exogenous corticosteroids is very low (25), but postmortem studies in humans treated with corticosteroids have shown that pharmacological doses similar to the ones the patients in the present study received are able to diminish expression of CRH in PVN neurons (26).

The effect of glucocorticoids that we observed in the present study is in agreement with rat studies showing decreased TRH mRNA in the PVN after dexamethasone treatment (11). In vitro, however, variable effects have been described. Both stimulation and inhibition of TRH mRNA in hypothalamic cells cultures were observed, dependent on the concentration of dexamethasone in the medium (9, 10). The discrepancy between inhibitory effects in vitro and stimulatory effects in vitro of corticosteroids on TRH mRNA may be partly explained by an indirect effect of glucocorticoids. The absence of afferent input to cultured neurons indicates that glucocorticoids may affect the PVN in vivo directly as well as indirectly, e.g., via the hippocampus (27).

In the present study, we found a decrease in TRH mRNA expression in the PVN of corticosteroid-treated patients. This may explain somewhat lower serum TSH in patients treated with pharmacological doses of corticosteroids. By inference, endogenous hypercortisolism as may be present in patients with Cushing’s syndrome, critical illness, and major depression (1–3) may also decrease TRH mRNA expression in the PVE. Indeed, decreased TRH mRNA has been reported by our group in the PVN of patients with nonthyroidal illness and in patients with major depression (7, 16). This decrease in TRH mRNA may be of importance in the pathogenesis of depression, which is often seen in patients treated with corticosteroids (28) and patients with Cushing’s syndrome (29, 30). Because intrathecal administration of TRH in refractory depression has marked beneficial effects (31), a diminish-


Erratum

In the article “Specificity and Regioselectivity of the Conjugation of Estradiol, Estrone, and Their Catechol-estrogen and Methoxyestrogen Metabolites by Human Uridine Diphospho-glucuronosyltransferases Expressed in Endometrium” by Johanie Lépine, Olivier Bernard, Marie Plante, Bernard Tétu, Georges Pelletier, Fernand Labrie, Alain Bélanger, and Chantal Guillemette (The Journal of Clinical Endocrinology & Metabolism 89:5222–5232, 2004), there are two errors in Table 3. The relative $V_{max}$ for the formation of 4-OH-E1-3G by UGT1A8 was reported as 1975 pmol/min/mg but should have been reported as 197.5 pmol/min/mg. Similarly, the catalytic efficiency ($V_{max}/K_{m}$) should be 1.07 μL/min/mg instead of 10.7 μL/min/mg. The authors regret the errors.