Different Effects of Pyridostigmine on the Thyrotropin Response to Thyrotropin-Releasing Hormone in Endogenous Depression and Subclinical Thyrotoxicosis

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Primary organic disorders of the thyroid gland must be excluded in interpreting the thyrotropin (TSH)-releasing hormone (TRH) test in affective disease. Both endogenous depression and subclinical thyrotoxicosis are frequently associated with low basal TSH levels and a blunted (<5 mIU/L) TSH response to TRH despite thyroid hormone levels within the normal range. The present study was performed to establish whether a reduction of the hypothalamic somatostatinergic tone by treatment with the acetylcholinesterase inhibitor pyridostigmine before TRH might be useful to distinguish endocrine from affective diseases. Twelve male depressed patients (aged 41.4 ± 3.1 years) and 12 men (aged 43.4 ± 4.1 years) with subclinical thyrotoxicosis because of autonomous thyroid nodules were selected according to the presence of a low basal TSH level and a blunted TSH response to 200 μg TRH intravenously (IV) (TSH increment was <5 mIU/L at 30 minutes [peak] after TRH) but thyroid hormone levels within the normal range. All patients were tested again with TRH 60 minutes after treatment with 180 mg pyridostigmine orally. Eleven normal men served as controls. Basal TSH levels were 0.2 ± 0.2 mIU/L (mean ± SE) in depression and 0.1 ± 0.2 in subclinical thyrotoxicosis (normal controls, 1.4 ± 0.3). In both groups, the mean peak response to TRH was significantly higher than baseline; however, according to selection, the TSH increase was less than 5 mIU/L. Pyridostigmine did not change basal TSH levels in any group, but significantly enhanced the TRH-induced TSH increase in normal controls and in depressed subjects (TSH increment became >7 mIU/L in all depressed subjects). In contrast, no significant change in the TSH response to TRH was observed in subclinical thyrotoxicosis after pyridostigmine treatment. Basal and TRH- and pyridostigmine + TRH-induced TSH levels were significantly higher in the normal controls than in the other groups. These data show a cholinergic involvement in the blunted TSH response to TRH in patients with endogenous depression, but not in subjects with subclinical thyrotoxicosis, suggesting that these diseases could be separated on the basis of the pyridostigmine + TRH-induced TSH response test.

ENDOGENOUS DEPRESSION is characterized by various alterations in hypothalamic-pituitary function. In fact, a frequently observed marker of depression is represented by reduced basal and thyrotropin (TSH)-releasing hormone (TRH)-stimulated TSH secretion despite circulating thyroid hormone levels within the normal range.[1-5] This phenomenon does not require therapeutic attention from an endocrine point of view, but makes euthyroid depressed subjects not easily distinguishable from patients affected by subclinical thyrotoxicosis, who show a similar hormonal pattern (ie, serum thyroid hormones within the normal range with low basal and TRH-induced TSH secretion).[6]

Hypothalamic control of TSH secretion is mediated mainly by the stimulating action of TRH and the inhibitory tone of somatostatin.[7] Acetylcholine is known to inhibit hypothalamic somatostatin release.[8] Pretreatment with the acetylcholinesterase inhibitor pyridostigmine has been shown to improve the TSH response to TRH in clinical conditions characterized by enhanced hypothalamic somatostatin activity, such as obesity[9] and Cushing's disease.[10] Also, in endogenous depression, an increased hypothalamic somatostatinergic tone is thought to be responsible for the reduced TSH response to TRH.[5] Therefore, also in depressed subjects, pyridostigmine might be expected to improve the TRH-induced TSH increase. To verify this hypothesis, in the present study, we measured the TSH response to TRH either in the presence or absence of pyridostigmine treatment in patients with a blunted TSH response to TRH because of endogenous depression or subclinical thyrotoxicosis due to autonomous thyroid nodules. The prevalence of endogenous subclinical thyrotoxicosis is highly dependent on the population studied; it appears to be highest in nodular goitrous disease.[11] Also, patients with Graves' disease may be affected by subclinical thyrotoxicosis; however, we chose to test patients with autonomous thyroid nodules, because this pathological condition is highly prevalent in our area.

SUBJECTS AND METHODS

Twelve depressed male inpatients of the Psychiatric Clinic of Parma Hospital (mean ± SE age, weight, and body mass index [BMI], 41.4 ± 3.1 years, 66.5 ± 1.6 kg, and 22.4 ± 0.4, respectively), 12 male inpatients of the Internal Medicine Division (age, 43.4 ± 4.1 years; weight, 68.0 ± 1.7 kg; BMI, 22.5 ± 0.3) with subclinical thyrotoxicosis because of an autonomously functioning thyroid adenoma (n = 4) or multinodular goiter (n = 8, diagnosed by ultrasonography and ^{131}I external scintiscanning), and 11 euthyroid men (age, 40.7 ± 3.9 years; weight, 67.4 ± 1.5 kg; BMI, 22.2 ± 0.4) participated in the study.

The psychiatric status of all subjects who participated in this study was evaluated by two independent psychiatrists. The presence of affective symptoms was excluded in normal men and patients with thyroid disease. Major depression was characterized according to

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Submitted February 6, 1997; accepted May 29, 1997.

Supported in part by grants from the Ministero dell'Università (V.C. and P.C.).

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0026-0495/98/4701-0010$03.00/0

DSM-III-R criteria.[12] The diagnosis was made with the aid of the Structured Clinical Interview for DSM-III-R–Patient Version.[13] The severity of depression was assessed with the Hamilton Rating Scale for Depression.[14] The presence of schizophrenia was excluded in all patients with the Schedule for Affective Disorders and Schizophrenia.[15] All depressed patients had been free of psychotropic medication (except for sporadic use of low-dosage benzodiazepines in three patients) for at least 1 month before the experimental day. In addition, all patients who had been treated during the previous year with electroconvulsive therapy, lithium, monoamine oxidase inhibitors, anticonvulsants, antipsychotics, or antidepressants were excluded from the study.

Patients with depression or subclinical thyrotoxicosis were included in the study after a screening performed according to the following criteria: serum free triiodothyronine (FT_3) and free thyroxine (FT_4) levels within the normal range for our laboratory (FT_3, 3.8 to 5.0 pg/mL; FT_4, 1.0 to 1.7 ng/mL), basal TSH less than 0.4 mIU/L[6,11,16] (normal range, 0.5 to 3.0), and maximal change in response to TRH not greater than 5 mIU/L.[6] Subjects of all groups showed normal clinical and laboratory parameters of liver, renal, and gastrointestinal function. Furthermore, exclusion criteria for the study included epilepsy, head injuries, endocrine diseases, diabetes mellitus, or other chronic organic diseases. None of the subjects had excessive alcohol consumption (<300 mL ethanol · wk^{-1}). All subjects were free of drugs known to interfere with endocrine function.

The Ethics Committee for Clinical Trials of the institutes reviewed the protocol of the study, and subjects provided informed consent. All subjects were tested twice, once with TRH + placebo and another time with TRH + pyridostigmine.

TRH Test

This test was the first to be performed. At 8:00 AM after an overnight fast, an intravenous (IV) catheter was inserted into an antecubital vein and kept patent by a slow infusion of normal saline (NaCl 0.9%). Three tablets of placebo were administered orally 30 minutes after insertion of the cannula (time −60), 1 hour before IV bolus injection of 200 μg TRH. Blood samples were taken at time −60, just before time 0, and 20, 30, 45, 60, 90, and 120 minutes after TRH injection.

TRH + Pyridostigmine Test

This test was performed at least 10 days after the TRH test. All subjects received three tablets (180 mg) of pyridostigmine orally (Mestinon; Roche, Basel, Switzerland) at time −60. The test followed the same procedure described for the TRH test.

Blood samples were centrifuged after each experiment. The serum was frozen until it was used for TSH assay. Serum TSH concentrations were evaluated with a sensitive immunoradiometric method (Serono, Milan, Italy). The sensitivity of the assay was 0.02 mIU/L. Intraassay and interassay coefficients of variation for TSH were 4.8% and 6.7%, respectively. Serum FT_4 and serum FT_3 levels were measured using lisophase kits (Sclavo, Siena, Italy). The presence of antithyroglobulin and antithyroid microsomal antibodies was detected using a hemoagglutinin technique (Wellcome Reagents, Pomezia, Italy; Thymus-T and -M).

The change in TSH was determined by subtracting the basal from the TRH-induced peak TSH value. All subjects with a change in TSH of 5 mIU/L or less were considered to have a blunted or decreased TSH response to TRH.

The data were analyzed statistically by Wilcoxon's pair-rank-sum test, Mann-Whitney *U* test, and ANOVA, as appropriate. All data are reported as the mean ± SE.

RESULTS

Clinical and biochemical data for all examined groups are shown in Table 1. Antithyroglobulin and antithyroid microsomal antibodies were negative in all subjects.

TRH administration induced a significant increase in serum TSH concentrations in all groups (mean peak at time 30 *v* time 0, $P < .01$ in normal subjects, $P < .02$ in depressed subjects, and $P < .02$ in patients with subclinical thyrotoxicosis); however, according to the inclusion criteria of this study, TRH-induced TSH increments were less than 5 mIU/L in patients with endogenous depression or subclinical thyrotoxicosis and more than 5 mIU/L in normal subjects (Fig 1). Mean peak TSH levels were observed at 30 minutes in all groups, but were significantly higher in normal subjects than in patients with endogenous depression ($P < .01$) or subclinical thyrotoxicosis ($P < .01$); these latter groups showed similar increments in the response to TRH (Fig 1; Mann-Whitney *U* test).

Administration of pyridostigmine induced a significant increase in the TRH-induced TSH response in normal subjects ($F = 10.4$, $P < .02$ *v* TRH test) and in patients with endogenous depression ($F = 14.3$, $P < .01$) (ANOVA), but not in patients with subclinical thyrotoxicosis. In the presence of pyridostigmine, the mean TRH-induced TSH peak level in depressed patients was still significantly lower than in normal controls ($P < .02$), but it was significantly higher than in subjects with subclinical thyrotoxicosis ($P < .01$, Mann-Whitney *U* test; Fig 1).

No side effects were observed in any subjects after TRH administration. In contrast, in all subjects, pyridostigmine induced a feeling of increased bowel movement, abdominal discomfort, restlessness, and muscular fasciculation of the extremities that started 80 to 90 minutes after drug administration and lasted 1 hour.

DISCUSSION

The data presented here confirm the hypothesis that an enhancement in endogenous cholinergic tone with the acetylcho-

Table 1. Clinical and Biochemical Data in Depressed (n = 12), Subclinically Thyrotoxic (n = 12), and Normal (n = 11) Subjects (mean ± SEM)

Group	Age (yr)	Weight (kg)	BMI (kg/m^2)	HDRS	FT_3 (pg/mL)	FT_4 (ng/mL)	Basal TSH (mIU/L)	Change in Maximal TSH (mIU/L)
Depressed patients (simple major depression)	41.4 ± 3.1	66.5 ± 1.6	22.4 ± 0.4	21.4 ± 0.4	4.0 ± 0.8	1.4 ± 0.4	0.2 ± 0.2	2.5 ± 0.5*
Subclinically thyrotoxic patients	43.4 ± 4.1	68.0 ± 1.7	22.5 ± 0.3	—	4.2 ± 0.7	1.5 ± 0.3	0.1 ± 0.2	2.3 ± 0.8*
Normal controls	40.7 ± 3.9	67.4 ± 1.5	22.2 ± 0.4	—	3.7 ± 0.5	1.4 ± 0.3	1.4 ± 0.3	7.2 ± 0.7

Abbreviation: HDRS, Hamilton Depression Rating Scale (17-item version).

*$P < .01$ *v* normal controls.

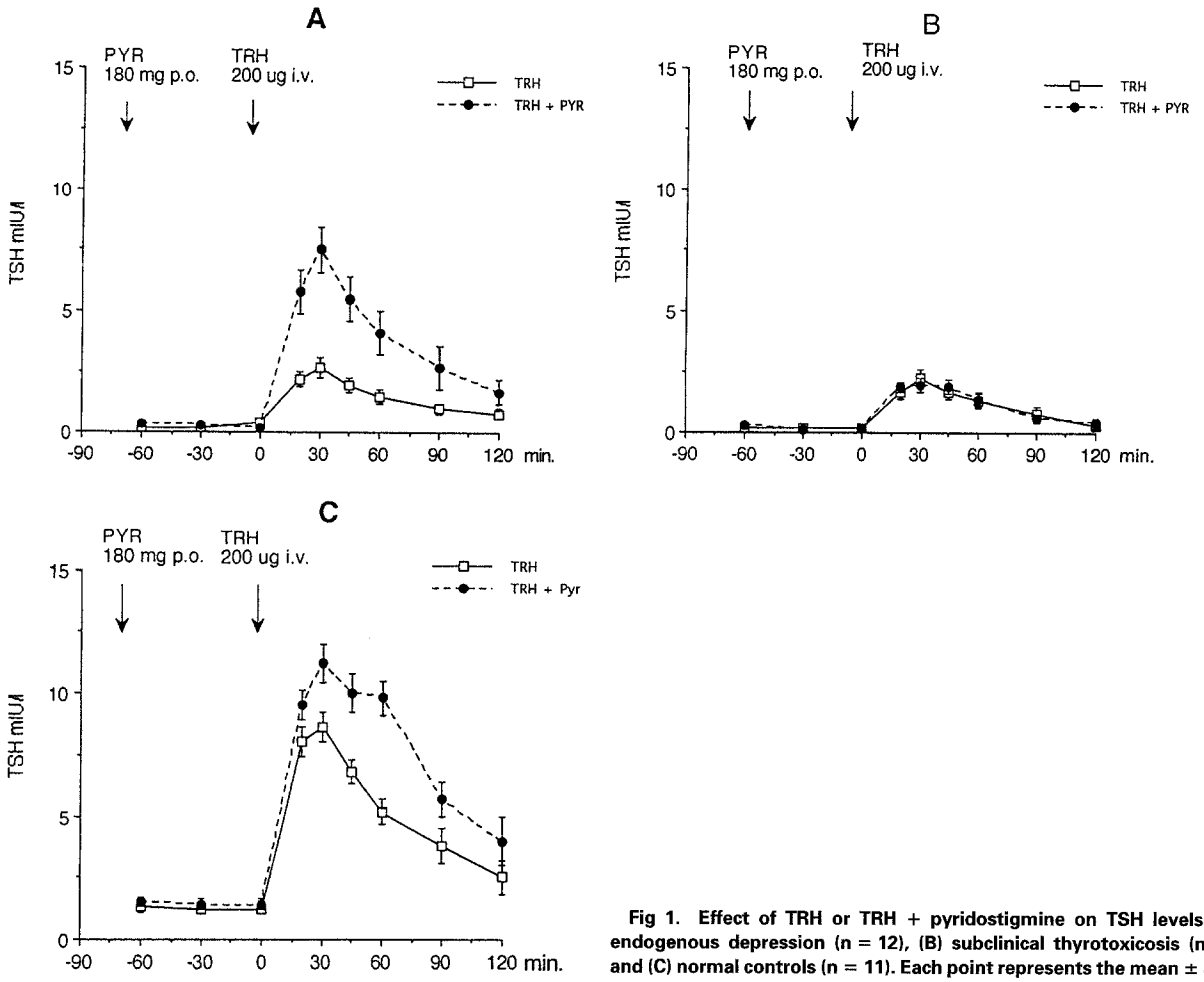

Fig 1. Effect of TRH or TRH + pyridostigmine on TSH levels in (A) endogenous depression (n = 12), (B) subclinical thyrotoxicosis (n = 12), and (C) normal controls (n = 11). Each point represents the mean ± SE.

linesterase inhibitor pyridostigmine significantly increases the TSH response to TRH in endogenous depression. It is likely that an enhancement in cholinergic activity induced by pyridostigmine inhibits hypothalamic somatostatinergic tone, and thus enhances TRH-induced TSH release.[17]

In contrast, pretreatment with pyridostigmine did not change the TSH response to TRH in patients with subclinical thyrotoxicosis. The drug is readily absorbed from the gastrointestinal tract and mainly excreted by the kidney.[18,19] Since subjects of all groups showed good nutritional status with similar parameters of gastrointestinal and renal function, it is unlikely that pyridostigmine failure in subclinically thyrotoxic patients was due to an increased drug metabolic rate and/or clearance. A more likely explanation is that the endogenous cholinergic tone was already maximal in our subjects with subclinical thyrotoxicosis, and thus pyridostigmine could not produce any further increment. This hypothesis is based on the well-known stimulatory effect exerted by thyroid hormones on cholinergic neurons.[20-23] In fact, thyroid hormones have been shown to increase cholinergic uptake, cholineacetyltransferase activity, and muscarinic cholinergic receptor number.[20]

At this point, a logical question concerns the reason why in subclinical thyrotoxicosis the TRH-induced TSH response is lower than normal rather than being increased by a hypothetical reduction of hypothalamic somatostatin release induced by increased cholinergic activity. A likely explanation is provided by the observation that the pituitary thyrotrope is exquisitely sensitive to the feedback inhibition of very slight increments in circulating thyroid hormone levels produced by administration of thyroid hormones, despite concentrations remaining within the normal range.[6] In other words, the reduced TSH response to TRH in patients with subclinical thyrotoxicosis is presumably caused by the ambient thyroid hormone levels, which are slightly elevated, although they are insufficient to cause clinical thyrotoxicosis. Furthermore, we cannot exclude the presence in these patients of a decreased set-point for thyroid hormone–mediated feedback inhibition of TSH secretion.[5,6,11]

In conclusion, different mechanisms underlie the blunted TSH response to TRH in subjects with subclinical thyrotoxicosis and in depressed patients, with an increased somatostatinergic tone the likely cause of this phenomenon in the latter but not in the former. Pretreatment with pyridostigmine before the TRH test might be a useful tool in the clinical differential diagnosis of these pathological conditions. Particularly, it might contribute to rapid diagnosis in depressed patients with low basal and TRH-induced TSH secretion, avoiding inappropriate use of antithyroid medications.

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