Acipimox Potentiates Growth Hormone Response to Growth Hormone–Releasing Hormone by Decreasing Serum Free Fatty Acid Levels in Hyperthyroidism

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Hyperthyroidism is associated with an impairment of growth hormone (GH) responses to secretagogues. The aim of this study was to evaluate the effect of acipimox, an antilipolytic agent able to decrease free fatty acids (FFA), on GH response to GH-releasing hormone (GHRH) in hyperthyroid and normal control subjects. We studied six men with hyperthyroidism; seven normal men served as control subjects. Each subject underwent treatment with (1) 2 tablets of placebo orally or (2) 500 mg acipimox orally, 120 minutes before intravenous (IV) injection of 1 μg/kg GHRH-(1-29)NH_2. GH response to GHRH in hyperthyroid patients was markedly reduced; the mean peak GH response (9.6 ± 1.0 μg/L) and the area under the GH response curve (12.9 ± 1.3 μg/L × 2 h) were lower than those of control subjects (25.7 ± 1.8 μg/L, $P < .05$; 28.7 ± 2.1 μg/L × 2 h, $P < .05$). Hyperthyroid patients had higher baseline levels of plasma FFA than control subjects (998.0 ± 38.9 *v* 498.0 ± 36.0 μEq/L, $P < .01$). Acipimox decreased FFA levels in both hyperthyroid and control subjects; the lowest FFA levels of hyperthyroid subjects induced by acipimox were similar to those of control subjects. After acipimox pretreatment, GH responses to GHRH increased significantly ($P < .05$); the mean peak plasma GH level (25.9 ± 4.6 μg/L) was similar to the peak GH levels of control subjects during the GHRH test, and the area under the GH response curve (41.1 ± 6.7 μg/L × 2 h) was even higher than that of control subjects with the GHRH test. However, the enhanced GH responses of hyperthyroid patients were still lower that those of control subjects during the acipimox plus GHRH test. We demonstrated that the decreased FFA levels induced by acipimox potentiate somatotrope responsiveness, likely acting at the pituitary level. Our results indicate that high FFA levels are responsible for impaired GH responses to GHRH in hyperthyroidism.

IT IS WELL KNOWN that thyroid hormones play a major role in the regulation of growth hormone (GH) synthesis and secretion both in vivo and in vitro. GH responses to GH-releasing hormone (GHRH), hypoglycemia, and arginine are impaired in hypothyroid patients.[1,2] These abnormalities are reversed after thyroxine (T_4) therapy. Although impairment of the cholinergic pathway has been suggested as a cause of low GH response in hypothyroidism,[3] the precise mechanism remains to be clarified. In vitro, thyroid hormones increase GH gene transcription and GH response to GHRH in somatotrope cells from animals.[4-6] A recent report[7] showed that hyperthyroid patients had an increase in GH pulsatility in both frequency and amplitude, leading to total 24-hour GH secretion that is more than three times greater than in normal subjects. However, despite high secretion of endogenous GH, surprisingly, GH responses to various GH stimuli such as hypoglycemia and GHRH were impaired.[8-11] These decreased responses to exogenous stimuli in hyperthyroidism could be suggestive of the existence of inhibitory factors on GH secretory responses of somatotrope cells. The basic mechanisms of this impaired GH responsiveness are not yet clarified.

Hyperthyroidism is frequently associated with high levels of plasma free fatty acids (FFA) as a result of the increased thyroid hormone–enhancing lipolysis.[12-14] FFA participate in the regulation of pituitary GH secretion. GH secretion is stimulated when plasma FFA levels are depressed, and increased FFA levels block GH secretion elicited by all stimuli.[15,16] Therefore, the high FFA levels of hyperthyroidism might be responsible for the impaired GH responsiveness.

The aim of this study was to evaluate the effect of acipimox, an antilipolytic agent able to decrease FFA, on GH response to GHRH in normal and hyperthyroid subjects.

SUBJECTS AND METHODS

Subjects

Six Korean men with hyperthyroid Graves' disease aged 24 to 33 years and seven normal men aged 24 to 30 years were studied. Hyperthyroidism was diagnosed both clinically and by elevated triiodothyronine ([T_3] 6.01 ± 0.45 nmol/L; normal range [mean ± 95% confidence interval], 1.23 to 2.46), T_4 (282.5 ± 11.0 nmol/L; normal range, 64.4 to 148.0), and free T_4 ([fT_4] 84.8 ± 11.6 pmol/L; normal range, 10.3 to 25.8) levels with undetectable thyrotropin (TSH) levels. Control subjects had normal levels of T_3 (1.94 ± 0.04 nmol/L), T_4 (115.1 ± 4.4 nmol/L), and fT_4 (15.9 ± 0.9 pmol/L). None of the control subjects had diabetes mellitus or other medical problems or had used any hormonal preparations within 60 days before the study. The study was approved by the Hospital Ethics Committee, and informed consent was obtained from each subject.

Methods

All subjects were asked to arrive at the hospital at 8 AM on the day of the study, after an overnight fast. Intravenous (IV) cannulas were placed in antecubital veins and provided subjects with a slow infusion of 0.9% NaCl. After a 1-hour rest period to minimize the effects of physical activity and nonspecific stress on GH levels, the tests were started. Subjects remained supine during the entire study. The following two tests were performed in random order 7

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days apart. (1) GHRH test: at 9 AM (time −120 minutes), 2 tablets of placebo were administered orally; 2 hours later (time 0), 1 μg/kg GHRH (GRF-(1-29)NH_2; BACHEM Feinchemikalien, Bubendorf, Switzerland) was injected as an IV bolus. (2) Acipimox plus GHRH test: at 9 AM (time −120 minutes), 500 mg acipimox (Olbetam; Farmitalia Carlo Erba, Milan, Italy) was administered orally; 2 hours later (time 0), 1 μg/kg GHRH was injected as an IV bolus. Blood samples were collected at −150, −120, −90, −60, −30, 0, 15, 30, 60, and 120 minutes.

Each specimen was centrifuged immediately, and the plasma was stored at −70°C until assayed.

Assays

GH level was measured by an immunoradiometric assay from Daiichi (Tokyo, Japan). Sensitivity was 0.1 μg/L and intraassay and interassay coefficients of variation (CVs) were 1.3% and 1.4% and 1.1% and 2.1% at dose levels of 1.92 and 30.2 μg/L, respectively. All samples from each subject were analyzed in duplicate at the same time. Concentrations of FFA were determined by calorimetry. Plasma T_3, T_4, fT_4, and TSH were assayed by enzyme-linked immunosorbent assay (Boehringer, Mannheim, Germany). The lower detection limit of TSH is 0.03 mU/L, normal ranges (mean ± 95% confidence interval) are 0.25 to 3.1 mU/L, and intraassay and interassay CVs were 10.9% and 4.2% at dose levels of 1.73 mU/L, respectively. Sensitivities were 0.38 and 7.7 nmol/L for T_3 and T_4, respectively. Intraassay and interassay CVs of T_3 were 5.7% and 4.9% at dose levels of 1.5 nmol/L, respectively. Intraassay and interassay CVs of T_4 were 7.9% and 6.5% at dose levels of 80.8 nmol/L, respectively. Sensitivity of fT_4 was 2.6 pmol/L. Intraassay and interassay CVs of fT_4 were 3.7% and 6.7% at dose levels of 12.0 pmol/L, respectively.

Statistical Analysis

Results are expressed as the mean ± SEM. Statistical comparisons were made using Mann-Whitney *U* tests between different groups and the Wilcoxon rank test between related groups because the assays produced no normally distributed results. Areas under the curve (AUCs) of GH secretion (micrograms per liter per 2 hours) were calculated by a trapezoidal method. Factors related to GH response and hyperthyroidism were analyzed using stepwise multiple regression. *P* less than .05 was accepted as the significance level.

RESULTS

In control subjects, GHRH administration induced a clear-cut increase in plasma GH levels. The mean peak GH level was 25.7 ± 1.8 μg/L at 30 minutes, and the area under the GH response curve was 28.7 ± 2.1 μg/L × 2 h (Fig 1). Acipimox decreased FFA levels; these reached the lowest levels at 30 and 60 minutes and were still being suppressed throughout the test (Fig 1). Acipimox pretreatment potentiated plasma GH response: mean peak plasma GH level was 50.8 ± 6.7 μg/L ($P < .05$; Fig 1), and the area under the GH response curve (65.1 ± 7.3 μg/L × h) increased nearly twofold ($P < .05$; Fig 2).

In hyperthyroid subjects, baseline GH levels before the GHRH test were similar to those of control subjects, but GH responses to GHRH administration were markedly reduced. The mean peak GH response (9.6 ± 1.0 μg/L) and the area under the GH response curve (12.9 ± 1.3 μg/L × 2 h) were lower than those of control subjects ($P < .05$ and $P < .01$, respectively; Figs 1 and 2). Baseline FFA levels of hyperthyroid subjects (998.0 ± 38.9 μEq/L) were higher than those of control subjects (498.0 ± 36.0 μEq/L, $P < .01$; Fig 1). Hyperthyroid subjects had higher levels of FFA than control subjects throughout the GHRH test. Acipimox decreased FFA levels; these reached the lowest levels at time 0 and 15 minutes, similar to those of

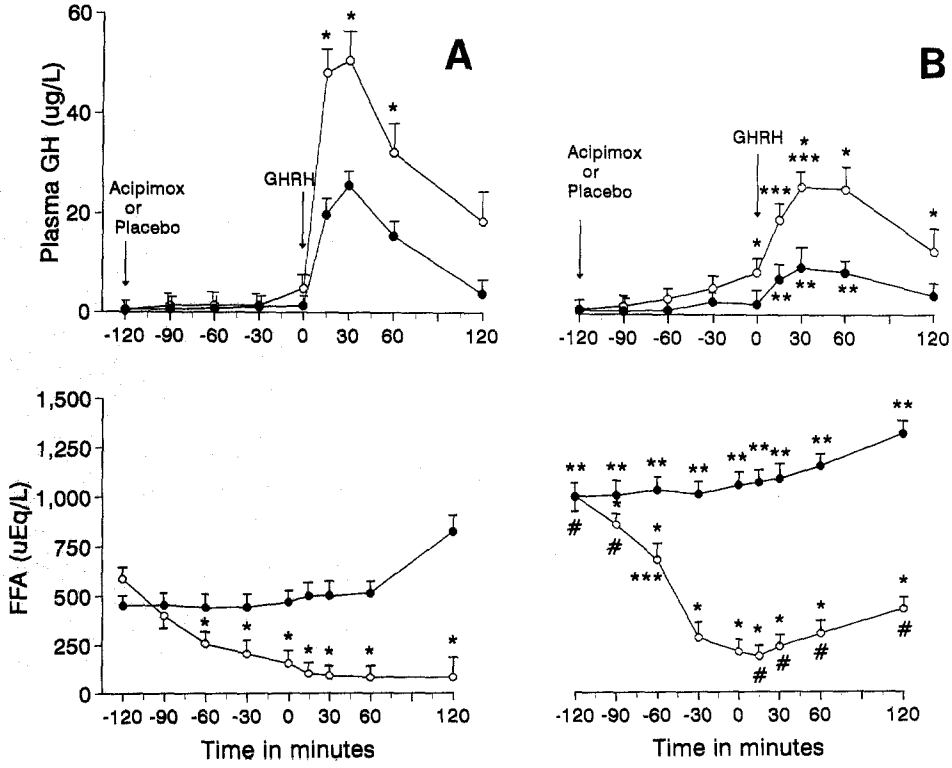

Fig 1. Mean ± SEM plasma GH (A) and FFA (B) concentrations after administration of placebo orally plus GHRH 1 μg/kg IV (●) or 500 mg acipimox orally plus GHRH (○) in 7 control subjects (left) and 6 hyperthyroid subjects (right). *$P < .05$ *v* GHRH; **$P < .01$ *v* control subjects with GHRH; ***$P < .05$ *v* control subjects with acipimox plus GHRH; #$P < .01$ *v* control subjects with acipimox plus GHRH.

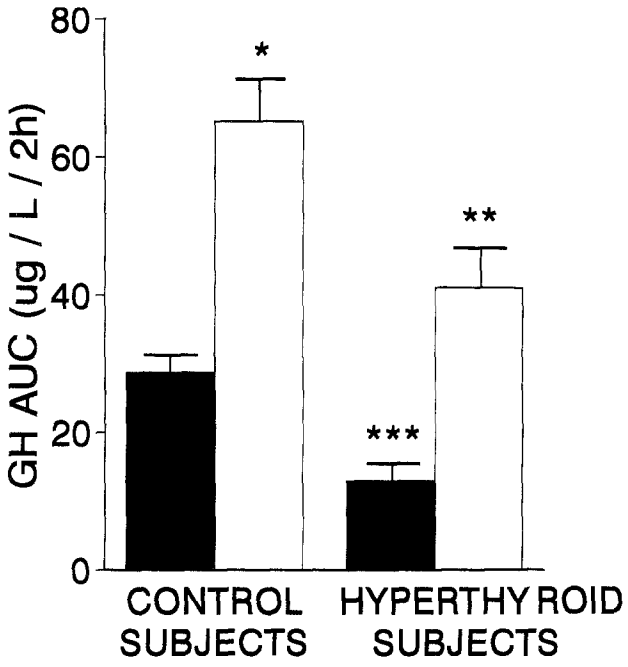

Fig 2. GH response AUCs after administration of placebo orally plus GHRH 1 μg/kg, IV (■) or 500 mg acipimox orally plus GHRH (□) in 7 control subjects and 6 hyperthyroid subjects. *$P < .05$ v GHRH; **$P < .05$ v acipimox plus GHRH in control subjects; ***$P < .05$ v GHRH in control subjects.

control subjects, and then increased progressively to the baseline levels of control subjects. After acipimox pretreatment, GH levels of hyperthyroid subjects were not significantly different from those of control subjects before GHRH administration, but GHRH increased GH responses significantly ($P < .05$). The mean peak plasma GH level (25.9 ± 4.6 μg/L) was similar to the peak GH level of control subjects during the GHRH test, and the area under the GH response curve (41.1 ± 6.7 μg/L × 2 h) was even higher than that of control subjects during the GHRH test (Figs 1 and 2). However, the enhanced GH responses of hyperthyroid patients were still lower that those of control subjects during the acipimox plus GHRH test. Stepwise multiple regression analysis showed a highly significant effect of FFA on the area under the GH response curve to the GHRH test ($39.58 - 0.026$ FFA, $P < .001$) and the acipimox plus GHRH test ($105.69 - 0.075$ FFA, $P < .05$).

DISCUSSION

The results of this study confirm that hyperthyroid subjects show a markedly blunted GH response to GHRH. We also demonstrated that the decreased FFA levels induced by acipimox potentiate somatotrope responsiveness, likely acting at the pituitary level, indicating that high plasma FFA levels are responsible for impaired GH response to GHRH in hyperthyroidism.

Decreased GH secretory response to exogenous stimuli in hyperthyroidism could be explained by several possible mechanisms, ie, defects of the hypothalamic controlling system or decreased responsiveness of pituitary somatotrope cells.

There have been previous reports[17,18] that the decrease in GH responsiveness was reversed during treatment with methimazole and remission of thyroid hyperfunction. The improvement of GH response to GHRH after the patient became euthyroid was related in time to a decrease in circulating levels of insulin-like growth factor-I (IGF-I). Serum IGF-I levels were elevated in hyperthyroid patients.[17,19] This might decrease somatotrope responsiveness to GHRH. However, in previous studies, there have been no reports about the relationship between plasma IGF-I levels and GH responses to GHRH in hypothyroid patients before and during treatment with T_4.[19,20] Furthermore, if the decreased GH response to GHRH in hyperthyroidism were only due to increased IGF-I levels, the decrease in spontaneous GH secretion could be suspected. But this is not the case, since recent reports showed that hyperthyroid patients had an increase in GH pulsatility in both frequency and amplitude, leading to total 24-hour GH secretion that is more than three times greater than in normal subjects.[7]

There is now evidence of tonic negative control of somatostatinergic neurons of the hypothalamus by the cholinergic pathway.[21,22] Activation of cholinergic neurotransmission leads to suppression of somatostatin release from the hypothalamus. In contrast, cholinergic blockade increases hypothalamic somatostatinergic tone.[23,24] Recent reports that activation of cholinergic pathways with the acetylcholinesterase inhibition pyridostigmine does not increase GH response to GHRH and that pyrenzepine, a cholinergic muscarinic receptor blocker, suppressed GH responses to GHRH in hyperthyroidism were suggestive of chronic hypersecretion of somatostatin or reduced activity of GHRH neurons as the basis for the derangement in GH secretion.[25] However, if the decreased GHRH tone and the increased somatostatinergic tone of the hypothalamus in hyperthyroidism were responsible for impaired GH responses to GHRH, the decrease in spontaneous GH secretion could also be suspected. But there have been no such reports. Accordingly, it is still possible that other controlling mechanisms are also altered in GH responsiveness in hyperthyroidism as a result of chronic exposure to increased thyroid hormone levels.

Thyroid hormones play a regulatory role in lipolysis. It has been reported that thyroid hormones attenuate insulin action in adipose tissue and enhance lipolytic activity of catecholamines.[4-6] Then elevated thyroid hormones in hyperthyroid patients enhance lipolysis, as well as triglyceride/fatty acid substrate cycling, leading to increased FFA levels. Increased FFA levels were significantly decreased after patients became euthyroid with antithyroid treatment.[5]

FFA participates in the regulation of pituitary GH secretion. GH secretion is stimulated when plasma FFA levels are depressed, and high FFA levels block GH secretion elicited by all stimuli. The site of FFA action is the pituitary.[15,16]

Acipimox was chosen to decrease plasma FFA levels in this study. The pharmacologic action of acipimox is to block

FFA release from adipose tissue,[25,26] leading to a decrease in plasma FFA levels. In previous reports,[27] acipimox enhanced GH response to GHRH in normal subjects. We found that acipimox potentiated GH response to GHRH in both normal and hyperthyroid subjects. The enhanced GH responses (peak) by acipimox in hyperthyroid subjects were especially similar to the levels of GH response to GHRH alone in control subjects. These data indicate that high plasma FFA levels play an important role in the impaired GH responsiveness, suggesting the existence of a somatotrope defect in hyperthyroidism.

In conclusion, our results indicate that GH responses to GHRH in hyperthyroid patients are recovered by decreasing plasma FFA levels with acipimox pretreatment. This suggests that high plasma FFA levels may inhibit somatotrophe responsiveness to GHRH in hyperthyroid patients, although other factors might also be involved in GH derangements.

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