Evidence for an Inhibitory Effect of Physiological Levels of Insulin on the Growth Hormone (GH) Response to GH-Releasing Hormone in Healthy Subjects

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ABSTRACT

It has been previously reported that in healthy subjects, the acute reduction of free fatty acids (FFA) levels by acipimox enhances the GH response to GHRH. In the present study, the GH response to GHRH was evaluated during acute blockade of lipolysis obtained either by acipimox or by insulin at different infusion rates. Six healthy subjects (four men and two women, 25.8 ± 1.9 yrs old, mean $\pm$ SE) underwent three GHRH tests ($50\ \mu g$ iv, at 1300 h) during: 1) iv 0.9% NaCl infusion (1200–1500 h) after oral acipimox administration (250 mg) at 0700 h and at 1100 h; 2) $0.1\ mU \cdot kg^{-1} \cdot min^{-1}$ euglycemic insulin clamp (1200–1500 h) after oral acipimox administration (250 mg at 0700 h and at 1100 h); 3) $0.4\ mU \cdot kg^{-1} \cdot min^{-1}$ euglycemic insulin clamp (1200–1500 h) after oral placebo administration (at 0700 and 1100 h).

Serum insulin (immunoreactive insulin) levels were significantly different in the three tests (12 ± 2, 100 ± 10, 194 ± 19 pmol/L, $P < 0.05$), plasma FFA were low and similar (0.04 ± 0.003, 0.02 ± 0.005, 0.02 ± 0.003, not significant), and the GH response to GHRH was progressively lower (4871 ± 1286, 2414 ± 626, $1076 \pm 207\ \mu g/L \cdot 120$ min), although only test 3 was significantly different from test 1 ($P < 0.05$). Pooling the three tests together, a significant negative regression was observed between mean serum immunoreactive insulin levels and the GH response to GHRH ($r = -0.629$, $P < 0.01$).

Our results indicate that in healthy subjects, acipimox and hyperinsulinemia produce a similar decrease in FFA levels and that at similar low FFA, the GH response to GHRH is lower during insulin infusion than after acipimox. These data suggest that insulin exerts a negative effect on GH release. Because the insulin levels able to reduce the GH response to GHRH are commonly observed during the day, for instance during the postprandial period, we conclude that the insulin negative effect on GH release may have physiological relevance. (*J Clin Endocrinol Metab* **82:** 2239–2243, 1997)

T HE ROLE OF metabolic/hormonal factors in the physiological regulation of GH release is only partially known. Free fatty acids (FFA) inhibit GH release via a negative feedback (1–7), thought to occur both at the pituitary (6) and at the hypothalamic level (5). In contrast, the role of insulin is less clear. Through hypoglycemia, insulin stimulates GH release, but several pieces of experimental data suggest possible direct effects of insulin on the GH axis; for instance, insulin receptors are present in rat hypothalamus (8), and insulin binding sites have been demonstrated in normal rat pituitary cells [though at extremely low concentrations (9)], in rat anterior pituitary adenoma cells (10), and in human pituitary adenoma cells (11). Furthermore, *in vitro* studies have shown that insulin decreases GH synthesis, release, and messenger RNA (mRNA) content of rat pituitary normal and adenoma cells (12–16) and suppresses GH release from human pituitary adenoma cells (11). Data available *in vivo* in normal subjects also suggest an inhibitory role of insulin on GH release: integrated 24-h GH concentrations are elevated during fasting, *i.e.* under conditions of low serum insulin levels (17); during euglycemic insulin clamp, a reduction of the GH response to GHRH has been observed (18), whereas during hypoglycemic insulin clamp, the GH response

to hypoglycemia is inversely related to the degree of hyperinsulinemia (19).

In studying the effect of insulin *per se* on GH release, one is faced with the problem that an increase in insulin levels is accompanied by a concomitant decrease in FFA levels, caused by the antilipolytic activity of insulin (20). Because an acute decrease in FFA levels enhances the GH responsiveness to GHRH in healthy subjects (7, 21), this may mask the concomitant effect of insulin on GH release. On the other side, the fall of FFA levels after pharmacologic blockade of lipolysis, by ameliorating insulin sensitivity, leads to a significant reduction of circulating insulin levels (22, 23), raising the question of whether the enhancement of GH release observed under those experimental conditions reflects not only low FFA, but also low insulin levels. To single out the effect of insulin *per se* on GH release, independently of FFA, we designed an experimental protocol in which GH responsiveness to GHRH was evaluated at low and similar FFA levels but progressively increasing insulin levels. The subjects underwent three GHRH tests after acipimox (a drug inducing an acute reduction of circulating FFA levels) (7, 21) and during two insulin infusions at increasing rates.

Subjects and Methods

Subjects

Six normal subjects (four men and two women, 25.8 ± 1.9 yrs old, mean $\pm$ SE; body mass index, $22.7 \pm 1.1\ kg/m^2$) were studied after giving

a written informed consent. The protocol of the study was approved by the Ethics Committee of the Istituto Scientifico San Raffaele. All subjects were in good health and had normal routine laboratory examination, normal endocrine function, normal glucose tolerance after an oral glucose load (75 g), and were taking no medications.

Experimental procedure

According to a single-blind, randomized cross-over protocol, the subjects underwent, three GHRH tests (50 μg iv, at 1300 h) during: 1) iv 0.9% NaCl infusion (1200 h to 1500 h), with acipimox (Olbetam, Pharmacia UpJohn, London, UK), 250 mg, being administered per os (p.o.) at 0700 h and at 1100 h; 2) 0.1 mU·kg^{-1}·min^{-1} euglycemic insulin clamp, started at 1200 h and continued until 1500 h, with acipimox, 250 mg, being administered p.o. at 0700 h and at 1100 h; 3) 0.4 mU·kg^{-1}·min^{-1} euglycemic insulin clamp, started at 1200 h and continued until 1500 h, with placebo being administered p.o. at 0700 and 1100 h. Acipimox is a nicotinic acid analog able to inhibit spontaneous, as well as norepinephrine-, isoprenaline-, and GH-induced lipolysis (24–26). The rates of insulin infusion were chosen to obtain steady-state serum insulin levels comparable with those physiologically reached in normal subjects during the day (27). Insulin infusion was started at 1200 h to obtain steady-state insulin levels and a significant decrease in plasma FFA levels by the time of GHRH injection (1300 h), as indicated by a prior pilot study and according to previous reports (28). In test 2, acipimox was administered to consistently decrease FFA levels to those observed in test 1 at the time of GHRH injection. In test 3, insulin clamp was performed under placebo, because in a pilot study, the high insulin infusion rate was consistently able to decrease FFA levels to those observed after acipimox.

All subjects were fasted overnight, and the tests were performed in the recumbent position. Blood samples for evaluation of plasma FFA, blood glycerol, serum insulin [immunoreactive insulin (IRI)], and serum GH levels were always drawn at −60 min and just before GHRH administration (time zero) and 10, 15, 30, 45, 60, and 120 min after, via an indwelling catheter inserted into a forearm vein at least half an hour before the beginning of the sampling period.

The euglycemic insulin clamp was performed as previously reported (29). Briefly, human insulin (Humulin R, Lilly, Indianapolis, IN) was given as a prime-continuous infusion by means of a Harvard pump (Model 975A, Millis, MA). A 20%-dextrose solution was infused by means of a Harvard servo-controlled infusion system (Model 2990). The glucose infusion rate adjustment was based on a feedback principle to maintain euglycemia. Blood samples for evaluation of blood glucose levels were collected every 5 min during the euglycemic clamp.

Assays

Plasma FFA levels were measured by a spectrophotometric method adapted to Cobas-Fara 2 (Roche, Basel, Switzerland) using kits supplied by Italfarmaco (Milano, Italy). Intra- and interassay coefficients of variation (CVs) were 2.3 and 3.1%, respectively. Blood glycerol levels were measured by a spectrofluorimetric method adapted to Cobas-Fara 2. Intra- and interassay CVs were 0.7 and 2.7%, respectively. Serum IRI levels were measured by RIA using kits supplied by IncStar (Stillwater, MN). The minimum sensitivity of the assay was 13 pmol/L, and intra- and interassay CVs were 3.9 and 8.9%, respectively. Serum GH levels were measured by RIA using kits supplied by Farmos Diagnostic (Turku, Finland). The minimum sensitivity of the assay was 0.2 μg/L, and the median intra- and interassay CVs for GH concentrations ranging from 0.2 to 50 μg/L were less than 9 and 10%, respectively. Blood glucose levels were measured by a glucose oxidase method (Beckman Glucose Analyzer II, Beckman Instruments, Fullerton, CA).

Calculations and statistical analysis

Each variable was expressed as the mean ± SE at each time point; the integrated GH response to GHRH (GH Δ area under the curve, 0–120 min) was calculated by the linear trapezoidal method. Statistical analysis was performed by the one-way ANOVA for repeated measures, followed by the Student-Newman-Keuls test. P-values less than 0.05 were considered statistically significant.

Results

No severe side effects were observed during the tests (only a moderate facial skin rash being evident in one subject after the first, but not after the second, capsule of acipimox. Figure 1 shows plasma FFA, blood glycerol, serum IRI, and serum
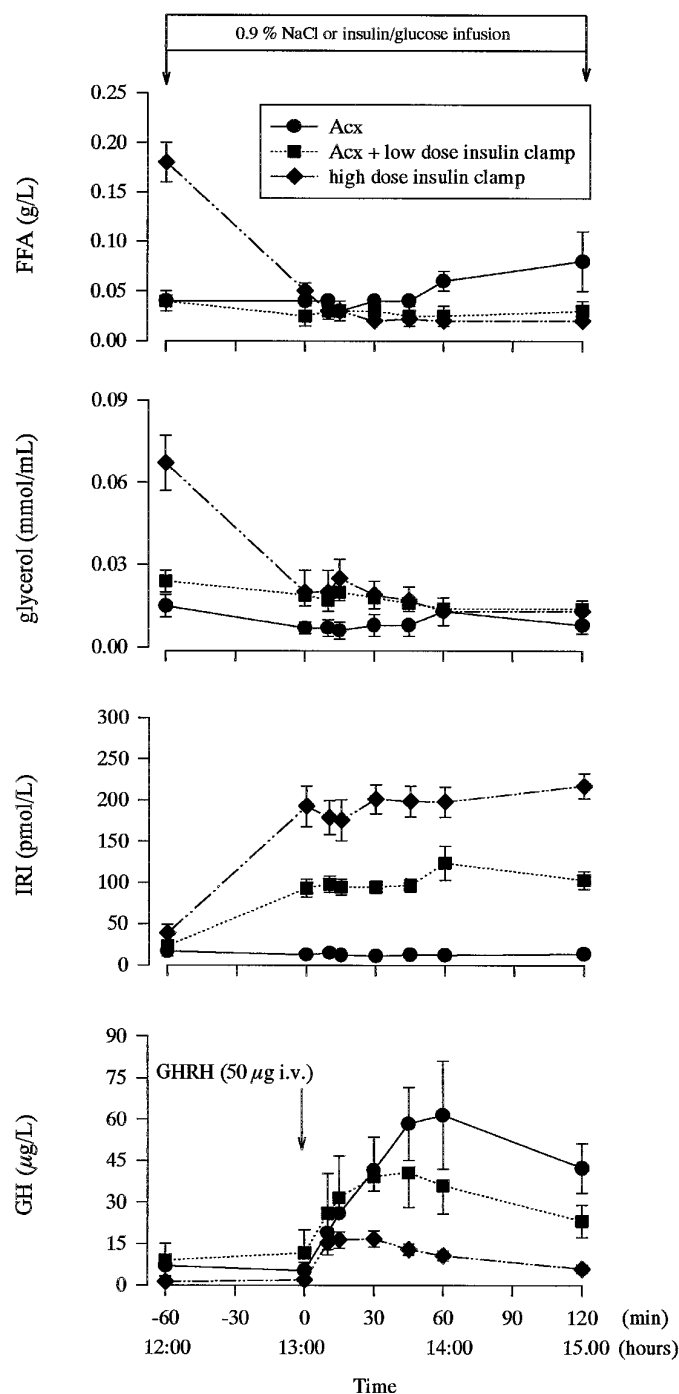

FIG. 1. Plasma FFA, blood glycerol, serum insulin (IRI), and serum GH levels in six healthy subjects: 1) after acipimox administration and during NaCl infusion; 2) after acipimox administration and during low-dose euglycemic insulin clamp; and 3) after placebo administration and during high-dose euglycemic insulin clamp. Each *time point* represents the mean ± SE. Acx, acipimox.

GH levels before and after GHRH in normal subjects: 1) after acipimox and during a 0.9% NaCl infusion; 2) after acipimox and during a 0.1 mU·kg^{-1}·min^{-1} insulin clamp; and 3) after placebo and during a 0.4 mU·kg^{-1}·min^{-1} insulin clamp, respectively. Mean levels of all the parameters of interest measured at time of GHRH injection (time zero) are reported in Table 1. At that time, plasma FFA, blood glycerol, and blood glucose levels were similar, and serum IRI levels were significantly different ($P < 0.05$) in the three tests. Serum GH levels were higher after acipimox (alone or combined with the low-dose insulin clamp) than during the high-dose insulin clamp, although not significantly because of high variability observed after acipimox.

TABLE 1. Mean plasma FFA, blood glycerol (glycerol), serum insulin (IRI), blood glucose, and serum growth hormone GH levels at the time of GHRH injection (0 min, 50 μg iv) in the six subjects. Each value represents the mean $\pm$ SE

Parameters	Treatments		
	Acx	Acx + low dose-insulin clamp	High-dose insulin clamp
FFA (g/L)	0.04 ± 0.003	0.02 ± 0.005	0.05 ± 0.008
Glycerol (mmol/mL)	0.02 ± 0.002	0.02 ± 0.004	0.03 ± 0.009
IRI (pmol/L)	12 ± 4	93 ± 11[a]	192 ± 24[b]
Glucose (mmol/L)	4.3 ± 0.1	4.5 ± 0.1	4.6 ± 0.1
GH (μg/L)	5.3 ± 2.9	11.7 ± 8.4	1.9 ± 0.9

[a] $p < 0.05$ *vs.* acx.
[b] $p < 0.05$ *vs.* acx and acx + low-dose insulin clamp.
Acx: acipimox.

Figure 2 shows mean plasma FFA, blood glycerol, serum IRI levels, and mean GH Δ areas during the 2 h after GHRH injection (0–120 min). At comparable low plasma FFA and blood glycerol levels, hyperinsulinemia progressively decreased the GH response to GHRH as compared with that observed after acipimox alone, although the difference was statistically significant only with the high-dose insulin clamp ($P < 0.05$ *vs.* acipimox alone); in fact, the low-dose insulin infusion reduced the GH response to GHRH only in four out of six subjects, whereas the high-dose insulin infusion was effective in all subjects, independently of sex. Mean blood-glucose levels were similar in all tests (4.4 ± 0.1 *vs.* 4.6 ± 0.1 *vs.* 4.5 ± 0.1 mmol/L, not significant). Considering the three tests together, a significant negative regression was observed between the mean serum IRI levels and the GH response to GHRH (GH Δ areas) (r = −0.629, $P < 0.01$).

Discussion

In lean subjects, pharmacologic blockade of lipolysis by acipimox enhances the GH response to GHRH (7, 21), caused by the acute reduction of circulating FFA levels and the removal of their negative feedback on GH release. Other direct effects of acipimox, not mediated by an FFA fall, are unlikely, as shown by Fulcher and co-workers, who observed no significant GH changes when acipimox was administered together with a lipid infusion able to maintain stable FFA levels (30). In the present study, we evaluated the GH response to GHRH under conditions of low FFA levels ob-
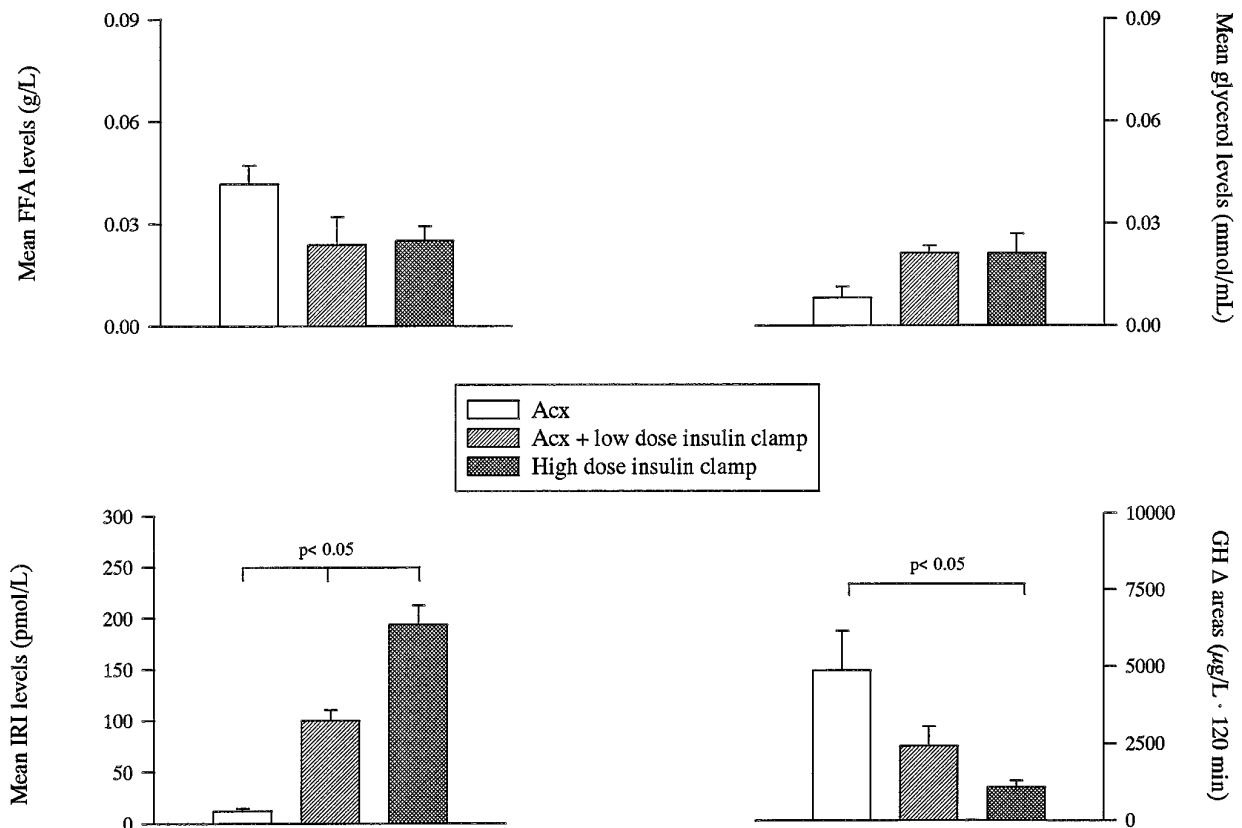

FIG. 2. Mean plasma FFA, blood glycerol, and serum insulin (IRI) levels, and mean GH Δ areas within the interval 0–120 min after GHRH injection in the three tests. Each *value* represents the mean ± SE. Acx: acipimox.

tained either by acipimox administration or by insulin infusion at different rates. The results of the present study indicate that when low plasma FFA levels are attained by insulin infusion, the GH response to GHRH is significantly lower than that observed after acipimox. Specifically, we observed a significant reduction of the GH response to GHRH at the insulin levels yielded by the high-dose insulin infusion (150–200 pmol/L), whereas at lower insulin levels (75–100 pmol/L), the GH response to GHRH, although lower, was not significantly different from that observed after acipimox administration. Our data, therefore, suggest that, independently of FFA, insulin *per se* is able to exert an inhibitory effect on GH release. This effect could have a physiological relevance, because it occurs at insulin levels (150–200 pmol/L) commonly observed under postprandial conditions (27), when GH and FFA levels are low. This finding is also in line with previous reports by Diamond and co-workers (19), who observed a progressive reduction of the GH response to hypoglycemia, with increasing insulin infusion rates.

Anatomical and experimental data, supporting a possible negative effect of peripheral insulin on GH release, already have been reported in the literature. Specific insulin binding sites have been found in normal rat adenoypophyseal cells (9), in rat anterior pituitary adenoma cells (10), and in human pituitary adenoma cells (11); inhibition of GH synthesis and release, and suppression of GH mRNA content also have been observed when pituitary cells are exposed to insulin (11–16). However, the physiological relevance of insulin action in the pituitary gland remains doubtful, in light of the low number of specific insulin receptors present in normal pituitary cells in comparison with those for insulin-like growth factors (9, 10). On the other side, insulin could affect GH release at the level of the central nervous system, *i.e.* of the hypothalamus. This hypothesis is supported by experimental evidence. First, the central nervous system can be reached by peripheral insulin (8), either via a specialized transport system across the blood brain barrier (8, 31) or by diffusion in circumventricular organs of the brain lacking blood brain barrier (8). Specifically, peripheral insulin could reach the hypothalamus, at least in part, by diffusion in the median eminence. Second, elevated insulin concentrations, insulin receptors, and insulin receptor substrate-1 have been found in the hypothalamus (8, 32–34). Insulin could affect GH release by increasing levels and release of hypothalamic catecholamines (35–38), which in turn, may stimulate SRIH release via β-adrenergic receptors (39). In this regard, the mediatory role of neuropeptide Y, which is known to stimulate (at least in the rat) hypothalamic SRIH release through an α_1- and β-adrenergic receptor-mediated mechanism (40), remains a matter of speculation. Finally, insulin could, at least in part, inhibit GH release through its influence on potassium homeostasis and circulating amino acid levels. In fact, by increasing the active membrane transport of potassium into the cells (41) via an activation of membrane (Na^+-K^+)-adenosine triphosphatase (ATPase) (42, 43), insulin could lead to membrane hyperpolarization and reduced GH release. Modulation of GH release during insulin infusion also could result from low circulating amino acid levels (44,

45), because amino acids are known to stimulate GH release (46).

Whichever the mechanism of insulin action on GH release may be, the data of the present study indicate that in healthy subjects: 1) acipimox and hyperinsulinemia produce a similar decrease in FFA levels caused by their antilipolytic activity; 2) under conditions of comparable low FFA levels, the GH response to GHRH is significantly lower during insulin infusion than after acipimox. Because acipimox has no direct effects on GH release (independent of its action on FFA), our data suggest the existence of an inhibitory effect of insulin on GH release. This effect may be of physiological relevance, because it occurs at insulin levels commonly observed during the day, for instance, during the postprandial period.

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