Metabolism
Clinical and Experimental

VOL 53, NO 10 OCTOBER 2004

Insulin-Induced Hypoglycemia Suppresses Plasma Parathyroid Hormone Levels in Patients With Adrenal Insufficiency

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Hypoglycemia has been reported to cause suppression of parathyroid hormone (PTH) levels in serum in normal subjects. It is possible that increasing cortisol levels in response to hypoglycemia was responsible. To examine this possibility the acute PTH response to insulin administration and resulting hypoglycemia was examined in patients with adrenal insufficiency. The possible acute impact of insulin-induced hypoglycemia on bone formation and bone resorption in the absence of an endogenous cortisol response was also examined. A prospective open study was undertaken to examine the acute effects of insulin and resulting hypoglycemia on PTH levels, on bone formation as indicated by serum levels of aminoterminal propeptide of type 1 procollagen (PINP), and on bone resorption as indicated by serum levels of β carboxy terminal telopeptide of type 1 collagen (β-CTx). Seven patients with adrenal insufficiency participated. These patients were studied on 3 occasions under different conditions: (1) when insulin was administered to induce hypoglycemia while the patients received their routine glucocorticoid replacement; (2) when the patients received their routine glucocorticoid replacement, but were not rendered hypoglycemic; and (3) when they did not receive glucocorticoid replacement and were not rendered hypoglycemic, ie, untreated. This facilitated isolation of the PTH response to insulin and hypoglycemia from the effects of the normal increase in endogenous cortisol levels in response to hypoglycemia. Blood samples were taken at baseline and after 3 hours while the subjects continued fasting for measurement of plasma glucose, serum ionized calcium (Cai), magnesium, phosphate, PINP, PTH, and β-CTx. Insulin 0.075 IU/kg body weight was given intravenously after the first blood sample. The usual morning glucocorticoid replacement dose was given 20 minutes after the baseline blood sample was obtained. After the administration of insulin, plasma glucose decreasedl from 4.8 ± 0.5 to 2.7 ± 0.5 mmol/L, mean ± SD ($P < .0001$). PTH was not influenced by time or glucocorticoid treatment, but decreased in response to insulin-induced hypoglycemia ($P < .05$). Serum levels of PINP and β-CTx decreased when untreated between 9 AM and 12 PM ($P < .05$), but were not independently influenced by insulin-induced hypoglycemia or glucocorticoid treatment. Serum levels of Cai increased and serum phosphate levels decreased in response to insulin-induced hypoglycemia, while serum phosphate levels were also independently influenced by time decreasing between 9 AM and 12 PM ($P < .05$). There was no effect of time, insulin-induced hypoglycemia, or glucocorticoid treatment on serum levels of magnesium. Possible mechanisms involved in the acute decrease in serum PTH observed include a direct effect of insulin or hypoglycemia or an indirect effect, eg, increased sympathomimetic activity on PTH secretion or on calcium or phosphate intercompartmental shifts.

DURING INSULIN-INDUCED hypoglycemia in normal human subjects, plasma parathyroid hormone (PTH) concentrations have been reported to be either stimulated,[1] suppressed,[2] or unchanged.[3] The normal endogenous cortisol response to hypoglycemia may also affect PTH secretion and bone turnover. Treatment with inhaled glucocorticoid has been reported to produce a tendency to suppression of serum PTH levels by some,[4] but not all investigators.[5,6] The effect of near physiologic replacement of glucocorticoid on PTH secretion has not been definitely established. Serum levels of the aminoterminal propeptide type 1 procollagen (PINP) and the β-carboxy terminal telopeptide of type 1 collagen (β-CTx) have been used as indices of bone formation and resorption, respectively, and thus provide insight into the process of bone turnover.[7,8] The present study was undertaken to identify the impact of hypoglycemia on PTH secretion in a manner so it would be possible to distinguish between the effects of insulin and hypoglycemia and glucocorticoid. Patients with adrenal insufficiency provide a model in which the normal

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Submitted June 9, 2003; accepted January 24, 2004.

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0026-0495/04/5310-0040$30.00/0
doi:10.1016/j.metabol.2004.01.005

endogenous glucocorticoid response to hypoglycemia does not occur. By examining the acute PTH, PINP, and β-CTx responses to hypoglycemia in patients with adrenal insufficiency, it was possible to separate the specific effects of insulin-induced hypoglycemia and glucocorticoid on PTH, PINP, and $\beta\beta$-CTx.

MATERIALS AND METHODS

Patients and Treatment Schedules

Seven patients, 6 women and 1 man (52 ± 8.4 years, mean age ± SD), with adrenal insufficiency participated. Six patients had primary adrenal insufficiency. One female patient had secondary adrenal insufficiency. Each patient received their usual glucocorticoid treatment by mouth: 4 patients took hydrocortisone 10 mg with breakfast and 5 mg with lunch and one of these also took 5 mg with the evening meal. Two patients took dexamethasone 0.5 mg and 1 patient took dexamethasone 0.25 mg with breakfast. All 7 patients were taking fludrocortisone replacement 0.1 to 0.05 mg daily. Three women were receiving hormone replacement therapy for menopausal symptoms, 2 patients were taking replacement with L-thyroxine 100 μg/d. Blood samples were obtained for measurement of serum PTH, PINP, β-CTx serum ionized calcium, serum magnesium, and serum phosphate at 9 AM and at 12 PM. Baseline values are shown in Table 1. On 2 of the 3 study days, hydrocortisone replacement was given at 9:20 AM, and on one of the study days, this followed insulin, 0.075 IU/kg body weight, administered intravenously at 9 AM, and on another study day when insulin was not given. On a third study day, neither glucocorticoid therapy nor insulin was given. Otherwise patients were fasting during the study. None of the patients had any serious side effects. This study was approved by the Research and Ethics Committee St. Vincent's University Hospital, and all patients gave informed written consent.

Analytical Methods

Serum ionized calcium (Cai) (reference range, 1.19 to 1.35 mmol/L) was measured using an ion-selective electrode (Radiometer). Plasma glucose levels were measured on a Beckman CX7, as were levels of serum magnesium (reference range, 0.7 to 1.0 mmol/L) and serum phosphate (reference range, 0.80 to 1.40 mmol/L). The following analytes were measured by immunoassay: serum PTH was assayed by 2-site immunoradiometric analysis (Allegro Nichols, San Juan, Capistrano, CA). The assay sensitivity was 1.1 ng/mL and intra-assay and interassay coefficients of variation (CV) were 6.7% and 8.1%, respectively, while the reference range was 2 to 52 ng/mL. PINP was measured by radioimmunoassay (Orion Diagnostica, Espoo, Finland). The assay sensitivity was 2 μg/L and intra-assay and interassay CV were 4.1% and 5.7%, respectively, while the normal reference range (combined male and female) was 27.2 to 72 μg/L. β-CTx was measured by an electrochemiluminescence immunoassay (Roche Elecsys 1010). The assay sensitivity was 0.01 μg/L and intra-assay and interassay CV were 1.6% and 1.9%, respectively, while the reference range for premenopausal females was 0.025 to 0.573 μg/L and for postmenopausal females 0.014 to 1.008 μg/L and males, 30 to 50 years, 0.016 to 0.584 μg/L.

Statistical Analysis

Data that was not normally distributed was log-transformed prior to analysis. Paired *t* tests were used to determine whether variables changed between 9 AM and 12 PM on each study day. To determine the independent effects of time (baseline *v* 3 hours), insulin-induced hypoglycemia and glucocorticoid administration on each of the studied variables, multiple regression analysis was performed in which time, insulin, and glucocorticoid were independent variables, and each of the studied biochemical values were the dependent variables. Statistical significance was considered to be present when the *P* value was $<.05$.

Table 1. Baseline Biochemical Variables on 3 Study Days in Which Seven Patients With Adrenal Insufficiency Received GC and Underwent IH + GC, Received GC, or Received No Intervention

	IH + GC	GC	No Treatment
PTH (ng/mL)	22.5 ± 7.3	24.1 ± 17.7	21.6 ± 11.8
PINP (μg/L)	37.8 ± 25.1	36.6 ± 16.7	38.0 ± 19.4
β-CTx	0.29 ± 0.07	0.30 ± 0.07	0.27 ± 0.07
Ionized calcium (mmol/L)	1.19 ± 0.03	1.20 ± 0.03*	1.19 ± 0.05
Serum magnesium (mmol/L)	0.85 ± 0.04	0.85 ± 0.06	0.84 ± 0.04†
Serum phosphate (mmol/L)	1.23 ± 0.23	1.25 ± 0.15	1.31 ± 0.15†

Abbreviations: IH + GC, insulin-induced hypoglycemia and glucocortoid replacement.

*Measurement of serum ionized calcium when HC was given, but insulin was not given (N = 5).

†Measurement of serum magnesium and phosphate under control conditions (N = 5).

RESULTS

Serum Glucose, PTH, PINP, and β-CTx Levels After Insulin Administration

After the administration of insulin, plasma glucose decreased from 4.8 ± 0.5 to 2.7 ± 0.5 mmol/L, mean ± SD ($P < .0001$). PTH levels decreased significantly between 9 AM and 12 PM only on the study day when insulin and glucocorticoid replacement were both given ($P < .005$ (Fig 1). Multiple regression analysis revealed that PTH was not influenced by time or glucocorticoid treatment, but decreased in response to insulin-induced hypoglycemia.

Serum PINP decreased between 9 AM and 12 PM on all 3 study days, although this narrowly failed to reach significance on the day in which insulin and glucocorticoid were both administered ($P = .069$ (Fig 1). Multiple regression analysis revealed that PINP was influenced by time, but not by insulin-induced hypoglycemia or glucocorticoid administration (Fig 1). Serum β-CTx also proved to be independently influenced by time, levels decreasing between 9 AM and 12 PM (Fig 1).

Serum Phosphate, Calcium, and Magnesium Levels After Insulin Administration

On each of the 3 study days, serum phosphate levels decreased significantly ($P < .05$), while there was no significant change in Cai and serum magnesium levels between 9 AM and 12 PM (Fig 1). Multiple regression analysis revealed that serum levels of Cai increased, and serum phosphate levels decreased in response to insulin-induced hypoglycemia, while serum phosphate levels were also independently influenced by time, decreasing between 9 AM and 12 PM. There was no effect of any variable on serum magnesium.

DISCUSSION

In the present study, 7 patients with adrenal insufficiency were studied with and without glucocorticoid replacement therapy. In this way we could eliminate the effects of glucocorticoid on PTH. In addition, it was possible to deduce the effect of insulin-induced hypoglycemia on PTH and

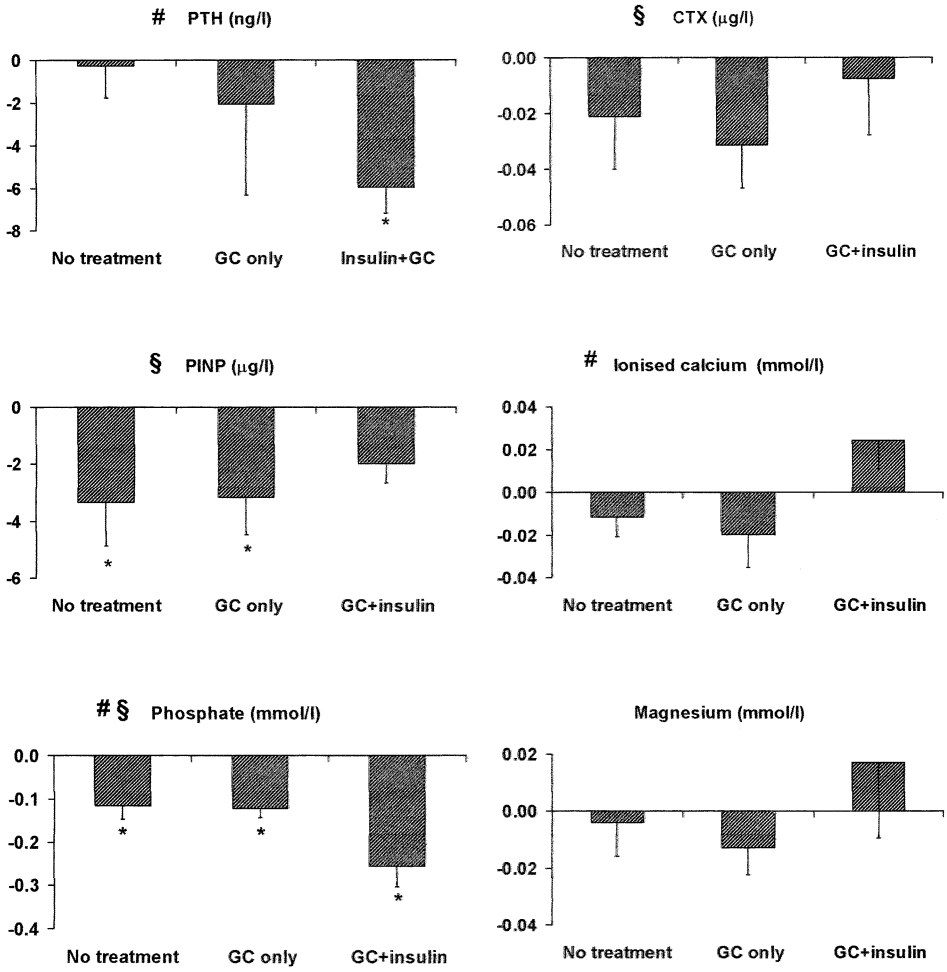

Fig 1. Change from baseline of biochemical variables on 3 study days in which 7 patients with adrenal insufficiency received glucocorticoid replacement and underwent insulin-induced hypoglycemia (GC + insulin), received glucocorticoid replacement only (GC only), or received no intervention (no treatment). *$P < .05$ *v* baseline, #$P < .05$ independent effect of insulin-induced hypoglycemia, §$P < .05$ independent effect of time.

PINP in the absence of endogenous cortisol secretion. Our results indicate that PTH levels decrease significantly in response to insulin administration and/or associated hypoglycemia. This response was not seen when the patients received glucocorticoid replacement without having been rendered hypoglycemic or when glucocorticoid treatment and insulin induced-hypoglycemia were withheld. This observation is in agreement with the results obtained by Shearing et al.[2] The exact mechanism for the decrease of PTH levels in serum in response to insulin administration and hypoglycemia is not known. The major factor controlling PTH secretion is the Cai level. In our study, regression analysis revealed an increase in serum Cai associated with insulin administration and hypoglycemia when there was a significant decrease in PTH levels. Shearing et al,[2] who reported a decrease in PTH in response to hypoglycaemia, also observed a significant, but transient, increase in Cai levels 15 minutes after hypoglycemia. D'Erasmo et al[9] reported decreased PTH and decreased total and Cai levels in serum induced by hyperglycemia and hyperinsulinemia following oral glucose loading in healthy human subjects. Ohno et al,[10] using the euglycemia hyperinsulinemic clamp technique, observed a decrease in intact PTH levels in serum and increased fractional excretion of calcium in young male subjects. In our study, a very early change in serum Cai cannot be outruled. Both the D'Erasmo et al[9] and Ohno et al[10] studies tend to attribute the PTH decline to hyperinsulinemic factors in their hyperglycemic[9] and euglycemic[10] subjects. Serum magnesium levels also affect serum PTH levels, but serum magnesium levels did not change significantly on any of the 3 study days. Serum phosphate levels decreased significantly during all 3 study days, but this decrease was exaggerated when insulin was administered. It is possible that the decrease in serum PTH levels in response to insulin administration and hypoglycemia could be a direct effect of hypoglycemia or insulin on PTH secretion or could be mediated through the sympathetic nervous system.[11,12] Intravenous infusion of epinephrine in humans has been reported to cause either transient increase in PTH secretion[13] or no response.[14] When Shearing et al[2] pretreated normal subjects with an α- or β-adrenoreceptor blocker, the PTH levels decreased in response to hypoglycaemia, but to a lesser extent than without pretreatment. Furthermore, Cai levels did not increase when adrenoreceptor blockers were used.

It is possible that the decrease in PTH levels came about

when the Cai level was supported by a non-PTH-dependent mechanism. Theoretically, a shift of calcium into the vascular space as a consequence of mechanisms induced by insulin or hypoglycemia or increased sympathetic tone would allow a decrease in PTH levels no longer required to maintain calcium within the physiologic range. It is possible that the primary effect of insulin or hypoglycemia was to bring about a decrease in phosphate levels with consequent decrease in the complexed fraction and an increase in the ionized fraction of plasma calcium and thereby suppression of PTH secretion. However, it is not possible to draw a conclusion on the precise mechanism underlying the decrease in PTH levels.

The present study indicates that insulin administration and/or mild hypoglycemia is associated with an acute decrease of PTH levels in serum. Because this decrease in PTH levels did not occur when glucocorticoid replacement therapy was given in the absence of treatment with insulin and the associated hypoglycemia, it can be concluded that insulin and/or hypoglycemia and not glucocorticoid replacement was related to the decrease in serum PTH levels. Furthermore, elevated endogenous glucocorticoid levels achieved in normal subjects after induction of hypoglycemia could not have accounted for the decrease of PTH values seen in our patients who had adrenocortical insufficiency. Candidate mechanisms for the decrease in PTH levels include a direct effect of insulin or hypoglycemia on PTH secretion. Alternatively, the decrease in serum PTH levels could be mediated indirectly by levels of catecholamines increasing in response to hypoglycemia. Such effects may be exerted as an immediate effect on PTH secretion. Alternatively, shifts in the compartmental distribution of calcium and phosphate leading to a decrease in serum phosphate and an increase in serum calcium would cause suppression of PTH secretion.

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