Insulin Suppresses Growth Hormone Secretion by Rat Pituitary Cells

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Abstract. The effects of insulin on basal and hydrocortisone-induced growth hormone (GH) secretion were studied in rat pituitary tumor cells (GH_3). Cells were grown in monolayer culture and exposed to exogenously added insulin for up to 8 d. Basal GH secretion was inhibited by insulin (0.7 nM) after a 48-h lag period by ~50% ($P < 0.01$, vs. untreated control cells). The suppression of GH secretion was reversible, as removal of added insulin resulted in return of GH secretion to normal levels after 24 h. Maximal suppression of basal GH secretion was achieved by 0.7 nM insulin, and these effects were prevented by simultaneous exposure of the cells to guinea pig anti-insulin serum (1:2,000). No effects of insulin on cell replication were evident, and glucose concentration in the medium did not differ in control or insulin-treated wells. Insulin (7 nM) significantly suppressed the fivefold hydrocortisone-induced GH stimulation during 5 d of incubation with up to 1,000 nM of the steroid ($P < 0.001$). These inhibitory effects were similarly observed in glucose- and pyruvate-free medium, and in the presence of 2-deoxyglucose. Insulin also reversed the suppression of prolactin (PRL) secretion induced by hydrocortisone (1 uM), and actually stimulated basal PRL secretion by over 50%. Insulin did not alter the inhibitory effect of hydrocortisone on GH_3 cell proliferation. Although higher doses (13 nM) of insulin-like growth factor (IGF-I) also suppressed basal GH secretion, IGF-I did not alter the GH and PRL secretory changes induced by hydrocortisone. The results show that insulin exerts a direct, specific inhibitory effect on basal and hydrocortisone-induced GH secretion by GH_3 cells unrelated to glucose utilization by the cells.

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Introduction

Although insulin and glucose play a significant role in the modulation of growth hormone (GH)[1] secretion (1, 2), specific action of insulin on the pituitary are difficult to demonstrate in vivo. The cellular effects of insulin include a rapid modulation of membrane-related transport systems, as well as a longer-term effect on regulation of gene expression and protein synthesis independent of its effects on glucose and amino acid transport (3–6). Insulin was shown to regulate carbohydrate metabolism in the rat anterior pituitary (7), but its role in directly modulating anterior pituitary hormone secretion generally, and GH specificially, has not been extensively studied. Pituitary GH content of rats rendered diabetic by alloxan was significantly lower than in nondiabetic rats (8). As pituitary GH content was inversely correlated with the severity of diabetes in these animals, a role for insulin in modulating GH synthesis was proposed (8). Although insulin stimulated new protein synthesis by the rat anterior pituitary during 3 h of in vitro exposure to the hormone (9), no specific effects on incorporation of radiolabeled precursor into GH or prolactin (PRL) were noted during this short treatment.

Recently, the presence of specific binding sites for insulin have been characterized on rat pituitary homogenatees (10), pituitary microsomal membranes (11), and rat pituitary tumor cells (GH_3, GH_1, and GC) (12, 13). The dissociation constant (K_D) for insulin binding in these studies ranged from 2 to 0.3 nM, which is of a similar order of magnitude seen in other insulin-responsive tissues (14). Biological effects of insulin on pituitary cell hormone secretion were not reported in these two preliminary studies (12, 13).

In view of the above findings, experiments were performed to further evaluate the effects of insulin on in vitro GH secretion. GH_3 cells, a cloned rat pituitary tumor cell line secreting both

1. *Abbreviations used in this paper:* ANOVA, analysis of variance; dbCAMP, dibutyryl-cyclic AMP; GH, growth hormone, IGF-I, insulin-like growth factor-I; IGF-II, insulin-like growth factor-II; PRL, prolactin.

GH and PRL, were used (15). The hormonal secretory patterns of these cells have been well characterized (13, 16). Hydrocortisone stimulates GH secretion in these cells (17, 18), and inhibits PRL secretion (19). The steroid causes increased GH messenger RNA (20–22) and GH gene transcription (23, 24). This response has also recently been confirmed in vivo (24), supporting the physiologic relevance of hydrocortisone-induced GH stimulation.

The studies reported here show that physiological concentrations of insulin suppress basal GH secretion and high physiologic doses inhibit the hydrocortisone-induced stimulation of GH by these cells.

Methods

Materials. Bovine pancreatic insulin and hydrocortisone were purchased from Sigma Chemical Co., St. Louis, MO, and guinea pig antibovine insulin serum from Miles Biochemicals, IN. Partially purified somatomedin (25) was kindly provided by Dr. Ron Rosenfeld, Stanford University. 1 mg of this preparation contained 50 μg insulin-like growth factor-I (IGF-I) and 22.7 μg insulin-like growth factor-II (IGF-II) as measured by specific radioimmunoassay (RIA) (26, 27). Doses of somatomedin are expressed as IGF-I (nanograms per milliliter). Fetal calf serum was obtained from Sterile Systems, Logan, UT. Unless otherwise stated, cell culture materials were obtained from Irvine Scientific Co., Santa Ana, CA.

Cells. GH_3 cells, a cloned line of rat pituitary tumor cells secreting GH and PRL (16), were obtained from the American Type Culture Collection, Rockville, MD. Cells were maintained in serial monolayer culture as described (28). The cells have been routinely grown in Ham's F10 medium supplemented with fetal calf serum (2.5%), horse serum (15%), glutamine (5 mM), and antibiotics (culture medium) in a humidified atmosphere of 95% air:5% CO_2. Glucose concentration, as measured by glucose oxidase method in a Beckman glucose analyzer (Beckman Instruments, Inc., Fullerton, CA), was 110 mg/dl. Insulin concentration, determined by RIA (29), was undetectable (<5 μU/ml) in the culture medium.

For experiments utilizing glucose-free medium, cells were grown in deficient (glucose-free) Dulbecco's Modified Eagle medium enriched with dialyzed horse serum (10%), glutamine (10 mM), and antibiotics (glucose-free culture medium). This culture medium contained <5 mg/dl glucose. Sodium pyruvate (10 mM) or 2-deoxyglucose (20 mg/dl) were added to glucose-free medium for some experiments.

Experimental procedure. All experiments were performed on cells obtained from the same parent culture flask (75 cm^2, Costar, Cambridge, MA). Approximately 50,000 cells were seeded in Multiwell tissue culture plates (35 mm diameter, Costar) in 2 ml of culture medium. Cells were either pretreated with insulin before seeding into experimental wells for the times indicated, or insulin and drugs were added immediately after seeding of cells. At the designated time intervals, medium was aspirated, centrifuged at 800 *g* for 10 min, and frozen at −20°C for hormone assay. Cells from each well were taken up in 2 ml isotonic saline and counted in an Automatic Cell Counter (Coulter Electronics Inc., Hialeah, FL).

Insulin degradation. In order to assess the degradation of insulin under the culture conditions employed, exogenously added insulin was examined by TCA precipitability of ^{125}I-labeled insulin. ^{125}I-labeled insulin was prepared by enzymatic iodination using Enzymobead reagent (Bio-Rad Laboratories, Richmond, CA) (30). More than 95% of the radioactivity of ^{125}I-labeled insulin (80–100 μCi/μg sp. act.) was precipitable by ice cold 12% TCA and was also recovered in the intact insulin peak when ^{125}I-insulin was subjected to gel filtration over G-50 Sephadex. After 72 h of exposure to the cells in culture, 60% of media ^{125}I-insulin was precipitable and 40% of the tracer was soluble in TCA. Therefore, in all experiments, 40% of the initial insulin dose was replenished after 72 h in order to maintain the required concentrations of the hormone.

RIAs. Rat GH was iodinated using chloramine T and sodium metabisulfate as described (31). More than 95% of the radioactivity was precipitable by TCA and was also recovered in the intact rat GH peak when subjected to gel filtration over G-75 Sephadex. Rat GH and PRL were measured by RIA using materials supplied by the National Pituitary Agency, National Institutes of Arthritis, Diabetes, Digestive and Kidney Diseases, Bethesda, MD. All samples from each experiment were assayed in the same RIA. Samples were assayed in duplicate with at least three dilutions in order to yield accurate extrapolations from the standard curve. Insulin was assayed by RIA (29). None of the medium or reagents employed in the experiments caused a cross-reaction in the RIAs used.

Statistics. Results are expressed as mean±SEM unless otherwise indicated. Differences were assessed by nonpaired *t* testing, or two-way analysis of variance (ANOVA) with Scheffe's multiple comparison procedure (32).

Results

Insulin effects on GH degradation. In order to test whether insulin altered the rate of extracellular degradation of GH, cells were incubated in the presence of ^{125}I-GH and insulin, and aliquots of medium were removed periodically to assess the TCA precipitability of the added tracer. 95% of ^{125}I-GH present in the medium prior to incubation (zero-time) was precipitable after 96 h of incubation in wells not containing cells (cell-free). During this period, 80% of the zero-time tracer counts were precipitable when ^{125}I-GH was incubated in wells containing GH_3 cells growing in culture medium. There was no difference in stability of the ^{125}I-GH in the presence of 0.7 and 7 nM added insulin, as compared with control wells containing cells with no added insulin. Insulin therefore did not alter the degradation of GH, at least as assessed by TCA precipitability of the ^{125}I-GH tracer.

Basal GH secretion. Insulin did not inhibit GH hormone release in the medium during the first 24 h of incubation (Fig. 1, insert). Basal GH secretion was inhibited by 0.7 nM insulin over 50% during 48 h of incubation (Fig. 1, bottom). Even when a higher dose (7 nM) of insulin was used, no inhibition of GH was seen during the first 24 h of incubation. This inhibition was significant throughout the 8-d duration of these experiments, with basal hormone secretion being suppressed by 40–50% at each time point. Using a higher dose of insulin (7 nM), GH secretion was further suppressed at each point. Fig. 1 shows data from a single representative experiment. Because of the wide inter-experimental variation in control and GH secretion, data from four separate experiments were pooled and expressed as a percentage of control values. After 48 h, GH secretion was 61±2 and 40±6% of controls in the presence of 0.7 and 7 nM

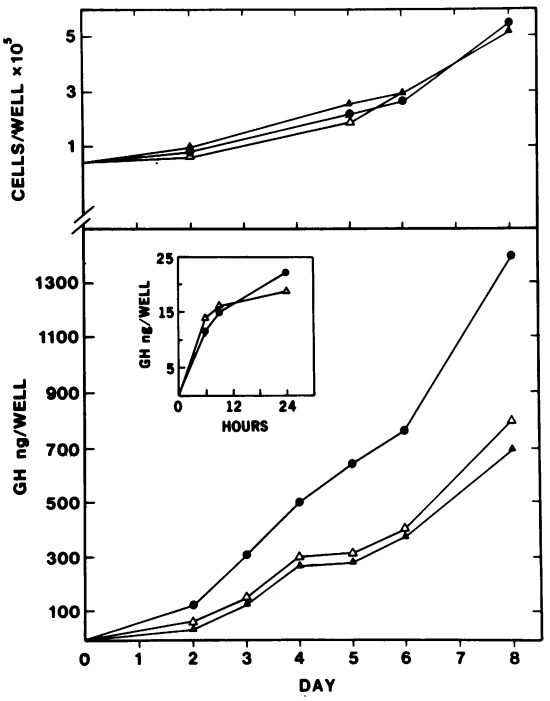

Figure 1. Time course of cell replication (top panel) and GH secretion (bottom) in presence of insulin. About 5×10^4 cells were incubated in 36 multiwells on day 0 in culture medium containing no added insulin (controls, — ● —), or insulin, 0.7 nM (— △ —) or 7 nM (— ▲ —). At each time point, medium from duplicate wells was aspirated for RIA, and cells counted. Insert shows the time-course for 0–24 h.

insulin, respectively. After 8 d, GH suppression was similar in the two insulin-treated groups, 65±4 and 63±4% of controls. No differences in cell number were observed in insulin-treated vs. untreated wells throughout 8 d of incubation (Fig. 1, top).

The reversibility of the inhibition of GH induced by insulin was tested by growing cells in the presence or absence of 0.7 nM insulin for 48 h. Medium was then completely aspirated, fresh medium containing no added insulin was replaced in all the wells, and the incubations continued. Fig. 2 shows that inhibition of GH secretion by cells previously exposed to insulin was present for up to 24 h after insulin was removed; thereafter, secretion recovered to attain similar levels to those seen in cells which were previously not exposed to insulin.

The dose-responsiveness of basal GH suppression by insulin is shown in Fig. 3. Maximal suppression was achieved by 0.7 nM insulin. To confirm the specificity of the observed suppression of basal GH secretion, cells were incubated with insulin with and without guinea pig anti-insulin serum (1:2,000). Cells in control wells were also exposed to similar titers of antiserum. The suppression of basal GH secretion by 0.7 nM insulin was neutralized by simultaneous exposure of cells to insulin antiserum (Fig. 4). Insulin antiserum alone, when added to control wells, did not alter basal GH secretion. Although the same titer

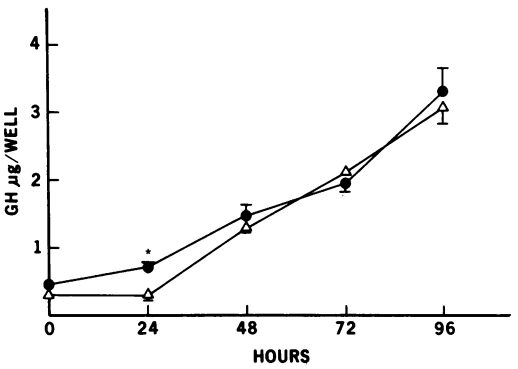

Figure 2. Recovery of GH secretion. GH_3 cells ($\sim 10^5$) were incubated in the absence (— ● —) or presence (— △ —) of insulin (0.7 nM) for 48 h in 2 ml of medium. At time 0, medium was changed and replaced with fresh medium containing no added insulin, and incubations continued for the indicated times. Each point represents mean of triplicate wells±SEM. After 24 h, there was no subsequent difference between control and insulin-treated GH secretion ($P < 0.05$). Cell counts in treated and untreated wells were not different.

(1:2,000) antiserum was able to significantly reverse the suppression of 7 nM insulin, GH secretion did not attain control, untreated values. A higher titer of guinea pig antiserum proved toxic to the cells and was therefore unable to be tested against the higher insulin dose (7 nM).

Hydrocortisone effects. As hydrocortisone has previously been shown to stimulate GH secretion by GH_3 cells (17, 18), the effects of insulin were tested on this response. For these experiments, GH_3 cells were preincubated in the presence or

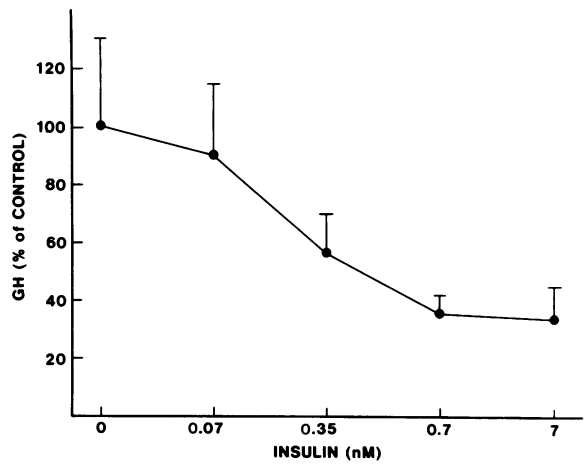

Figure 3. Dose-response of inhibition of GH secretion by insulin. Cells were incubated in the presence of the indicated doses of insulin for 48 h. Data from four experiments were pooled and expressed as a percentage of GH secreted in control wells receiving no added insulin. Control wells secreted 995±299 ng GH/10^6 cells per 48 h (mean±SD).

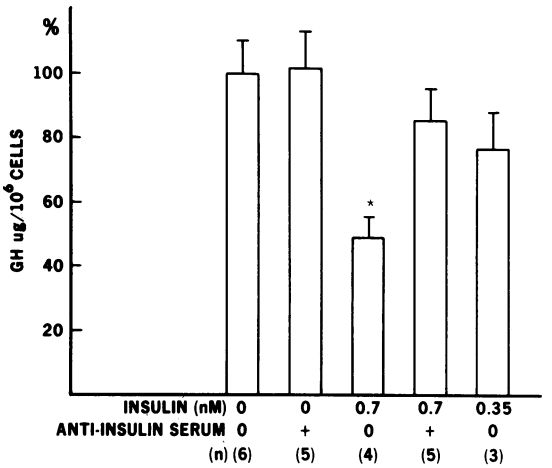

Figure 4. Effect of guinea-pig anti-insulin serum on GH secretion. GH_3 cells were incubated for 48 h in medium with or without added insulin and/or insulin antiserum (mean±SEM). *, $P < 0.01$ vs. controls and vs. insulin and antiserum.

absence of insulin for 48 h, and then seeded into multiwells in the presence of hydrocortisone (0–1,000 nM). The same doses of insulin were used for the preincubations and the hydrocortisone-treated incubations. Fig. 5 shows that hydrocortisone (100 nM) stimulated GH secretion fivefold during 5 d of incubation. By two-way ANOVA, the response to hydrocortisone was significantly inhibited at each dose tested by preincubation and simultaneous exposure of cells to 7 nM insulin ($P < 0.001$). In the presence of insulin, 10 nM hydrocortisone did not stimulate GH secretion, whereas 1 nM hydrocortisone doubled GH secretion when no insulin was added. Insulin (0.7 nM) was able to significantly suppress the stimulatory effect of up to 10 nM hydrocortisone ($P < 0.01$). The higher dose of insulin (7 nM) suppressed by half the GH stimulation seen with up to 1 μM hydrocortisone. That this action of insulin was a specific antagonism to the steroid effect rather than a general cellular effect was further confirmed by the observation that the suppressed PRL secretion induced by hydrocortisone was also reversed by insulin (Fig. 6). Insulin stimulated both basal PRL secretion as well as the suppressed PRL secretion caused by hydrocortisone. The typical effects of insulin and/or hydrocortisone on GH secretion were also seen in these same cells.

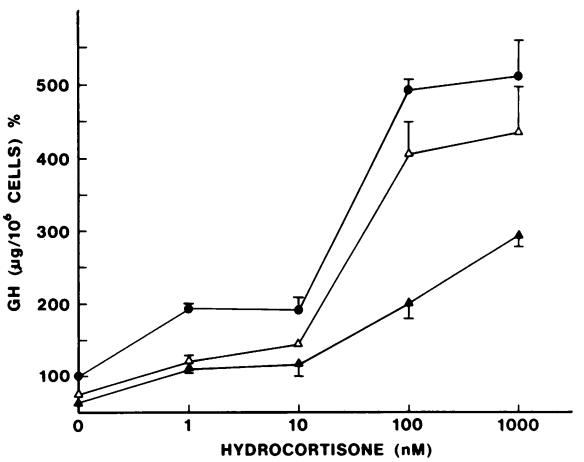

Figure 5. Effects of insulin on hydrocortisone-induced stimulation of GH secretion. GH_3 cells were grown in culture medium without (— ● —) or with added insulin, 0.7 nM (— △ —) or 7 nM (— ▲ —), for 48 h in 75 cm^2 tissue culture flasks. About 4×10^4 cells were then seeded in 30 multiwells containing 2 ml of medium without (control) or with added insulin, as well as the indicated concentrations of hydrocortisone. After 5 d, medium was aspirated for RIA, and cells counted. Each point represents mean (±SEM) of 4–6 wells pooled from two separate experiments. By two-way ANOVA, GH secretion in the wells treated with insulin, 7 nM, were significantly different from control wells ($P < 0.001$).

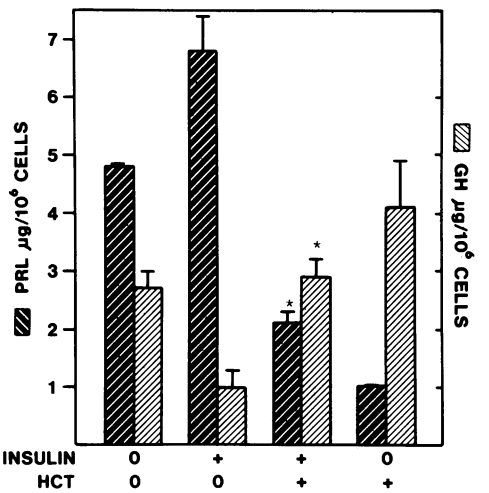

Figure 6. Effect of hydrocortisone (*HCT*) on GH and PRL secretion by GH_3 cells. Cells were incubated in multiwells for 5 d in the presence of HCT (1 μM) and/or insulin (0.7 nM), at which time medium was aspirated and cells counted. Each bar represents mean of triplicate wells (±SD). Data are representative of a single experiment performed independently eight times. (*, $P < 0.001$ vs. HCT alone or insulin alone).

Hydrocortisone clearly suppressed the growth of GH_3 cells (Table I). Although insulin modulated the secretory response of the cell to hydrocortisone, it had no effect on the suppression of cell growth induced by the steroid. This further confirmed the specificity of the insulin effect on hormone secretion.

Relationship of glucose availability to the insulin effects. Glucose, in different concentrations, was added to the medium in order to assess whether the observed insulin effects on GH secretion were related to glucose availability and utilization by the cells. Cells pretreated with insulin for 72 h were cultured in the presence of three different starting concentrations of glucose (110, 160, or 210 mg/dl). After 4 d incubation, glucose was measured in the medium. Table II shows that medium glucose levels fell after 4 d in both treated and untreated wells.

*Table I. Effects of Hydrocortisone and Insulin on GH_3 Cell Replication during 5 d**

Insulin		Hydrocortisone (*nM*) 0	1	10	100	1000	10,000
	nM						
Experiment 1	0	4.5±0.9	3.8±0.1	3.3±0.1	2.7±0.1	2.2±0.1	1.3±0.1
	0.7	3.9±0.4	3.5±0.04	2.9±0.1	2.9±0.2	2.1±0.04	1.4±0.01
Experiment 2	0	2.6±0.2	2.3±0.2	2.0±0.18	1.6±0.18	1.6±0.04	
	7	2.5±0.05	2.2±0.15	2.4±0.1	2.0±0.1	1.6±0.09	

* GH_3 cells were incubated for 5 d in the presence of hydrocortisone at the indicated doses with or without insulin. About 45,000 and 25,000 cells were initially seeded in experiments 1 and 2, respectively. Each point is mean of triplicate wells × 10^5±SD.

Insulin treatment of the cells did not alter the recovery of medium glucose as compared with control medium from untreated cells. Higher concentrations of glucose (>210 mg/dl) actually suppressed cell growth and were therefore not able to be tested.

To ascertain whether the observed effects of insulin on GH secretion were related to a metabolic effect of insulin on cellular glucose consumption, cells were tested in giucose-free medium containing either added glucose, 2-deoxy-glucose and Na-pyruvate, Na-pyruvate, or glutamine (Table III). For these experiments, cells which had either been pretreated with insulin (3.5 nM) for 72 h or not (controls) were plated into multiwells containing normal culture medium. After 24 h, this medium was aspirated and replaced with glucose-free medium containing the respective substrate and cells exposed to insulin and/or hydrocortisone for the ensuing 3 d.

Hydrocortisone (0.1 and 1 μM) stimulated GH secretion 3–5-fold in these experiments, but only a doubling of GH was seen when cells growing in glucose-free medium containing Na-pyruvate and dialyzed horse serum (10%) were exposed to the steroid (Table III). Nevertheless, insulin (3.5 nM) clearly suppressed hydrocortisone-induced GH secretion by at least half in the presence of all four substrates tested. Basal GH secretion was lower in cells exposed to 2-deoxyglucose (the cell number in this group was about 50% of glucose-treated values), nevertheless this was halved by insulin ($P < 0.025$). 2-deoxyglucose-treated cells responded to hydrocortisone with an almost 3-fold increase in GH secretion and this was clearly suppressed by insulin ($P < 0.001$).

Table II. Glucose Concentrations in Culture Medium

Medium glucose (*mg/dl*)*		
	Recovered	
Starting	Control	Insulin-treated
110	86±4	90±2
160	136±4	136±3
210	174±6	182±5

* Mean±SD of triplicate wells incubated for 4 d in the presence or absence (controls) of insulin (7 nM). Differences in glucose measured in control and insulin-treated wells were not significant for any of the three glucose doses.

Effects of insulin-like growth factor. In order to assess the effects of insulin-like growth factors on the GH response in these cells, they were exposed to varying doses of a partially purified somatomedin preparation containing IGF-I (0–500 ng/ml) for 4 d. The dose response shown in Fig. 7 shows that maximal (~60% of controls) inhibition of GH secretion was achieved by 250 ng/ml of IGF-I. Although IGF-I (100 ng/ml) suppressed basal GH secretion to 70% of controls ($P < 0.05$), no suppression of hydrocortisone-stimulated GH secretion was seen (Table IV). Similarly, IGF-I did not reverse the suppression of PRL induced by hydrocortisone (Table IV). No differences in results were observed when these experiments were repeated twice on cells which had been pretreated with the respective dose of IGF-I for 72 h.

Discussion

These experiments demonstrate a direct inhibitory effect of insulin on basal and hydrocortisone-stimulated GH secretion by GH_3 rat pituitary tumor cells. The suppressive effects of insulin on GH secretion appear to be unrelated to glucose utilization by the cells.

The intracellular turnover of GH and PRL by GH_3 cells is extremely rapid (<60 min) and the cells do not store large pools of intracellular growth hormone (13, 18, 33). Therefore, hormone release into the medium during these longer-term incubations may be regarded as a true reflection of hormone synthesis (18). Extracellular effects of insulin on GH stability during the time-course of these experiments were excluded, inasmuch as ^{125}I-GH tracer remained stable in the presence of insulin under experimental conditions.

The specificity of the GH response to insulin was suggested by the observation that insulin did not significantly alter basal GH_3 cell replication, nor did insulin antagonize the hydrocor-

Table III. Effects of Insulin on GH Secretion (nanograms per 10^6 cells) by Cells Grown in Glucose-free Medium for 3 d

Experiment	Added substrate	Insulin	Hydrocortisone 0	Hydrocortisone 1	Hydrocortisone 0.1
		nM	*μM*	*μM*	*μM*
I	Glucose (100 mg/dl)	0	1167±64	4210±225	5411±850
		3.5	706±129	1705±294	1681±177
		P	<0.025	<0.005	<0.01
II	2-deoxyglucose (20 mg/dl) and Na-pyruvate (10 mM)	0	78±8	224±13	221±8
		3.5	38±9	62±6	59±2
		P	<0.025	<0.001	<0.001
III	Na-pyruvate (10 mM)	0	998±133	2001±296	1404±157
		3.5	446±138	671±108	755±78
		P	<0.025	<0.01	<0.02
IV	Na-pyruvate (10 mM)	0	1090±9	3888±523	5053±296
		3.5	741±102	1417±156	1436±103
		P	<0.02	<0.01	<0.001
V	Glutamine (10 mM)	0	1700±16	6010±274	4283±284
		3.5	1374±71	3312±562	2512±218
		P	<0.01	<0.01	<0.01

GH_3 cells were grown in normal culture medium for 24 h, whereafter medium was aspirated and replaced with glucose-free medium (deficient Dulbecco's Modified Eagle medium, dialyzed horse serum (10%), glutamine (5 mM), and antibiotics), replenished with either glucose, 2-deoxyglucose and Na-pyruvate, Na-pyruvate, or glutamine as indicated. In experiment IV, horse serum (10%) was nondialyzed. Each value represents mean±SEM of at least triplicate wells.

tisone-induced suppression of cell growth. Kiino and Dannies (34), in fact, showed a slight decrease in the rate of GH_4C_1 cell replication in the presence of insulin and estradiol.

The time course of the insulin-induced effects on these experiments had a lag period of 48 h. When insulin was added to rat anterior pituitary incubations, for 3 h, no changes in incorporation of ^{14}C-lysine into the GH peak on polyacrylamide gel electrophoresis was noted (9). This relatively short incubation

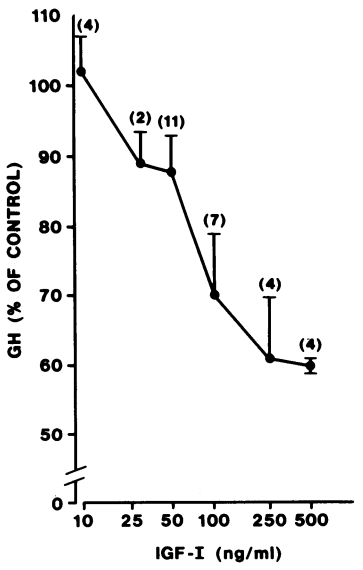

Figure 7. Dose response of inhibition of GH secretion by IGF-I. Cells were incubated for 4 d in the presence of the indicated doses of IGF-I. Figures in parentheses indicate total number of wells in each group. Data were pooled from four independent experiments. Secretion of GH in 16 control wells was 1030±321 ng/10^6 cells (mean±SEM).

Table IV. Effects of IGF on Hydrocortisone (1 μM)-mediated GH and PRL Secretion

	GH*	PRL*
Controls (12)	451±21	36±10
IGF-I (50 ng/ml) (9)	455±50	40±12
IGF-I (100 ng/ml) (7)	652±24	33±3

* Percent of hormone secretion by control wells not exposed to hydrocortisone or IGF.

Cells were treated as indicated for 5 d. GH and PRL values (mean±SEM) from three independent experiments were pooled and normalized as a percentage of mean hormone secretion by at least triplicate control wells not exposed to hydrocortisone or IGF in each experiment. Secretion of GH and PRL in 19 such control wells was 1188±129 and 11778±593 ng/10^6 cells, respectively.

time may have precluded the observation of insulin effects on protein synthesis.

Most of the cellular effects of insulin appear to be mediated by a specific surface receptor possessing a K_D of about 1 nM (14). Insulin receptors have been characterized on pituitary cells and their K_D was 2–0.3 nM (12, 13). GH_3 cells were reported to contain 10,000 specific binding sites per cell (12). The concentration of insulin employed here, which achieved maximal GH suppression, correlated well with the observed affinity of insulin for the GH_3 cell receptor.

Although insulin antiserum was able to block the inhibitory effects of insulin on GH secretion, an effect of insulin mediated via an insulin-like growth factor receptor (35) is unlikely at the doses employed. Usually, much larger doses of insulin than those employed here are necessary to activate other growth factor receptors and responses (36–40). The dose response of GH suppression by insulin clearly differs from that of IGF-I in these cells. Both structural and functional homology of insulin, IGF-I and IGF-II, and their respective receptors do exist (3), but the affinity of these compounds for each others' receptors differ markedly. Half-maximal displacement of ^{125}I-IGF-I from IGF-I receptors on IM-9 lymphocytes was achieved by 3.6 nM IGF-I and only by 500 nM insulin (38). In the experiments using IGF, the suppression of basal GH secretion was only achieved by relatively high doses of IGF-I (100 ng/ml or about 13 nM). Furthermore, IGF did not inhibit the hydrocortisone-stimulated GH secretion nor did it reverse the suppression of PRL caused by the steroid. Insulin, at lower doses on a molar basis, clearly reversed both the GH and PRL changes induced by hydrocortisone.

Somatomedin-C (equivalent to IGF-I, 25 ng/ml), but not proinsulin, inhibited dibutyryl-cyclic AMP(dbCAMP)-stimulated GH release by primary cultures of rat pituitary cells by ~90% during a 24-h incubation but had no effect after 4 h (41). Somatomedin-C did not alter basal GH secretion. In this interesting study, a dual feedback of somatomedin-C on both the hypothalamus and pituitary was described. When a similar dose of porcine insulin (25 ng/ml) was tested, it did not suppress basal GH but did inhibit the dbCAMP-stimulated GH secretion by 35% during 24 h (41). Lower doses of insulin were not tested. The dose of insulin used was equivalent to almost 4.2 nM, which is about sixfold greater than the dose of bovine insulin required in these studies to achieve about 50% inhibition of basal GH secretion by GH_3 pituitary tumor cells.

The suppressive effects of IGF-I on basal GH secretion by GH_3 cells were observed at higher doses than those employed in a recent study showing that 0.5–10 ng/ml IGF-I was required to inhibit both basal and growth hormone-releasing factor-stimulated GH secretion by rat pituitary cells (42). In the latter study, effects of insulin were not reported. The discrepancies in responses of basal in vitro GH secretion to somatomedin treatment (41, 42) as well as the differing dose requirements in the latter two studies and the present study may possibly be ascribed to the different somatomedin preparations used in all three studies, and also to the fact that the previous two studies employed primary rat pituitary cultures, whereas in the present study a rat cell line was used. In vivo studies have indicated a suppressive effect of intracerebral IGFs on GH secretion (43).

Hydrocortisone has been shown to stimulate GH (17, 18) and suppress PRL (19) in these pituitary cells, i.e., effects opposite to those observed for insulin in these experiments. Cortisol also stimulated GH secretion by monkey pituitary tissue (44). Dexamethasone has also been shown to stimulate pituitary GH gene transcription in vivo (24), and to enhance pituitary GH response to growth hormone-releasing factor (45). These experiments confirm that hydrocortisone stimulates GH and inhibits PRL secretion. The stimulation of GH secretion by GH_3 cells was shown to be independent of glucose utilization since it seemed to occur equally well in the presence or absence of glucose. This is in agreement with previous findings (46). In the present studies, insulin suppressed this enhanced GH secretion regardless of the presence of glucose in the medium. The basal secretion of GH was far lower in the cells exposed to 2-deoxyglucose (Table III) than in the other groups. Cell replication was also far slower in these cells, consistent with previously described effects of 2-deoxyglucose on GH cells (46). Nevertheless, the effects of insulin and hydrocortisone were observed regardless of the varying basal GH secretion in the different groups. Insulin is known to antagonize other cellular effects of hydrocortisone, especially in liver (47), adipose (47), and lung tissue (48). Interestingly, there has also been a preliminary report that dexamethasone increases the number of insulin receptors on GH_1 cells (12). In an earlier study using GH_1 cells, low doses (5–50 ng/ml) of insulin actually potentiated the GH response to cortisol (49). The induction of rat GH protein synthesis by dexamethasone (1 μM) in GC and GH_3 cells grown in a chemical defined serum-free medium (50) was inhibited by relatively high concentrations of insulin (10 ug/ml) added to the medium for at least 48 h. Insulin, in this system, inhibited the steroid inducibility of both rat GH and p16 protein synthesis as visualized by two-dimensional gel electrophoresis, but had no effect on the suppression of rat PRL synthesis caused by dexamethasone treatment of the cells. In the present studies, insulin was able to inhibit the effects of up to 1 μM hydrocortisone by >30%. That these effects were specific for hormone gene expression was confirmed by two other observations. First, insulin did not alter the suppressed GH_3 cell replication rate induced by hydrocortisone. The inhibitory effect of hydrocortisone on cell replication and total protein synthesis has been described in previous studies (17, 18). Using GH_1 cells, however, both low (5–50 ng/ml) and very high (2.5 μg/ml) doses of porcine insulin did stimulate the suppressed [^{3}H]thymidine incorporation caused by cortisol, while intermediate doses had no effect (49). Although insulin antagonized lecithin synthesis induced by cortisol in fetal lung cells, it also did not alter the inhibition of lung cell growth induced by cortisol (48). Secondly, insulin also reversed the suppression of PRL secretion induced by hydrocortisone (Fig. 6). In fact, the basal PRL secretion by GH_3 cells was markedly stimulated by over 50% in the presence of insulin and no added hydrocortisone. This is in agreement with similar previous observations where exposure of rat pituitary

tumor cells to insulin (1.8×10^{-7} M) and estrogens caused increased intracellular PRL concentrations in GH_4C_1 rat pituitary tumor cells (34), and this effect was not due to any cell cycle changes induced by insulin (51).

The discordant response of GH and PRL to insulin is further evidence for independent hormonal control of GH and PRL secretion by GH_3 cells (13, 15), although a reciprocal linkage cannot be excluded.

The results shown in this study as well as the inhibitory effect of insulin on GH gene expression (50) and on dbCAMP-stimulated GH secretion (41) suggest that insulin may play a specific direct negative feedback role on GH secretion.

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