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# Growth Hormone Inhibition of Glucagon- and cAMP-Induced Lipolysis by Chicken Adipose Tissue *in Vitro* (42500)

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*Abstract.* The ability of growth hormone (GH) to inhibit the early (first hour) lipolytic response to glucagon and cAMP was investigated using chicken adipose tissue explants *in vitro.* In the first hour of incubation, GH inhibited glucagon, 8-bromo-3′,5′-cyclic adenosine monophosphate (8-bromo-cAMP), and 1-isobutyl-3-methyl-xanthine (IBMX) induced glycerol release. The antilipolytic effect of GH was dose dependent, with inhibition of glucagon and 8-bromo-cAMP observed in the presence of as little as 100 ng/ml GH. In the fourth hour of incubation (late lipolytic response), GH (10, 100, or 1000 ng/ml) enhanced the lipolytic action of glucagon. 

Growth hormone (GH) has been demonstrated to exert both lipolytic and antilipolytic effects in rat adipose tissue *in vitro.* These effects are separated temporally, with inhibition of lipolysis occurring in the first 1–2 hr of incubation while stimulation of lipolysis is observed later (3–4 hr) (1, 2). The early antilipolytic effect involves GH inhibiting either epinephrine (3–6)- or norepinephrine (7)-stimulated lipolysis by rat adipose tissue segments or adipocytes. This antilipolytic action appears to be a function of age, since GH does not affect epinephrine-induced lipolysis when adipose tissue from young (weanling) rats is employed (8).

Recently, bovine or chicken GH, of either pituitary or bacterial derivation, have been shown to stimulate lipolysis by chicken adipose tissue *in vitro* (9). In the present study, the potential antilipolytic effects of GH are investigated with chicken adipose tissue explants. Unlike rat adipose tissue, chicken adipose is relatively insensitive to lipolytic stimulation by epinephrine or norepinephrine (10–12). Glucagon is the major hormonal stimulator of lipolysis in the chicken (11, 13, 14). The lipolytic action of glucagon in the chicken is presumably mediated by cAMP (3′,5′-cyclic adenosine monophosphate) (15, 16), similar to the situation observed with epinephrine-induced lipolysis in the rat (17, 18). The present study examines the ability of GH to inhibit glucagon- and cAMP-induced lipolysis by chicken adipose tissue *in vitro.*

**Materials and Methods.** Adipose tissue from adult (6–9 months) male chickens of the White Leghorn strain were employed. All birds received a commercial diet (Chick Grower, Agway) and water *ad libitum* prior to experimentation. Explants of abdominal adipose tissue from six (Table I) or three chickens (Table II) were used. Explants (7–10 per vial; 50–100 mg total wt) were randomly distributed. The incubation media was Krebs–Ringer–Hepes, containing 15 mM glucose, 1% bovine serum albumin (Armour, Fraction V), and 2.54 mM $CaCl_2$ (pH 7.45). All incubations were carried out in a shaking water bath (37.5°C, 70 oscillations/min) under an atmosphere of 95% $O_2$/5% $CO_2$. Following a 1-hr preincubation period, the adipose tissue explants were incubated for four successive 1-hr periods, with both media and treatment being replaced at the end of each hour. Glycerol release into the media (an index of lipolysis) was determined by a fluorometric enzyme method (19) for the first- and fourth-hour periods only.

The following GH preparations were employed: biosynthetic bovine somatotropin (GH, kindly donated by Eli Lilly Research Laboratories), native bovine GH (Miles Laboratories), native chicken GH (purified by the method of Harvey and Scanes, (20)). Porcine glucagon, 8-bromo-cAMP, and IBMX (1-isobutyl-3-methyl-xanthine) were obtained from Sigma Chemicals (St. Louis, MO). Statistical differences between means were determined by analysis of variance (ANOVA), followed by least significant differences (LSD).

**Results.** Table I shows the effects of various concentrations of GH on glucagon (1 ng/ml)-stimulated lipolysis. Major differences were



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TABLE I. EFFECT OF VARIOUS CONCENTRATIONS OF BIOSYNTHETIC BOVINE GH ON GLUCAGON-INDUCED LIPOLYSIS BY CHICKEN ADIPOSE TISSUE *in Vitro*

| Treatment | Glycerol release during incubation (% control ± SEM) First hour | Fourth hour |
|---|---|---|
| Control | 100.0 ± 1.7[a] | 100.0 ± 1.7[a] |
| GH (10 ng/ml) | 105.5 ± 4.8[a] | 129.1 ± 16.5[b] |
| GH (100 ng/ml) | 140.7 ± 9.6[b] | 169.8 ± 6.6[c] |
| GH (1000 ng/ml) | 150.7 ± 3.3[b,c] | 194.2 ± 3.9[c,d] |
| Glucagon (1 ng/ml) | 298.0 ± 17.8[e] | 201.8 ± 7.0[d] |
| GH (1 ng/ml) + GH (10 ng/ml) | 260.8 ± 16.4[d] | 230.9 ± 13.2[e] |
| GH (1 ng/ml) + GH (100 ng/ml) | 198.2 ± 18.1[c] | 242.9 ± 8.7[e,f] |
| Glucagon (1 ng/ml) + GH (1000 ng/ml) | 184.6 ± 17.9[c] | 260.0 ± 11.2[f] |

*Note.* Values represent means ± SEM of three independent experiments, with six replicates per treatment group/experiment. Control glycerol release values (nmole/g tissue ± SEM) were 224 ± 17 and 226 ± 17 for first and fourth hours, respectively. Tissue was incubated for four successive 1-hr periods. At the end of each period, the media was removed (for glycerol analysis) and replaced with fresh media and treatment.

[a-f] Means with different superscript letters are significantly different ($P < 0.05$) by ANOVA and LSD, as analyzed for each hour (within each column).

observed at the two time periods examined (first and fourth hours). In the first hour of incubation, a small elevation in glycerol release was observed in the presence of 100 and 1000 ng/ml GH, while glucagon (1 ng/ml) evoked a larger increase. When administered in combination, GH inhibited the early lipolytic effect of glucagon at all (GH) doses employed. In the fourth hour of incubation, GH stimulated lipolysis to a greater extent than in the first hour. The effect of glucagon, however, was much less in the fourth hour. No antilipolytic effect of GH was observed in the fourth hour; with GH (10, 100, and 1000 ng/ml) enhancing (nonadditively) the lipolytic effect of glucagon in the fourth hour of incubation.

The decreased lipolytic activity of glucagon per se over a 4-hr incubation period may represent adipose tissue refractoriness to glucagon or changes in tissue viability. Tissue integrity may be compromised by 4 hr of incubation (i.e., adipose tissue explants incubated for 4 hr may not be equally viable and hence as responsive as explants incubated for only 1 hr). Therefore, to investigate this question, the effect of pretreatment (1–3 hr) and subsequent treatment (fourth hour) on adipose tissue response to glucagon (1 ng/ml) was examined. Glycerol release due to glucagon present only in the fourth hour of incubation (with no pretreatment 1–3 hr) (731 ± 21 (6) nmol/g tissue ± SEM ($n = 6$)) was not different from that observed with glucagon present in the first hour of incubation (728 ± 27 (6) nmol/g). The continued presence of glucagon (pretreatment for 1–3 hr followed by fourth hour treatment) was required to observe a decreased response (to glucagon) in the fourth hour (glycerol release in the fourth hour being 510 ± 19 (6) nmol/g).

The *in vitro* lipolytic action of GH can be demonstrated in chicken adipose tissue using either biosynthetic (bacterially derived) or native (pituitary-derived) GH preparations (9). The antilipolytic effects of native bovine GH (1 μg/ml), native chicken GH (1 μg/ml) and biosynthetic bovine GH (1 μg/ml) were compared. All three GH preparations inhibited the

TABLE II. EFFECT OF BIOSYNTHETIC BOVINE GH ON 8-BROMO-cAMP OR 3-ISOBUTYL-1-METHYL-XANTHINE (IBMX) STIMULATED LIPOLYSIS BY CHICKEN ADIPOSE TISSUE *in Vitro*

| Treatment | Glycerol release during incubation (% control ± SEM) First hour | Fourth hour |
|---|---|---|
| Control | 100.0 ± 2.2[a] | 100.0 ± 2.5[a] |
| GH (1 μg/ml) | 169.2 ± 16.8[b] | 206.3 ± 16.0[b] |
| 8-bromo-cAMP (0.1 mM) | 194.5 ± 5.1[b] | 124.7 ± 12.3[a] |
| IBMX (0.05 mM) | 183.9 ± 24.0[b] | 97.9 ± 11.2[a] |
| 8-bromo-cAMP (0.1 mM) + GH (1 μg/ml) | 126.1 ± 6.0[a] | 197.5 ± 10.4[b] |
| IBMX (0.05 mM) + GH (1 μg/ml) | 91.9 ± 6.0[a] | 194.7 ± 37.5[b] |

*Note.* Values represent means ± SEM of three independent experiments, with six replicates per treatment group/experiment. Control glycerol release values (nmol/g tissue ± SEM) were 262 ± 22 and 259 ± 25 for first and fourth hours, respectively. Tissue was incubated for four successive 1-hr periods. At the end of each period, the media was removed (for glycerol analysis) and replaced with fresh media and treatment.

[a-b] Means with different superscript letters are significantly different ($P < 0.05$) by ANOVA and LSD, as analyzed for each hour (within each column).

response of glucagon (1 ng/ml) in the first hour of incubation (native bovine GH, by 24 ± 1.2%; native chicken GH, by 29 ± 2.2%; biosynthetic bovine GH, by 38 ± 1.4% that of glucagon-treated (289 ± 16 (6) nmol/g)). The greater antilipolytic effect of biosynthetic bovine GH, compared to either native bovine or chicken GH, was probably due to a greater relative purity of the biosynthetic preparation (9).

Glucagon is thought to activate lipolysis via a cAMP-dependent process in both rat (17, 21) and chicken (16, 22) adipose tissue. To determine whether GH similarly antagonizes cAMP-induced lipolysis, the effect of GH (1 μg/ml) on 8-bromo-cAMP-stimulated lipolysis was investigated (Table II). 8-Bromo-cAMP (0.1 m*M*) stimulated glycerol release in the first hour of incubation. As with the lipolytic effect of glucagon, the early response (first hour) to 8-bromo-cAMP was inhibited by GH. Lipolytic stimulation by 8-bromo-cAMP alone was much less in the fourth hour as compared to the first hour (Table II). In a further study, the ability of lower doses of GH to inhibit 8-bromo-cAMP (0.1 m*M*)-induced lipolysis was examined. At concentrations of 10 and 100 ng/ml, GH inhibited (by 13 ± 2.4 and 28 ± 2.7%, respectively) the first hour response to 8-bromo-cAMP (glycerol release being 372 ± 14 (6) μmole/g tissue). No antilipolytic effect of GH on 8-bromo-cAMP was observed in the fourth hour of incubation.

Endogenous levels of cAMP may also be elevated by decreased (cAMP) degradation (via phosphodiesterase). The effects of GH (1 μg/ml) on IBMX (a phosphodiesterase inhibitor)-stimulated lipolysis are seen in Table II. In the first hour of incubation, IBMX (0.05 m*M*) elevated glycerol release. This first hour lipolytic effect was completely inhibited by GH. In additional studies, a lower dose of GH (100 ng/ml) inhibited (by 26 ± 2.5%) the first hour response to IBMX (0.05 m*M*) (450 ± 22 (6) nmole/g tissue). In the fourth hour of incubation, no lipolytic stimulation by IBMX was observed.

**Discussion.** The present report establishes that GH can exert both lipolytic and antilipolytic effects on chicken adipose tissue *in vitro*. In rat adipose tissue or adipocytes *in vitro*, GH antagonizes epinephrine- or norepinephrine-stimulated lipolysis (3–7). Similarly, GH antagonizes glucagon-induced lipolysis in the first hour of incubation when chicken adipose tissue explants are employed. This antilipolytic effect of GH was observed using concentrations as low as 100 ng/ml, which falls within the physiological range for normal circulating GH levels in adult chickens (23).

The observation that GH inhibits the early lipolytic response to 8-bromo-cAMP and IBMX suggests that the control point for antilipolytic action by GH exists distal to cAMP synthesis and degradation. These findings are consistent with those reported by Birnbaum and Goodman (4), who surmised that the antilipolytic effect of GH on epinephrine-stimulated lipolysis might be due, at least in part, to decreased protein kinase activity. It was also noted that changes in cAMP accumulation or phosphodiesterase activity in rat adipose tissue could not fully account for the antilipolytic effects of GH. Another group of investigators (7) observed a decrease in phosphorylation of hormone-sensitive lipase by norepinephrine in the presence of GH. These studies lend evidence for control of antilipolysis at the level of protein kinase and/or hormone-sensitive lipase (possibly lipase phosphatase) in rat adipose tissue. However, the complete mechanism by which GH inhibits epinephrine-, norepinephrine-, or glucagon-stimulated lipolysis is yet to be elucidated.

The time courses of glucagon-, 8-bromo-cAMP-, and IBMX-stimulated lipolysis differed radically from that of GH. Within a 4-hr incubation period, chicken adipose tissue explants become refractory to the lipolytic influence of glucagon administered alone. Explants also become refractory to 8-bromo-cAMP- or IBMX-induced lipolysis (postreceptor lipolytic stimulation). Thus, down regulation of glucagon receptors cannot fully account for the decline in glucagon-stimulated lipolysis over a 4-hr time period. Refractoriness to glucagon appears to be controlled at a point distal to cAMP synthesis/degradation (protein kinase, hormone-sensitive lipase). The refractoriness observed *in vitro* may simulate the reduced lipolytic response to glucagon with adipocytes from fasted chicks as compared with that of fed chicks (24). Fasting elevates plasma glucagon levels and may act analogously to "pretreat" the adipose tissue *in vivo* with glucagon.

Since fasting (25) and chronic protein deprivation (26) elevate circulating GH levels in

chickens, a possible physiological role for GH and glucagon interaction might be hypothesized. *In vitro,* GH produces a progressive increase in lipolysis over a 4-hr period, whereas glucagon yields a large increase in lipolysis within 1 hr, with response decreasing over 4 hr. Administered together, GH acts to lower the magnitude of the initial glycerol release by glucagon (antilipolytic effect) and as time progresses, GH increases glycerol release, thereby reducing refractoriness to glucagon (lipolytic effect). It is then conceivable that GH may function *in vivo* to modulate the release of glycerol (and free fatty acid) from adipose tissue, particularly in times of chronic dietary stress. Such modulation of glucagon-induced lipolysis by GH would ensure a stable release of glycerol and free fatty acids over more extended periods of time.

It might be noted that GH is secreted episodically in chickens with peaks occurring approximately every hour (27). This time frame of release correlates well with the administration of GH and/or treatment in our adipose tissue explant system. Therefore, these circhoral peaks of GH may be involved in this control of lipolysis *in vivo.*

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