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Inhibition of Growth Hormone-Induced Lipolysis by 3′5′-Guanosine Monophosphate in Chicken Adipose Tissue *in Vitro* (42820)

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Abstract. The influence of cyclic 3′5′-guanosine monophosphate (cGMP) on the lipolytic and antilipolytic (inhibition of glucagon-stimulated lipolysis) responses to GH (1 μg/ml) was examined in chicken adipose tissue *in vitro*. Both 8-bromo-cGMP (0.1 m*M*) and sodium nitroprusside (1 m*M*) (a guanyl cyclase stimulator) completely inhibited the lipolytic effect of GH. A cGMP-lowering agent, LY83583 (10 μ*M*), reversed the inhibitory effect of sodium nitroprusside on GH-stimulated lipolysis. Furthermore, the suppressive effects of insulin (100 ng/ml), insulin-like growth factor I (IGF-I) (100 ng/ml), or insulin-like growth factor II (IGF-II/MSA) (100 ng/ml), but not somatostatin (1 ng/ml), on GH-stimulated lipolysis were prevented by LY83583 addition. Neither 8-bromo-cGMP, sodium nitroprusside, nor LY83583 altered GH-induced inhibition of glucagon (1 ng/ml)-stimulated lipolysis. It is proposed that cGMP may mediate inhibitory control of GH-stimulated lipolysis by insulin, IGF-I, and IGF-II in chicken adipose tissue.

Experimental evidence *in vitro* for cyclic 3′5′-guanosine monophosphate (cGMP) involvement in adipose tissue lipolysis remains equivocal. In rat adipocytes, cGMP concentrations are elevated by insulin, carbachol, oleic acid (1,2), and also by epinephrine (3). Although cGMP has been suggested to mediate the antilipolytic effects of insulin (i.e., inhibition of epinephrine-stimulated lipolysis) (1), neither cGMP nor dibutyryl cGMP affect epinephrine-stimulated lipolysis in rat adipose tissue *in vitro* (4). With chicken adipose tissue homogenates, it has been shown that cGMP, but not cAMP, can (in the presence of purified lung cGMP-dependent protein kinase) activate triglyceride lipase, and hence lipolysis (5). Thus, there is evidence, albeit limited, that cGMP has both stimulatory and inhibitory influences on lipolysis *in vitro*.

Growth hormone (GH) experts both lipolytic and antilipolytic (inhibition of epinephrine- (rat (6–8)) or glucagon (chicken (9–11))-induced lipolysis) effects on adipose tissue *in vitro*. Moreover, the lipolytic, but not the antilipolytic, effect of GH appears to be calcium dependent (12). In rat adipocytes, "low" media calcium concentrations (1–300 μ*M*) produce an increase in intracellular concentrations of cGMP (13–15). It is possible that the absence of lipolytic stimulation by GH on chicken adipose tissue in the presence of low calcium reflects induction of cGMP. The present studies examine the ability of cGMP to affect the lipolytic and antilipolytic responses to GH in chicken adipose tissue *in vitro*.

Materials and Methods. Adipose tissue explants were prepared as previously described (10) from adult (6–9 months) White Leghorn male chickens. Prior to experimental trials, all chickens were provided a commercial diet (Chick Grower, Agway) and water *ad libitum*. Adipose tissue was rapidly excised from the abdominal region of two to four chickens per trial and manually diced into explants. The adipose tissue explants were then randomly distributed into vials (7–10 explants per vial; 50–100 mg total weight) containing 1 ml Krebs–Ringer–Hepes medium (pH 7.4) supplemented with 15 m*M* glucose, 1% bovine serum albumin (Armour, Fraction V), and 2.54 m*M* $CaCl_2$. Incubations were conducted in a shaking water bath (37.5°C, 70 oscillations/min) under a 95% O_2/5% CO_2 atmosphere. Following a 1-hr preincubation period, the "preincubation" media was discarded and replaced with fresh Krebs–Ringer–Hepes media. All adipose tissue explants were then incubated for 1 hr in the presence or absence of the specified treatments. Glycerol release

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TABLE I. EFFECT OF CYCLIC 3'5'-GUANOSINE MONOPHOSPHATE (cGMP) ON THE LIPOLYTIC AND ANTILIPOLYTIC RESPONSES TO GH BY CHICKEN ADIPOSE TISSUE EXPLANTS

	Glycerol release during incubation (nmol/g tissue ± SEM (N = 3)*)	
	No addition	cGMP (0.1 mM)
Control	213.9 ± 16.5[a]	225.3 ± 15.4[a]
GH (1 μg/ml)	354.0 ± 31.5[b]	256.7 ± 20.0[a]
Glucagon (1 ng/ml)	617.3 ± 28.0[c]	609.7 ± 25.5[c]
GH (1 μg/ml) + glucagon (1 ng/ml)	364.2 ± 26.3[b]	358.9 ± 16.5[b]

[a–c] Means with similar superscript letters are not statistically different ($P < 0.05$) by analysis of variance, followed by least significant differences.

* Values represent means ± SEM of three independent trials, with six replicates per treatment group per trial. Chicken adipose tissue explants were incubated for 1 hr in Krebs–Ringer–Hepes (KRH) media. Following this preincubation period, the media was removed and replaced with fresh KRH media and specified treatments. All explants were then incubated (37.5°C) for 1 hr.

into the media (a relative index of lipolysis) was determined by an enzymatic/fluorometric method (16).

Biosynthetic bovine somatotropin (GH) and LY83583 (3-anilino-5,8 quinolinedione) were generously provided by Eli Lilly Research Laboratories (Indianapolis, IN), and biosynthetic human insulin-like growth factor I (IGF-I) by Kabi Laboratories (Stockholm, Sweden). Buffalo rat hepatocyte (line BRL-3A) CR multiplication stimulating activity (MSA or IGF-II), homologous to human IGF-II (17), was obtained from Collaborative Research, Inc. (Bedford, MA). Cyclic 3'5'-guanosine monophosphate, bovine insulin, sodium nitroprusside, and somatostatin (synthetic 1–14) were obtained from Sigma Chemical (St. Louis, MO). Statistical differences between means were determined by analysis of variance, followed by least significant differences. The tables presented are representative of three independent experimental trials.

Results. The effect of 8-bromo-cyclic 3'5'-guanosine monophosphate (8-bromo-cGMP) on the lipolytic (GH alone) and antilipolytic (inhibition of glucagon-stimulated lipolysis) responses to biosynthetic bovine growth hormone was investigated (Table I). Glucagon (1 ng/ml) or GH (1 μg/ml) each stimulated glycerol release in chicken adipose tissue explants, (by 189% and 65% that of the control, respectively). Addition of 8-bromo-cGMP (0.1 mM) did not influence basal or glucagon-stimulated glycerol release. However, GH-induced lipolysis was completely inhibited by 8-bromo-cGMP. Glucagon-stimulated lipolysis was reduced by GH (by 41%). However, this antilipolytic response to GH was not affected by the presence of 8-bromo-cGMP.

In order to establish a role for cGMP in the cellular control of lipolysis, the effects of elevating endogenous concentrations of cGMP were compared to those of exogenous 8-bromo-cGMP. This was examined using sodium nitroprusside, a guanyl cyclase activator (18). The effects of sodium nitroprusside on the lipolytic and antilipolytic responses to GH are shown in Table II. Sodium nitroprusside (1 mM), like 8-bromo-cGMP, blocked the lipolytic response to GH (1 μg/ml). Sodium nitroprusside, however, did

TABLE II. INFLUENCE OF SODIUM NITROPRUSSIDE ON THE LIPOLYTIC AND ANTILIPOLYTIC EFFECTS OF GH IN CHICKEN ADIPOSE TISSUE EXPLANTS

	Glycerol release during incubation (nmole/g tissue ± SEM (N = 3)*)	
	No addition	Sodium nitroprusside (1 mM)
Control	222.8 ± 20.4[a]	217.4 ± 17.8[a]
GH (1 μg/ml)	339.6 ± 16.5[b]	219.3 ± 18.0[a]
Glucagon (1 ng/ml)	672.2 ± 51.4[d]	644.8 ± 48.7[d]
GH (1 μg/ml) + glucagon (1 ng/ml)	461.9 ± 25.9[c]	477.8 ± 26.4[c]

[a–d] Means with similar superscript letters are not statistically different ($P < 0.05$) by analysis of variance, followed by least significant differences.

* Values represent means ± SEM of three independent trials, with six replicates per treatment group per trial. Chicken adipose tissue explants were incubated for 1 hr in Krebs–Ringer–Hepes (KRH) media. Following this preincubation period, the media was removed and replaced with fresh KRH media and specified treatments. All explants were then incubated (37.5°C) for 1 hr.

TABLE III. LY83583 REVERSAL OF SODIUM NITROPRUSSIDE-INDUCED BLOCKADE OF THE LIPOLYTIC RESPONSE TO GH BY CHICKEN ADIPOSE TISSUE *IN VITRO*

	Glycerol release during incubation (nmole/g tissue ± SEM (N = 3)*)	
	No addition	LY83583 (10 μM)
Control	192.6 ± 11.1[a]	216.3 ± 18.0[a]
GH (1 μg/ml)	332.4 ± 22.9[b]	339.8 ± 17.0[b]
Sodium nitroprusside (1 mM)	188.4 ± 13.7[a]	204.0 ± 16.8[a]
GH(1 μg/ml) + sodium nitroprusside (1 mM)	196.0 ± 22.5[a]	366.5 ± 31.4[b]

[a,b] Means with similar superscript letters are not statistically different ($P < 0.05$) by analysis of variance, followed by least significant differences.

* Values represent means ± SEM of three independent trials, with six replicates per treatment group per trial. Chicken adipose tissue explants were incubated for 1 hr in Krebs–Ringer–Hepes (KRH) media. Following this preincubation period, the media was removed and replaced with fresh KRH media and specified treatments. All explants were then incubated (37.5°C) for 1 hr.

not affect either basal or glucagon-stimulated glycerol release. Similarly, GH-induced inhibition of glucagon (1 ng/ml)-stimulated lipolysis was not influenced by the presence of sodium nitroprusside (Table II).

It follows that if the effect of sodium nitroprusside (blockade of GH-stimulated lipolysis) was specific for guanyl cyclase, then a cGMP "inhibitor" should be able to prevent this effect. LY83583 is an agent capable of lowering intracellular cGMP concentrations, without affecting cAMP concentrations (19, 20). The influence of LY83583 (10 μM) on sodium nitroprusside (1 mM)-induced blockade of the lipolytic response to GH (1 μg/ml) is summarized in Table III. Sodium nitroprusside-induced inhibition of GH-stimulated lipolysis was not observed in the presence of LY83583. It should be noted that LY83583 did not affect basal, glucagon-stimulated, or GH-stimulated glycerol release by chicken adipose tissue *in vitro*.

Somatostatin (SRIF), insulin, IGF-I and IGF-II can abolish the lipolytic effect of GH by chicken adipose tissue explants (21). The mechanism by which each of these hormones inhibit GH-induced lipolysis *in vitro*. is unknown. It is conceivable that cGMP might mediate the inhibitory effects of any of these hormones on GH-stimulated lipolysis. If this were the case, the addition of a cGMP-lowering agent, LY83583, might reverse the inhibitory action of hormones acting via cGMP elevation. The effect of LY83583 (10 μM) on SRIF (Table IV), IGF-I (Table IV), IGF-II (Table V), or insulin (Table V) blockade of GH-stimulated lipolysis was examined. In the presence or absence of LY83583, SRIF (1 ng/ml) inhibited the lipolytic effect of GH. Blockade of GH-stimulated lipolysis by IGF-I (100 ng/ml) was partially prevented by LY83583 (62% that of GH alone). Similarly, the inhibitory effects of IGF-II (100 ng/ml) or insulin (100 ng/ml) were completely blocked by LY83583 addition.

Discussion. The influence of cGMP on the lipolytic or antilipolytic response to GH has not been previously investigated in any species. The lipolytic, but not the antilipolytic (inhibition of glucagon-stimulated lipolysis),

TABLE IV. EFFECT OF LY83583 ON SRIF- OR IGF-I-INDUCED INHIBITION OF THE LIPOLYTIC RESPONSE TO GH BY CHICKEN ADIPOSE TISSUE *IN VITRO*

	Glycerol release during incubation (nmole/g tissue ± SEM (N = 3)*)	
	No addition	LY83583 (10 μM)
Control	252.1 ± 6.2[b]	247.6 ± 6.7[b]
GH (1 μg/ml)	431.0 ± 18.8[d]	426.2 ± 11.7[d]
SRIF (1 ng/ml)	185.3 ± 5.3[a]	186.0 ± 10.5[a]
GH (1 μg/ml) + SRIF (1 ng/ml)	248.1 ± 17.1[b]	225.6 ± 11.8[b]
IGF-I (100 ng/ml)	250.4 ± 7.3[b]	224.6 ± 16.8[b]
GH (1 μg/ml) + IGF-I (100 ng/ml)	246.8 ± 8.2[b]	358.8 ± 12.6[c]

[a–b] Means with similar superscript letters are not statistically different ($P < 0.05$) by analysis of variance, followed by least significant differences.

* Values represent means ± SEM of three independent trials, with six replicates per treatment group per trial. Chicken adipose tissue explants were incubated for 1 hr in Krebs–Ringer–Hepes (KRH) media. Following this incubation period, the media was removed and replaced with fresh KRH media and specified treatments. All explants were then incubated (37.5°C) for 1 hr.

TABLE V. EFFECT OF LY83583 ON IGF-II/MSA- OR INSULIN-INDUCED INHIBITION OF THE LIPOLYTIC RESPONSE TO GH BY CHICKEN ADIPOSE TISSUE *IN VITRO*

	Glycerol release during inhibition (nmole/g tissue ± SEM (N = 3)*)	
	No addition	LY83583 (10 μM)
Control	290.4 ± 4.8[b]	289.6 ± 5.9[b]
GH (1 μg/ml)	453.4 ± 10.0[c]	474.5 ± 14.8[c]
IGF-II (100 ng/ml)	191.9 ± 14.4[a]	192.6 ± 9.8[a]
GH (1 μg/ml) + IGF-II (100 ng/ml)	307.8 ± 7.2[b]	485.8 ± 8.0[c]
Insulin (100 ng/ml)	299.8 ± 15.1[b]	292.3 ± 11.1[b]
GH (1 μg/ml) + insulin (100 ng/ml)	304.3 ± 10.7[b]	474.5 ± 12.9[c]

[a–c] Means with similar superscript letters are not statistically different ($P < 0.05$) by analysis of variance, followed by least significant differences.

* Values represent means ± SEM of three independent trials, with six replicates per treatment group per trial. Chicken adipose tissue explants were incubated for 1 hr in Krebs–Ringer–Hepes (KRH) media. Following this preincubation period, the media was removed and replaced with fresh KRH media and specified treatments. All explants were then incubated (37.5°C) for 1 hr.

effect of biosynthetic bovine GH is blocked by 8-bromo-cGMP or sodium nitroprusside (a stimulator of cGMP synthesis) by chicken adipose tissue explants. Blockade of GH-induced lipolysis by sodium nitroprusside can be overcome by LY83583, a cGMP-lowering agent. Therefore, the lipolytic response to GH can be blocked by increased cGMP concentrations (exogenous or endogenous); reduction of cGMP concentrations prevents this inhibition of GH-induced lipolysis. Although not examined in adipose tissue, GH greatly enhances cGMP synthesis in various rat tissues (liver, heart, lung, kidney, pancreas, and skeletal muscle) *in vitro.* (22). It is speculated that cGMP may function as a physiological "negative feedback" inhibitor of the lipolytic response to GH in chicken adipose tissue. However, it should be noted that the lipolytic effect of GH does not appear to be under tonic cGMP inhibition, for LY83583 does not enhance basal or GH-induced lipolysis in the absence of other agents (Table III).

The lipolytic effect of glucagon in avian adipose tissue is mediated by cAMP (23–25). Neither 8-bromo-cGMP, sodium nitroprusside, nor LY83583 affected basal or glucagon-stimulated glycerol release by chicken adipose tissue *in vitro.* (Tables I–III). In view of this, cyclic GMP does not appear to be involved in the control of basal or glucagon-stimulated lipolysis. The present observations also confirm that LY83583 does not interfere (nonspecifically) with cAMP-dependent events.

Low concentrations of SRIF, insulin, IGF-I, or IGF-II inhibited GH-stimulated glycerol release by chicken adipose tissue explants (Tables IV and V) (21). It appears that the inhibitory action of SRIF on the GH lipolytic response does not involve cGMP as LY83583, a cGMP-lowering agent, did not ameliorate SRIF inhibition of GH-induced lipolysis. As reported previously (21), SRIF reduced basal glycerol release (by 26%); this effect was unaltered by LY83583 addition to the incubation medium (Table IV). It may be concluded that the antilipolytic effect of SRIF on basal lipolysis does not involve elevation of cGMP concentrations. However, LY83583 did partially restore the lipolytic response to GH in the presence of IGF-I, and completely restored the effect of GH in the presence of IGF-II/MSA and insulin (Tables IV and V). This is the first report whereby reversible (i.e., by addition of LY83583) inhibition of GH-induced lipolysis by IGF-I or IGF-II has been demonstrated. Thus, it is suggested that cGMP may be an integral component of the mechanism by which insulin, IGF-I, and IGF-II inhibit GH-induced lipolysis. The inability of LY83583 to completely restore the GH response may be dose dependent. Alternatively, cGMP may require an additional intracellular cofactor to mediate this suppression of GH-stimulated lipolysis. Cyclic GMP alone, however, does possess the ability to abolish the lipolytic effect of GH. The ability of LY83583 to block the antilipolytic effects of insulin, IGF-I, and IGF-II is consistent with the suggestion that these agents act in this system via a single receptor and cell mechanism.

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