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Pharmacological Investigations on the Lipolytic and Antilipolytic Effects of Growth Hormone (GH) in Chicken Adipose Tissue *in Vitro:* Evidence for Involvement of Calcium and Polyamines (42725)

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Abstract. The involvement of RNA/protein synthesis, calcium, calmodulin, protein kinase C, and polyamines in the lipolytic and antilipolytic (inhibition of glucagon-stimulated lipolysis) responses to GH have been investigated employing chicken adipose tissue *in vitro*. The lipolytic, but not the antilipolytic, effect of GH was blocked by inhibitors of RNA/protein synthesis (actinomycin D, cycloheximide, and puromycin) and calcium uptake (verapamil) and low calcium concentrations (0.28 m*M* $CaCl_2$). The antilipolytic, but not the lipolytic, effect of GH was blocked by α-difluoromethylornithine (DFMO), a polyamine synthesis inhibitor. DFMO-induced blockade of the antilipolytic GH response was reversed by the addition of spermidine. The lipolytic and antilipolytic effects of GH were not influenced by chlorpromazine (a calmodulin inhibitor) or phorbol 12-myristate 13-acetate (PMA) (an activator of protein kinase C).

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Growth hormone (GH) exerts both lipolytic and antilipolytic effects in chicken (1, 2) and rat (3, 4) adipose tissue *in vitro*. The "antilipolytic response" to GH is characterized by a transient (1–2 hr) inhibition of epinephrine [rat (3, 4)]- or glucagon [chicken (2)]-induced lipolysis. Under certain conditions GH stimulates lipolysis in rats [for example, over longer incubation periods (3–4 hr): in the presence of theophylline with adipose tissue from hypophysectomized rats (5), or in the presence of dexamethasone with adipocytes from intact rats (3)]. GH has been demonstrated to stimulate lipolysis by adipose tissue explants from hypophysectomized or intact chickens in the absence of such potentiating agents (1). GH-induced stimulation of chicken adipose tissue lipolysis is manifested in the first hour of incubation, but the magnitude of lipolytic response increases with time (up to 4 hr) (1). This highly reproducible *in vitro* system has been employed to examine the mechanism(s) by which GH affects lipolysis.

The mechanism(s) by which the lipolytic and antilipolytic effects of GH are exerted remains unclear. The role of cyclic adenosine 3′,5′-monophosphate (cAMP) has been examined with somewhat equivocal results. In short-term incubations (10–60 min) of rat adipocytes, GH reduces the lipolytic effects of epinephrine (6) or norepinephrine (7), which are presumed to be cAMP-mediated (8, 9). Similarly, GH inhibits glucagon-stimulated (cAMP-mediated) lipolysis by chicken adipose tissue explants during a 1-hr incubation period (2). The antilipolytic effect of GH, however, does not correlate temporally with depressed cAMP accumulation or enhanced phosphodiesterase activity in rat adipose tissue *in vitro* (6). Evidence suggests that decreased cAMP-dependent protein kinase activity (6) and/or triglyceride lipase phosphorylation (7) may account for the lipolytic response to GH. The antilipolytic effect of GH is similarly thought to exist distal to cAMP synthesis/degradation in chicken adipose tissue, as GH inhibits the lipolytic effects of 8-bromo-cAMP and IBMX (a phosphodiesterase inhibitor) *in vitro* (2).

Inhibitors of RNA and protein synthesis have been demonstrated to block the lipolytic (10), but not the antilipolytic (4), effect of GH in rat adipose tissue or adipocytes. It is hypothesized that the mechanisms by which GH exerts its lipolytic and antilipolytic actions are distinct. Hence, it should be possi-

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ble to separate these effects by selective pharmacological blockade. In the present studies, the involvement of RNA/protein synthesis and of putative "second messengers" (calcium, calmodulin, protein kinase C, and polyamines) in the lipolytic and antilipolytic actions of GH has been examined with chicken adipose tissue *in vitro*.

Materials and Methods. In all studies, adipose tissue from adult (6–11 months) male chickens (strain: White Leghorn) was utilized. Chickens were housed in brooder pens and provided a commercial diet (Chick Grower, Agway) and water *ad libitum* prior to experimentation. Explants of abdominal adipose tissue were prepared as previously described (1). Adipose tissue explants (excised from two to four chickens per trial) were randomly distributed into vials (7–10 explants per vial; 50–100 mg total wt) 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* $CaCl_2$ (unless otherwise noted, see Table II). 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 preincubation media were discarded and replaced with fresh Krebs–Ringer–Hepes media. The adipose tissue explants were then incubated for one (Tables I–VI) or four 1-hr periods (with media and treatment being replaced following each hour period; as noted) in the presence of the specified treatments. Incubations were terminated by rapid freezing. Glycerol release into the media (an index of lipolysis) was determined fluorometrically (11).

Biosynthetic bovine somatotropin (GH), verapamil, and α-difluoromethylornithine (DFMO) were kindly donated, respectively, by Eli Lilly Research Laboratories (Indianapolis, IN), Boehringer–Ingleheim (West Germany), and Merrell Dow (Cincinnati, OH). Porcine glucagon, A23187, chlorpromazine, phorbol 12-myristate 13-acetate (PMA), actinomycin D, cycloheximide, puromycin, putrescine, spermidine, and spermine were obtained from Sigma Chemicals (St. Louis, MO). A23187 was dissolved in 0.1% dimethyl sulfoxide (DMSO). As determined in preliminary studies, this concentration of DMSO did not affect glycerol release. Statistical differences between means were determined by analysis of variance (ANOVA), followed by least significant differences (LSD) as a post hoc test. All tables represent pooled data from three independent trials.

Results. The ability of inhibitors of RNA and protein synthesis to block the lipolytic effect of GH and the antilipolytic effect of GH (on glucagon-stimulated lipolysis) was examined. Lipolytic stimulation by GH was abolished by actinomycin D (100 n*M*) or cycloheximide (20 μg/ml) addition (Table I). Identical blockade of GH (1 μg/ml)-induced lipolysis was achieved with puromycin (100 μM) [glycerol release being (control): 230 $\pm$ 12.4 (n = 3); (GH): 346 $\pm$ 13.3 (n = 3); (puromycin): 210 $\pm$ 17.9 (n = 3); (GH + puromycin): 216 $\pm$ 9.8 (n = 3); nmoles/g tissue/hr]. The depression of glucagon-stimulated lipolysis by GH was not altered by either actinomycin D or cycloheximide (Table I).

The effects of various media calcium concentrations (0.28, 0.84, or 2.54 m*M* $CaCl_2$) on the lipolytic and antilipolytic responses to GH are illustrated in Table II. As has been observed previously (1), GH stimulated glycerol production by chicken adipose tissue explants in the presence of 2.54 m*M* $CaCl_2$-supplemented media. The effect of GH (1 μg/ml) was less than glucagon (1 ng/ml) (57% as compared to 196%, above that of the control) in 1 hr of incubation. This lipolytic effect of GH was observed with a lower concentration of calcium (0.84 m*M* $CaCl_2$, but not with 0.28 m*M* $CaCl_2$). In contrast, neither basal nor glucagon-stimulated lipolysis was affected by lower levels of calcium (0.28 or 0.84 m*M* $CaCl_2$) in the incubation media. GH also exhibited an "antilipolytic" effect, reducing glucagon-stimulated lipolysis (by 38%). This antilipolytic effect was observed at all calcium concentrations tested (0.28, 0.84, and 2.54 m*M* $CaCl_2$).

To determine whether calcium entry into the adipose tissue was required for GH-induced lipolysis, the effect of verapamil (a calcium channel blocker) was examined (Table III). Verapamil (10 μM) inhibited the lipolytic, but not the antilipolytic, response to GH (1 μg/ml). Neither basal nor glucagon-stimu-

TABLE I. EFFECT OF ACTINOMYCIN D OR CYCLOHEXIMIDE ON THE LIPOLYTIC AND ANTILIPOLYTIC RESPONSES TO GH BY CHICKEN ADIPOSE TISSUE *in Vitro*

	Glycerol release* during incubation (nmoles/g tissue ± SEM, n = 3)		
	No addition	Actinomycin D (100 nM)	Cycloheximide (20 μg/ml)
Control	251 ± 2.8^{a}	242 ± 10.3^{a}	225 ± 19.2^{a}
GH (1 μg/ml)	390 ± 8.0^{b}	264 ± 15.8^{a}	211 ± 15.9^{a}
Glucagon (1 ng/ml)	789 ± 42.2^{d}	807 ± 46.7^{d}	789 ± 38.7^{d}
GH (1 μg/ml) + glucagon (1 ng/ml)	526 ± 21.1^{c}	559 ± 20.2^{c}	516 ± 28.6^{c}

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

$^{a-d}$ Means with similar superscript letters are not statistically different ($P < 0.05$) by analysis of variance (ANOVA), followed by least significant differences (LSD).

lated lipolysis was affected by the presence of verapamil. A23187 (10 μM) (a divalent cation ionophore) augmented basal (by 34%), but not GH (1 μg/ml)-stimulated, lipolysis in 1 hr of incubation [glycerol release being (control): 229 ± 10.0 (n = 3); (GH): 364 ± 13.8 (n = 3); (A23187): 307 ± 16.1 (n = 3); (GH + A23187): 397 ± 15.5 (n = 3); nmoles/g tissue/hr]. Following longer incubation periods with A23187 (4 hr), no enhancement of GH-induced lipolysis was observed [glycerol release in the fourth hour being (control): 246 ± 13.5 (n = 3); (GH): 513 ± 25.5 (n = 3); (A23187): 383 ± 33.1 (n = 3); (GH + A23187): 624 ± 42.7 (n = 3); nmoles/gm tissue/hr]. Although there was no evidence for synergism between GH and A23187 at either time period, an effect may have been masked by the calcium concentration employed (2.54 mM $CaCl_2$). It is possible that more dramatic changes may occur with lower calcium concentrations (e.g., 0.28 or 0.84 mM $CaCl_2$), where GH effects are inhibited.

With GH-induced lipolysis appearing to require exogenous calcium it was pertinent to investigate the involvement of calmodulin. Furthermore, as intracellular calcium mobilization could not be ruled out as a possible mediator of antilipolytic GH actions,

TABLE II. INFLUENCE OF VARIOUS CALCIUM CONCENTRATIONS ON THE LIPOLYTIC AND ANTILIPOLYTIC EFFECTS OF GH BY CHICKEN ADIPOSE TISSUE EXPLANTS

	Glycerol release* during incubation with various media calcium chloride ($CaCl_2$) concentrations (nmoles/g tissue ± SEM, n = 3)		
	0.28 mM $CaCl_2$	0.84 mM $CaCl_2$	2.54 mM $CaCl_2$
Control	210 ± 15.8^{a}	261 ± 25.4^{a}	280 ± 24.8^{a}
GH (1 μg/ml)	250 ± 20.6^{a}	385 ± 46.4^{b}	439 ± 40.8^{b,c}
Glucagon (1 ng/ml)	743 ± 23.9^{d}	806 ± 35.6^{d}	829 ± 60.9^{d}
GH (1 μg/ml) + glucagon (1 ng/ml)	497 ± 36.5^{c}	524 ± 35.0^{c}	512 ± 22.1^{c}

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

$^{a-d}$ Means with similar superscript letters are not statistically different ($P < 0.05$) by analysis of variance (ANOVA), followed by least significant differences (LSD).

TABLE III. EFFECT OF VERAPAMIL ON THE LIPOLYTIC AND ANTILIPOLYTIC RESPONSES TO GH BY CHICKEN ADIPOSE TISSUE EXPLANTS

	Glycerol release during incubation (nmoles/g tissue ± SEM, n = 3)*	
	No addition	Verapamil (10 μM)
Control	232 ± 13.1[a]	228 ± 4.3[a]
GH (1 μg/ml)	368 ± 16.1[b]	228 ± 9.6[a]
Glucagon (1 ng/ml)	781 ± 69.9[d]	692 ± 41.2[d]
GH (1 μg/ml) + glucagon (1 ng/ml)	536 ± 47.1[c]	480 ± 9.6[c]

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

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

an antilipolytic role for calmodulin was equally conceivable. Chlorpromazine (10 μM) did not affect either the lipolytic or antilipolytic responses to GH [glycerol release being (control): 232 ± 13 (n = 3); (10 μM chlorpromazine): 254 ± 8 (3); (GH): 373 ± 12 (3), (GH + chlorpromazine): 366 ± 3 (3); (glucagon): 719 ± 9 (3); (glucagon + chlorpromazine): 740 ± 25 (3), (glucagon + GH): 486 ± 17 (3); (glucagon + GH + chlorpromazine): 450 ± 32 (3); nmoles/g tissue/hr]. The lipolytic and antilipolytic effects of GH, as well as basal and glucagon-stimulated glycerol release, were also unaffected by PMA (an activator of protein kinase C) addition [glycerol release being (control): 232 ± 13 (n = 3); (100 nM PMA): 252 ± 28; (GH): 373 ± 12 (3); (GH + PMA): 356 ± 7 (3); (glucagon): 719 ± 9 (3); (glucagon + PMA): 712 ± 19486 ± 17 (3); (glucagon + GH + PMA): 450 ± 23 (3); nmoles/g tissue/hr].

The possible involvement of polyamines in the lipolytic/antilipolytic effects of GH was examined. Inhibition of ornithine decarboxylase by DFMO (5 mM) did not affect the lipolytic response to GH (μg/ml) but blocked the inhibitory effect of GH on glucagon-stimulated lipolysis. Lipolysis was not affected by DFMO alone. Furthermore DFMO did not influence glucagon-induced lipolysis (Table IV).

The ability of DFMO to inhibit the antilipolytic response to GH suggests that GH

TABLE IV. EFFECT OF PUTRESCINE (0.5 mM) OR DFMO (5 mM) ON THE LIPOLYTIC AND ANTILIPOLYTIC RESPONSES TO GH BY CHICKEN ADIPOSE TISSUE *in Vitro*

	Glycerol release during incubation (nmoles/g tissue ± SEM, n = 3)*		
	No addition	Putrescine	DFMO
Control	304 ± 9.8[a]	313 ± 40.1[a]	308 ± 23.9[a]
GH (1 μg/ml)	463 ± 32.8[b]	462 ± 26.8[b]	524 ± 59.5[b,c]
Glucagon (1 ng/ml)	901 ± 27.8[d]	908 ± 25.5[d]	888 ± 33.1[d]
GH (1 μg/ml) + glucagon (1 ng/ml)	600 ± 15.3[b,c]	594 ± 11.9[c]	858 ± 31.9[d]

* Values represent means ± SEM of three independent trials, with six replicates per treatment group/trial. Chicken adipose tissue explants were incubated for 1 hr in Krebs–Ringer–Hepes (KRH) media. Following this preincubation period, the media were removed and replaced with fresh KRH media and specified treatments. DFMO was also added during the last 15 min of the preincubation period. All explants were then incubated (37.5°C) for 1 hr.

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

TABLE V. EFFECT OF SPERMINE (0.5 m*M*) OR SPERMIDINE (0.5 m*M*) ON THE LIPOLYTIC AND ANTILIPOLYTIC RESPONSES TO GH BY CHICKEN ADIPOSE TISSUE *in Vitro*

	Glycerol release during incubation (nmoles/g tissue ± SEM, n = 3)*		
	No addition	Spermine	Spermidine
Control	264 ± 23.4^{a}	244 ± 19.5^{a}	305 ± 22.1^{a}
GH (1 μg/ml)	427 ± 18.2^{b}	415 ± 18.1^{b}	412 ± 22.4^{b}
Glucagon (1 ng/ml)	875 ± 59.8^{d}	880 ± 43.1^{d}	892 ± 32.5^{d}
GH (1 μg/ml) + glucagon (1 ng/ml)	508 ± 27.7^{b,c}	562 ± 18.9^{c}	526 ± 22.4^{c}

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

$^{a-d}$ Means with similar superscript letters are not statistically different ($P < 0.05$) by analysis of variance (ANOVA), followed by least significant differences (LSD).

exerts its antilipolytic effect by stimulating ornithine decarboxylase. Thus, polyamines would be expected to block glucagon-induced lipolysis. However, incubation of adipose tissues with putrescine (0.5 m*M*) failed to mimic the antilipolytic action of GH as anticipated (Table IV). Similarly, spermidine or spermine alone (0.5 m*M*) did not affect glucagon-stimulated lipolysis by chicken adipose tissue (Table V). While polyamines do not mimic the antilipolytic effects of GH, polyamines may still serve an obligatory function for GH to exert its antilipolytic action. Alternatively, DFMO may be having a nonspecific effect. To test this latter hypothesis, the antilipolytic response to GH (GH + glucagon) was first blocked by DFMO, and then the polyamine was introduced into the incubation medium. DFMO blockade of the antilipolytic GH effect was reversed in the presence of spermidine (0.5 m*M*) (Table VI). Unexpectedly, DFMO and spermidine administered together (but not individually, Tables IV, VI) elevated basal and glucagon-stimulated glycerol release. Furthermore, in the presence of both DFMO and spermidine, no lipolytic effect of GH was observed.

Discussion. Lipolytic and antilipolytic effects of GH can be readily demonstrated with chicken adipose tissue *in vitro* [(1, 2) and

TABLE VI. REVERSAL OF DFMO (5 m*M*)-INDUCED BLOCKADE OF THE ANTILIPOLYTIC RESPONSE TO GH BY SPERMIDINE (0.5 m*M*) *in Vitro*

	Glycerol release during incubation (nmoles/g tissue ± SEM, n = 3)*			
	No addition	DFMO	Spermidine	DFMO + spermidine
Control	227 ± 9.9^{a}	230 ± 15.8^{a}	262 ± 18.8^{a}	400 ± 24.4^{b,c}
GH (1 μg/ml)	355 ± 18.6^{d}	391 ± 44.4^{b,c}	354 ± 18.9^{b}	395 ± 35.6^{b,c}
Glucagon (1 ng/ml)	731 ± 9.5^{e}	737 ± 20.1^{e}	755 ± 16.1^{e}	883 ± 43.2^{f}
GH (1 μg/ml) + glucagon (1 ng/ml)	457 ± 17.0^{c}	709 ± 14.3^{e}	453 ± 20.3^{c}	540 ± 17.8^{d}

* Values represent means ± SEM of three independent trials, with five replicates per treatment group/trial. Chicken adipose tissue explants were incubated for 1 hr in Krebs–Ringer–Hepes (KRH) media. Following this preincubation period, the media were removed and replaced with fresh KRH media and specified treatments. DFMO was also added during the last 15 min of the preincubation period. All explants were then incubated (37.5°C) for 1 hr.

$^{a-f}$ Means with similar superscript letters are not statistically different ($P < 0.05$) by analysis of variance (ANOVA), followed by least significant differences (LSD).

Table I]. In chicken (Table I) and rat (3, 4, 10) adipose tissue, the lipolytic, but not the antilipolytic, effect of GH is blocked by inhibitors of RNA or protein synthesis. The differential blockade of these responses by RNA/protein synthesis inhibitors has led to the belief in two independent, but potentially interactive, mechanisms of action. Based upon this proposition, we have been able to achieve some pharmacological separation of the lipolytic and antilipolytic effects of GH.

The protein(s) implicated in the lipolytic action of GH may be associated with receptor and/or postreceptor events. It is possible that the blockade of GH-induced lipolysis (by protein synthesis) inhibitors may be crude inhibition of the GH receptor itself. Recent evidence indicates a rapid (45 min) GH receptor turnover rate in rat adipocytes (12) which can be regulated by GH (13, 14). Preincubation of rat fat cells with cycloheximide or puromycin results in decreased binding of [^{125}I]-hGH to these GH receptors (15). It is then conceivable that the inhibitory effects of cycloheximide on lipolysis could be due to a reduction in GH receptor synthesis and/or by inhibition of a protein required for GH binding (15). According to this hypothesis, the antilipolytic effect of GH (which is unaffected by cycloheximide) does not correlate with the same receptor-mediated event.

The influence of calcium in the lipolytic and/or antilipolytic response to GH has not been previously examined in any species. GH-induced lipolysis was not observed when calcium concentrations were low (0.28 m*M* $CaCl_2$) or in the presence of verapamil, a calcium channel blocker (Tables II, III). While the effect of GH on intracellular calcium concentrations was not determined in these studies, it is indicated that (extracellular) calcium uptake is required for the lipolytic activity of GH. Calmodulin, a calcium binding protein, has been proposed to mediate various calcium-related hormone actions (16, 17). Calmodulin binding to rat adipocyte membranes is calcium- and time-dependent (18) and can be hormonally regulated (19). Calmodulin appears not to be required for calcium mediation of GH-induced lipolysis, as indicated by the lack of a calmodulin-inhibitor (chlorpromazine) effect. Protein kinase C activation, leading to intracellular calcium mobilization, has also been proposed as a mechanism of hormonal signal transduction (20, 21). Protein kinase C is present in adipose tissue (22, 23) and can be stimulated by phorbol esters (e.g., phorbol 12-myristate 13-acetate) (24, 25). A role for protein kinase C in the lipolytic action of GH is not suggested, since a phorbol ester (PMA) did not influence GH-stimulated glycerol release.

The antilipolytic effect of GH (i.e., inhibition of glucagon-stimulated lipolysis) was not affected by low calcium concentrations (0.28 m*M* $CaCl_2$), verapamil, chlorpromazine, or PMA (Tables II, III). Therefore, unlike the lipolytic effect of GH, the antilipolytic effect appears to be calcium-independent.

The effect of calcium on GH-induced lipolysis appears to be quite specific. Basal glycerol release was unaffected by reduced calcium concentrations (<2.54 m*M* $CaCl_2$), verapamil, or chlorpromazine (Tables II, III) in agreement with reports from other laboratories (26–30). Furthermore, glucagon-stimulated lipolysis is not altered by reduced calcium concentrations (<2.54 m*M* $CaCl_2$), verapamil, or chlorpromazine (Tables II, III). Protein kinase C also does not appear to be involved in basal or glucagon-stimulated lipolysis, as PMA did not affect the response to these parameters. The lack of an effect of PMA on chicken adipose tissue lipolysis is similar to previous studies with rat adipocytes, where PMA did not influence either basal or catecholamine-induced lipolysis by rat adipocytes (31, 32).

Thus far, pharmacological blockade of RNA/protein synthesis and calcium uptake could be preferentially employed to inhibit the lipolytic, but not the antilipolytic, response to GH (Tables I–III). In fact, no data exist in any species describing pharmacological blockade of the antilipolytic response to GH. Like GH, endogenous polyamines (putrescine, spermidine, and spermine) are known to exert antilipolytic effects in rat adipocytes, reducing epinephrine-stimulated glycerol production (33). α-Difluoromethylornithine is a selective, irreversible inhibitor of polyamine synthesis (i.e., ornithine decarboxylase) (34). DFMO was found to be capable of preventing the antilipolytic effect of GH (Table IV). Polyamines are not solely

responsible for antilipolytic effects of GH, as putrescine, spermidine, and spermine were unable to inhibit glucagon-stimulated lipolysis (Tables IV, V). It is suggested that polyamines may be one component of the transduction mechanism. Reversal of DFMO-induced antilipolytic blockade in the presence, but not absence, of GH suggests a cooperative effect of another mediator in conjunction with polyamines (Table VI). However, as inhibitors of RNA/protein synthesis do not suppress the antilipolytic effect, production of polyamines and the putative other mediator (if protein) by a process involving protein synthesis is contraindicated. Consequently, shifts in activation state (e.g., ADP-ribosylation, phosphorylation) or compartmentalization of preexisting polyamines and mediator(s) appears to be more likely.

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