Failure of Growth Hormone Alone to Potentiate Epinephrine-Induced Lipolysis[1] (34315)

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Although growth hormone is generally thought to be a lipid-mobilizing hormone (1), its mode of action in this regard is poorly understood. Because the lipolytic effects of growth hormone are most evident in the presence of some other stimulus for fat mobilization such as fasting (2), or theophylline (3), it was suggested that growth hormone may not trigger lipolysis, but may potentiate endogenous signals for fat mobilization (3, 4). Hypophysectomy reduced lipolysis in response to lipolytic agents (5, 6) but chronic administration of growth hormone restored the capacity of epididymal fat to release FFA in response to epinephrine (5). Since no data on glycerol production were presented, however, the finding that growth hormone alone restored the output of FFA in response to epinephrine may have resulted from decreased reesterification of FFA secondary to inhibition of glucose utilization (7) rather than from facilitation of lipolysis. Growth hormone, in combination with dexamethasone, also increased the sensitivity of isolated adipose cells *in vitro* to the lipolytic effects of norepinephrine (8) and epinephrine (9). However, incubation with dexamethasone alone, but not growth hormone alone, increased the sensitivity of adipose tissue to catecholamines (9). The present experiments were undertaken to determine whether growth hormone alone administered *in vivo* has a role in catecholamine-induced lipolysis.

Materials and Methods. Hypophysectomized male rats, weighing 100–120 g, were obtained from the Charles River Breeding Labs and were fed a high carbohydrate, fat-free diet[3] from the day of surgery until studied 2–4 weeks later. Completeness of hypophysectomy was judged from failure of growth and testicular atrophy.

The response of adipose tissue to epinephrine was assessed *in vitro*. Epididymal fat was excised and handled as described previously (3). The incubation medium was Krebs–Ringer–bicarbonate buffer and contained 4% bovine serum albumin (Armour, fraction V); glucose, 1 mg/ml; and 0.1 mg/ml of ascorbic acid to retard oxidation of epinephrine. Glycerol and FFA were analyzed by the methods of Wieland (10) and Dole (11). The growth hormone preparations used in these studies were NIH GH S-7 and NIH GH B-10.[4]

Results. Pretreatment of hypophysectomized rats with a single intravenous injection of 100 mg of growth hormone 3.5 hr before sacrifice increased the net production of FFA in response to epinephrine (Table I). Net production of FFA was calculated by subtracting the FFA present in the tissues initially from the sum of the FFA found in the tissue and medium after incubation for 1 hr with epinephrine. Despite its effects on FFA production, growth hormone failed to modify glycerol production in response to epinephrine. Since the production of glycerol, and not FFA reflects the lipolytic rate, these experiments provide evidence only for an inhibitory effect of growth hormone on reesterification of FFA.

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TABLE I. Lipolysis in Response to Epinephrine in Adipose Tissue from Hypophysectomized Rats Injected with 100 μg of Growth Hormone 3.5 hr Earlier.

	Control rats	Growth hormone-treated rats	p
Glycerol production (μmoles/g/hr)			
Control	1.93 ± 0.32[a]	2.49 ± 0.29	NS[b]
Increase due to epinephrine			
0.05 μg/ml	5.37 ± 0.86	6.17 ± 0.76	NS
0.50 μg/ml	9.88 ± 0.78	11.66 ± 1.06	NS
Net production of FFA[c] (μeq/g hr)			
Control	2.55 ± 1.07	1.52 ± 0.82	NS
Increase due to epinephrine			
0.05 μg/ml	3.49 ± 0.58	6.08 ± 1.09	<.05
0.50 μg/ml	7.38 ± 0.95	11.61 ± 0.99	<.01

[a] Mean ± SEM, 8 observations.

[b] Not statistically significant ($p > .05$).

[c] Calculated by subtracting the initial concentration of FFA in the tissues from the sum of the final tissue content plus the FFA released into the medium.

In similar experiments, growth hormone administered acutely or chronically failed to increase lipolysis (glycerol production) in response to epinephrine, although growth hormone consistently increased lipolysis in response to theophylline in tissues from the same rats (Table II). Pretreatment with growth hormone for 3 days also failed to increase glycerol production in response to epinephrine in 2 additional experiments. When the data for all 3 experiments were pooled, the increment in glycerol production caused by 0.05 μg/ml of epinephrine was 4.80 ± 0.45 μmoles/g/hr for 27 control rats and 5.23 ± 0.43 μmoles/g/hr for the 27 rats treated with 100 μg of growth hormone/day ($p > .4$).

Discussion. Growth hormone administered *in vivo* increased the production of FFA by adipose tissue in response to epinephrine, but did not increase glycerol production. This observation confirms earlier results on FFA

TABLE II. The Effects of Growth Hormone on Lipolysis in Response to Theophylline and Epinephrine.

	Glycerol production (μmoles/g/hr)		
	Control rats	Growth hormone treated	p
Acute treatment[a]			
Basal	2.26 ± 0.43[b]	2.38 ± 0.28	NS
Increment due to:			
Theophylline (0.3 mg/ml)	1.99 ± 0.39	3.85 ± 0.42	<.01
Epinephrine (0.05 μg/ml)	5.52 ± 0.78	6.17 ± 0.70	NS
Chronic treatment[c]			
Basal	3.57 ± 0.30	3.00 ± 0.27	NS
Increment due to:			
Theophylline (0.3 mg/ml)	1.72 ± 0.27	3.09 ± 0.11	<.01
Epinephrine (0.05 μg/ml)	4.33 ± 0.50	5.03 ± 0.53	NS

[a] NIH GH S7: 100 μg/rat, 3.5 hr before sacrifice.

[b] Mean ± SEM.

[c] NIH GH B 10: 100 μg/rat/day, for 3 days before sacrifice.

production in response to epinephrine in tissues obtained from hypophysectomized rats after chronic treatment with growth hormone (5). The failure of growth hormone to induce a parallel increase in glycerol production indicates that increased production of FFA was achieved by slowing the rate of FFA reesterification rather than by accelerating lipolysis. It may be assumed that for each mole of glycerol liberated into the incubation medium, 1 mole of triglyceride was completely hydrolyzed to yield 3 moles of FFA. Since the amount of fatty acids oxidized in adipose tissue is small (12), the difference between the actual production of FFA and three times the production of glycerol must therefore represent the amount of FFA resynthesized into triglyceride. Hence in adipose tissue from untreated hypophysectomized rats three-fourths of the FFA produced under the influence of epinephrine must have been reconverted to triglyceride. Treatment with growth hormone reduced this figure to about two-thirds. Reesterification of FFA depends upon the availability of α-glycerol phosphate (13). Because adipose tissue has little capacity for phosphorylating glycerol (14), the α-glycerol phosphate required for reesterification must be produced from glucose. Since growth hormone reduces glucose metabolism several hours after administration *in vivo* (7), it is likely that reduced reesterification is secondary to the effects of growth hormone on glucose metabolism in adipose tissue.

In the present experiments, growth hormone did not increase the basal production of glycerol. This observation is consistent with earlier findings in this laboratory (7, 15). In no case did growth hormone by itself increase lipolysis in response to epinephrine. This observation seems in conflict with present and earlier (3) findings that growth hormone increased the lipolytic response to theophylline. Epinephrine probably exerts its action on lipolysis by increasing the activity of adenyl cyclase which catalyzes the formation of cyclic adenosine monophosphate (CAMP) which in turn is thought to activate lipase (16). Theophylline inhibits the enzyme phosphodiesterase which degrades CAMP (17) and presumably exerts its lipolytic action by allowing the accumulation of spontaneously formed CAMP. Both epinephrine and theophylline increase the concentration of CAMP in adipose cells (18), yet growth hormone potentiates the action of only theophylline. Current information on the lipolytic system is insufficient to explain these findings in any but a speculative way. This is especially true since "the measurement of cyclic AMP at the low intracellular concentrations which appear to be physiologically relevant would be at best very difficult even with the sensitive assays which are available" (18). Nevertheless, if growth hormone either increased the activity of CAMP, or decreased its rate of destruction, potentiation of both epinephrine and theophylline would be expected. It is possible, however, that growth hormone increases the rate of spontaneous formation of CAMP. This need not result in increased basal lipolysis if the rate of CAMP formation remains low with respect to the capacity of cells to degrade it. In most tissues, the total concentration of phosphodiesterase is considerably higher than adenyl cyclase (17, 19, 20). When phosphodiesterase activity is reduced with theophylline, however, the small increase in spontaneous CAMP formation caused by growth hormone might result in a considerably higher concentration of CAMP than that achieved in control tissues. In this way growth hormone might increase lipolysis in response to theophylline. If the amount of inactive adenyl cyclase is large compared to that which is spontaneously active, activation of adenyl cyclase by epinephrine would be relatively independent of the basal activity of the enzyme. Epinephrine increases the formation of CAMP many times higher than the basal rate, as evidenced particularly in adipose cells in which phosphodiesterase is blocked (18). In tissues exposed to epinephrine, the effects of growth hormone on the basal activity of adenyl cyclase might therefore be too small to affect the total production of CAMP to any significant extent, and a potentiation of the lipolytic effects of epinephrine by growth hormone might not be seen. This hypothesis has the sole virtue of

explaining apparently contradictory observations in a manner that is consistent with all of the data. Direct measurements of CAMP and adenyl cyclase will be required to test the validity of all of the assumptions made.

The data do not rule out the alternative possibility that theophylline might increase lipolysis by some action which is independent of CAMP and that growth hormone potentiates that action of theophylline. However, no evidence has yet been brought forward to support such an alternative.

The present findings indicate that growth hormone administered *in vivo* even over a period of 3 days does not increase the sensitivity of adipose tissue to epinephrine. When glucocorticoids are present, however, growth hormone clearly increases epinephrine-induced lipolysis (9). Since under physiological conditions glucocorticoids are present, and indeed are elevated by many of the same stimuli which increase growth hormone secretion, the present findings do not rule out a physiological role for growth hormone in epinephrine-mediated lipolysis.

Summary. Pretreatment of hypophysectomized rats with growth hormone 3.5 hr before sacrifice significantly increased free fatty acid (FFA) production by epididymal fat incubated *in vitro* in the presence of epinephrine. No change in glycerol production in response to epinephrine was seen even when rats were pretreated with growth hormone for 3 days. In replicate tissues from the same animals, pretreatment with growth hormone significantly increased glycerol production in response to theophylline. It is suggested that growth hormone may exert a small effect on the basal activity of adenyl cyclase.

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