The Effects of Epinephrine on Glycerol Production in Segments of Adipose Tissue Preincubated with Dexamethasone and Growth Hormone* (33684)

H. MAURICE GOODMAN[1]

Department of Physiology, Harvard Medical School, Boston, Massachusetts 02115

Fain (1) reported that incubation of isolated adipose cells with dexamethasone and growth hormone resulted in heightened sensitivity to the lipolytic effects of norepinephrine. We obtained similar results with segments of epididymal fat obtained from hypophysectomized rats (2), but in our hands, at least, attempts to demonstrate similar effects with tissue segments of normal rats were only partially successful. Inconsistent effects were encountered both in the basal production of glycerol after treatment with dexamethasone and growth hormone, and in the magnitude of the lipolytic response elicited by epinephrine. However, when sufficient data were collected, the apparent inconsistencies fell into a definite pattern.

Materials and Methods. Normal adult male rats (300–350 g) of the Holtzman strain were fed Purina lab chow and water *ad libitum* until the time of sacrifice. In all, 36 rats were studied on 5 experimental days. The rats were killed with an overdose of sodium pentobarbital and their epididymal fat rapidly excised. The thin, distal portions were divided into 8 segments weighing approximately 50 mg each. Four tissue segments from each rat were preincubated with 0.016 μg/ml dexamethasone (9 α-fluoro-11β, 17α, 21-trihydroxy-16α-methyl-1, 4-pregnadiene-2,20-dione) and 1 μg/ml of growth (NIHGH S7). The 4 remaining tissues from each rat were preincubated in the same medium without hormones. The incubation medium was Krebs–Ringer bicarbonate buffer and contained 1 mg/ml of glucose and 40 mg/ml of bovine serum albumin (Armour Fraction V, Lot no. B23407). After 3 hr of preincubation, the hormone-treated and control tissues were transferred to fresh medium and reincubated in the presence of various concentrations of epinephrine for an additional hour. In addition to albumin, glucose, and epinephrine, the reincubation medium also contained 0.1 mg/ml of ascorbic acid to retard oxidation of the epinephrine. All incubations were carried out at 37° in sealed vials under a gas phase of 95% oxygen: 5% carbon dioxide.

The release of glycerol into the incubation medium in the fourth hour of incubation was taken as an index of lipolysis. Glycerol was determined enzymatically according to the procedure of Wieland (3).

Results. Although variable responses to dexamethasone and growth hormone were encountered on each of the 5 experimental days, a significant increase in glycerol production in the absence of epinephrine became evident when all of the data from the 36 animals were pooled (Table I). The pooled data, however, failed to reveal any effect whatever of hormonal treatment on the sensitivity of adipose tissue to the lipolytic action of epinephrine.

In the absence of epinephrine, tissues from only about 1 rat in 3 produced glycerol at a markedly greater rate as a result of preincubation with dexamethasone and growth hormone. Data from those 13 rats in which dexamethasone and growth hormone produced a twofold or greater increase in basal lipolysis were pooled and tabulated (Table II). This group showed a mean increase in basal lipolysis of almost threefold. In replicate tissues from these animals, preincubation with the hormones actually decreased the lipolytic response to 0.001 μg/ml of epinephrine, but lipolysis in response to the remaining doses of epinephrine was unaffected. In the remaining 23 rats (Table III), preincubation with dexamethasone and growth hormone had no measurable effect on basal lipolysis, but sig-

* Supported by a grant, AM 06935, from the National Institutes of Health.

[1] Recipient of a Career Development Award, K3-AM-7797, from the National Institutes of Health.

TABLE I. The Effects of Preincubation with Dexamethasone (0.016 μg/ml) and Growth Hormone (1 μg/ml) on Lipolysis in Response to Epinephrine.

	Glycerol production (μmoles/g/hr)			
	Basal	Increase due to epinephrine		
Preincubation medium	(μg/ml):	0.1	0.01	0.001
Control	2.37 ± 0.17[a]	10.50 ± 0.84	3.45 ± 0.51	0.36 ± 0.21
Dexamethasone	3.95 ± 0.47	10.07 ± 0.75	4.46 ± 0.60	0.16 ± 0.44
+ growth hormone	$p < .01$	NS[b]	NS	NS

[a] Mean ± SEM, 36 observations.

[b] Not statistically significant, $p > .05$ when compared with corresponding values of control tissues.

nificantly increased glycerol production in response to 0.001 and 0.01 μg/ml of epinephrine. The 13 rats represented in Table II were distributed randomly among the 36, and at least 1 was encountered on each experimental day. All tissues were handled identically, and the albumin used in the medium was taken from the same bottle on all 5 days.

Discussion. Although little doubt is left that dexamethasone and growth hormone increase lipolysis, the nature of the participation of these hormones in the lipolytic process is poorly understood. In the experiments just described, the tissues from only about 1 rat in every 3 exhibited a pronounced increase in lipolysis after exposure to dexamethasone and growth hormone. In other experiments, especially when small (100 g) normal rats were studied, increased lipolysis was almost uniformly observed (4). However, when adipose tissue from small hypophysectomized rats was studied, a lipolytic response to dexamethasone and growth hormone was observed only rarely, and when present was small in magnitude (4). In Fain's experiments also (1) dexamethasone and growth hormone sometimes failed to increase lipolysis. He attributed this lack of response in some experiments to the action of an inhibitor present in some preparations of albumin (1). Inhibitory material present in albumin, however, probably cannot account for the present results since positive and negative responses were obtained with the same preparation of albumin. Furthermore, we have also incubated adipose tissue with dexamethasone and growth hormone for 3 hr in the absence of albumin, and still found that only tissues from some of the rats showed increased production of glycerol when transferred to albumin-containing medium and reincubated for an additional hour.

Dexamethasone and growth hormone potentiated the lipolytic action of epinephrine

TABLE II. Lipolysis in Response to Epinephrine in Adipose Tissue in which Basal Lipolysis was Increased by Preincubation with Dexamethasone and Growth Hormone.[a]

	Glycerol production (μmoles/g/hr)			
	Basal	Increase due to epinephrine		
Preincubation medium	(μg/ml):	0.1	0.01	0.001
Control	2.22 ± 0.27[b]	10.88 ± 1.66	3.86 ± 0.84	0.90 ± 0.41
Dexamethasone	6.42 ± 0.93	10.71 ± 1.80	3.51 ± 1.37	−1.65 ± 0.87
+ growth hormone	$p < .01$	NS[c]	NS	$p < .02$

[a] These data were included in the mean values presented in Table I.

[b] Mean ± SEM, 13 observations.

[c] Not statistically significant, $p > .05$ compared with corresponding values in control tissues.

TABLE III. Lipolysis in Response to Epinephrine in Adipose Tissue in which Basal Lipolysis was not Increased by Preincubation with Dexamethasone and Growth Hormone.[a]

	Glycerol production (μmoles/g/hr)			
	Basal	Increase due to epinephrine		
Preincubation medium	(μg/ml):	0.1	0.01	0.001
Control	2.45 ± 0.23[b]	9.69 ± 1.11	3.21 ± 0.66	0.05 ± 0.22
Dexamethasone	2.55 ± 0.18	9.74 ± 0.70	5.00 ± 0.49	1.18 ± 0.34
+ growth hormone	NS[c]	NS	$p < .05$	$p < .01$

[a] These data were included in the mean values presented in Table I.

[b] Mean ± SEM, 23 observations.

[c] Not statistically significant, $p > .05$ compared with corresponding values in control tissues.

only in those tissues which failed to show a marked increase in lipolysis. Similar observations were also reported by Fain (1). In the tissues in which basal lipolysis was increased by preincubation with dexamethasone and growth hormone, glycerol production in response to 0.001 μg/ml of epinephrine was actually reduced. When higher concentrations of epinephrine were added to these tissues, the lipolytic effects of dexamethasone plus growth hormone and those of epinephrine appeared to summate. In other experiments, particularly at high basal rates of lipolysis, glycerol production in response to epinephrine and to dexamethasone and growth hormone was less than the sum of the two lipolytic processes measured separately, even though the total glycerol production was less than maximal (Goodman, in preparation). Current knowledge appears still inadequate to explain these interactions.

The present observation that dexamethasone and growth hormone either increased basal lipolysis or potentiated the lipolytic action of epinephrine is compatible with the suggestion (4) that in increasing lipolysis, the combination of growth hormone and glucocorticoid may not act as the actual initiator of lipolysis, but rather might merely potentiate other endogenous lipolytic signals present in the tissues at the time of excision. The observations that dexamethasone and growth hormone can potentiate the lipolytic effects of norepinephrine and that RNA and protein synthesis are required for this effect led Fain (1) to conclude that "activation of lipolysis by growth hormone and glucocorticoids involves the synthesis of a protein which may facilitate the activation of adenyl cyclase." The hypothetical signals or activators of adenyl cyclase remain to be elucidated. Further experiments will be required to validate or refute this hypothesis and to explain why some rats increase basal lipolysis in response to growth hormone and dexamethasone while others only show a heightened response to lipolytic agents.

Summary. After preincubation with dexamethasone (0.016 μg/ml) and growth hormone (1 μg/ml), segments of epididymal fat were tested for sensitivity to the lipolytic effects of epinephrine. When the data from the entire group of 36 rats were pooled and analyzed, it appeared that dexamethasone and growth hormone significantly increased basal lipolysis, but had no significant effect on epinephrine-induced lipolysis. Tissues from only 13 of the 36 rats had increased basal lipolysis of the order of twofold or greater. When the data from these rats were pooled, it appeared that pretreatment with dexamethasone and growth hormone actually reduced lipolysis in response to 0.001 μg/ml of epinephrine and had no effect on higher concentrations of epinephrine. Pooling the data from the remaining 23 rats indicated that the preincubation procedure did not increase basal lipolysis, but significantly increased lipolysis in response to 0.001 and 0.01 μg/ml of epinephrine. The difference in

the response of the two groups of rats cannot be attributed to inhibitors present in the albumin solution.

The growth hormone (NIH GH S7) was a gift of the Endocrinology Study Section of the National Institutes of Health. The superb technical assistance rendered by Mrs. Rosalie Snyder is gratefully acknowledged.

1. Fain, J. N., Endocrinology **82**, 825 (1968).
2. Goodman, H. M., Proc. Third Intern. Congr. Endocrinol. Mexico City (1968). in press.
3. Wieland, O., Biochem. Z. **329**, 313 (1957).
4. Goodman, H. M., Endocrinology **83**, 300 (1968).

Received Oct. 30, 1968. P.S.E.B.M., 1969, Vol. 130.

Aldosterone Production in Chronic Renal Failure (33685)

JOHN P. HAYSLETT, JOHN E. BOYD, AND FRANKLIN H. EPSTEIN
Department of Internal Medicine, Yale University School of Medicine, New Haven, Connecticut

Changes in salt and water balance consequent to renal failure might be expected to involve changes in the secretion of sodium-retaining hormones. When renal mass is reduced by renal disease or by ablation of renal tissue, sodium excretion per nephron increases so that normal sodium blanace is maintained. Previous studies using micropuncture techniques in rats showed that the mechanism for this adjustment is not dependent on either the compensatory increase in glomerular filtration per remaining nephron which occurs in these circumstances, or on a reduction in intrinsic reabsorptive capacity in the proximal tubules (1). It seems likely, therefore, that the early adjustments in sodium excretion in renal failure take place in the distal tubule. One possible explanation is that aldosterone secretion is diminished. Another hypothesis is that distal absorption of sodium is decreased in renal insufficiency by an augmented load of urea per nephron. This too might modify aldosterone secretion, but in the opposite direction. For example, the obligatory losses of sodium imposed by a urea diuresis might be expected to deplete uremic individuals of salt, stimulate the secretion of aldosterone, and thus secondarily modify distal reabsorption of sodium so as to maintain sodium balance. The rate of aldosterone secretion in experimental renal failure is therefore of special interest.

Methods. Experiments were performed upon two groups of male Sprague-Dawley rats. One group of 10 rats with an initial weight of 212 ± 10 g (mean ± SE), served as the control group. The second group of 10 rats, initial weight 199 ± 5 g, were azotemic after ablation of approximately 85% of renal tissue. Reduction of nephron mass was accomplished in two stages. In the first stage the upper and lower poles of the left kidney were removed under ether anesthesia. Four to 5 days following this procedure, the right kidney was removed. Sham operations were performed in the control animals. Both groups were then placed on a normal diet containing 109 meq of sodium/kg and 120 meq of potassium/kg and tap water *ad libitum*. Animals from both groups were pair-fed to maintain an identical intake of sodium, since the intake of food by azotemic rats was reduced.

Fourteen days after the second stage of surgery, anesthesia was induced with sodium pentobarbital, blood was obtained from the abdominal aorta, and both adrenal glands were excised, cleaned of adherent fat, weighed, and quartered. The two adrenal glands from each rat were combined and incubated in Krebs–Ringer bicarbonate buffer with added glucose, 200 mg/100 ml, in an atmosphere of 95% O_2 and CO_2 at 36–37°. After preincubation for 30 min, the media was renewed and incubation was performed for 2 hr. The aldosterone content in the bathing medium was estimated by methods previously described (2). The rate of production