# Effect of Drugs on the Lipolytic Action of Hormones in Isolated Fat Cells

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(Received January 7, 1966)

SUMMARY

The effect of various drugs on the lipolytic action of growth hormone plus dexamethasone was compared with that on the action of ACTH and epinephrine in isolated fat cells from starved rats. Phenoxybenzamine reduced the acceleration of lipolysis by growth hormone plus dexamethasone but had no significant effect on that of either ACTH or epinephrine. Butoxamine inhibited the effect of growth hormone plus dexamethasone and of ACTH on lipolysis.

*dl*-β-hydroxy-*N*-*tert*-butyl-2,4-dichlorophenethylamine (DCB) stimulated lipolysis and did not prevent the acceleration of lipolysis by growth hormone and dexamethasone. There was no lipolytic effect of epinephrine and ACTH in the presence of DCB. Propranolol, which is a beta adrenergic blocking agent with little sympathomimetic activity, completely blocked the lipolytic effect of epinephrine. Propranolol did not inhibit the lipolytic action of either ACTH or growth hormone plus dexamethasone.

Nicotinic acid blocked the lipolytic effect of growth hormone plus dexamethasone, and theophylline potentiated their lipolytic action. Actinomycin D blocked the lipolytic effect of growth hormone and dexamethasone, but not of epinephrine.

## INTRODUCTION

The addition of both growth hormone and dexamethasone,[4] a potent synthetic glucocorticoid, accelerated lipolysis to a much greater extent than was seen after the addition of either hormone alone to incubated adipose tissue or isolated fat cells (1). In the presence of dexamethasone very low concentrations of growth hormone, 0.01–0.1 μg/ml, stimulated lipolysis, and the effects of these hormones were blocked by insulin. On the basis of these findings it was postulated that the accelerated release of fatty acid from adipose tissue of animals deprived of food or insulin might involve a direct stimulation of lipolysis by growth hormone and glucocorticoids (1). An elevated release of fatty acid by adipose tissue is thought to be largely responsible for diabetic ketosis, and it has been found that both ketosis and the release of fatty acid by adipose tissue from diabetic rats are almost completely abol-

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[4] Abbreviations and trivial names used are: FFA, free fatty acids; dexamethasone, 9α-fluoro-11β,17,21-trihydroxy-16α-methyl-1,4-pregnadiene-3,20-dione; DCB, *dl*-β-hydroxy-*N*-*tert*-butyl-2,4-dichlorophenethylamine; ACTH, adrenocorticotropic hormone; butoxamine, *N*-*tert*-butylmethoxamine; propranolol, 1-isopropylamine-3-(1-naphthyloxy)-2-propanol.

ished in the absence of the anterior pituitary (2).

However, it has been postulated that the increased mobilization of fatty acid from adipose tissue of diabetic animals is mediated by an adrenergic mechanism (3–5). This hypothesis was based on the finding that phenoxybenzamine and butoxamine,[4] which have been thought to act solely as adrenergic blocking agents, reduced fatty acid mobilization in diabetic animals. Salvador and associates (3, 4) administered butoxamine to diabetic dogs and found a decrease in the concentration of plasma FFA,[4] Wertheimer *et al.* (5) had earlier observed that the release of fatty acid by incubated adipose tissue from alloxan-diabetic rats was reduced in animals treated with phenoxybenzamine.

The present experiments were designed to investigate the possibility that phenoxybenzamine and butoxamine reduced fatty acid mobilization in diabetic animals by interfering with the acceleration of lipolysis by growth hormone and glucocorticoids. The effect of phenoxybenzamine and butoxamine on the lipolytic action of growth hormone plus dexamethasone was compared with their effect on that of ACTH and epinephrine. In addition the effect of DCB, propranolol, theophylline, and nicotinic acid on the lipolytic action of growth hormone and dexamethasone was examined.

All the present experiments were performed with fat cells isolated from rat parametrial adipose tissue by the procedure of Rodbell (6). The fat cells can be obtained free of the other cells in adipose tissue and have a greater lipolytic response to hormones than incubated tissue (6). We have found experiments with isolated fat cells to be more convenient and reproducible than those with incubated tissue. In addition the effect of the various hormones and drugs on lipolysis can be attributed to their direct action on the fat cell since the other cells which occur in adipose tissue are not present in fat cell suspensions (6; and unpublished observations).

## MATERIALS AND METHODS

Female Sprague-Dawley rats (130–150 g), which had been fed a high fat (40%) diet (7) for 5–14 days before the experiments, were deprived of food for 18 hr.

Isolated fat cells were prepared by a modification of the procedure of Rodbell (6) in which parametrial adipose tissue was incubated for 1 hr in albumin–bicarbonate buffer (8) media containing bacterial collagenase. Glucose was not present in the buffer solution which contained collagenase or in the solution used to wash the cells. A 4% solution of bovine, fraction V albumin (Armour lot No. 22,312) in buffer solution was made up fresh each day. The pH of the albumin solution, which contained 0.2–0.3 μmole of FFA per milliliter, was adjusted to pH 7.4 in an atmosphere of 5% $CO_2$, 95% $O_2$.

The isolated fat cells were incubated for 4 hr at 37.5° in 1-ounce polyethylene bottles.[5] Initial control values were obtained with fat cells incubated for 5 min. Incubations were performed in duplicate in each experiment. All statistical evaluations are based on paired comparisons.

At the end of the experiments an aliquot of the medium was analyzed for glycerol (9). Total lipid and FFA were extracted from the remaining medium plus cells by a modification of the procedure of Dole and Meinertz (10) in which hexane was substituted for heptane. An aliquot of the hexane extract was evaporated to dryness, 1 ml of Nile Blue A indicator solution was added and the FFA content determined by titration. Another aliquot of the hexane extract was taken for determination of total fatty acid content (6). The total fatty acid content of the flasks was used to measure the cells present in each flask; 1 g of adipose tissue is assumed to contain approximately 3 mmoles of fatty acid.

*dl*-β-Hydroxy-*N*-*tert*-butyl-2,4-dichlorophenethylamine (DCB), obtained through the courtesy of Dr. I. H. Slater of Lilly Research Laboratories; *N*-*tert*-butylmethoxamine (butoxamine), obtained through the courtesy of Dr. J. J. Burns of Wellcome Research Laboratories, and propranolol,[6] obtained through the courtesy of

[5] The Nalgene® plastic bottles were rinsed with water because certain lots of these bottles contained small amounts of glycerol.

[6] Inderal®.

TABLE 1

*Effect of phenoxybenzamine on lipolysis in fat cells*

Free fat cells (17.1 mg/flask) were incubated for 4 hr in 2 ml of medium containing glucose (2.4 mM). The difference due to hormone addition is shown as the mean ± standard error of 6 paired replications.

| Phenoxybenzamine (μg/ml) | Basal release | Increase due to dexamethasone, 0.016 μg/ml, + growth hormone, 1.0 μg/ml | Increase due to ACTH, 0.002 μg/ml | Increase due to epinephrine, 0.1 μg/ml |
|---|---|---|---|---|
| | | *Glycerol release (μmoles/g)* | | |
| 0 | 30 | +70 ± 20 | +80 ± 20 | +85 ± 20 |
| 1 | 35 | +44 ± 15 | +60 ± 20 | +85 ± 20 |
| 10 | 45 | +20 ± 10 | +85 ± 30 | +85 ± 15 |
| | | *Fatty acid release (μmoles/g)* | | |
| 0 | 40 | +165 ± 40 | +180 ± 50 | +190 ± 35 |
| 1 | 30 | +155 ± 20 | +195 ± 50 | +225 ± 30 |
| 10 | 70 | +45 ± 25 | +110 ± 30 | +165 ± 40 |

Dr. Alex Sahagian-Edwards of Ayerst Laboratories, were added to the incubation medium as the water-soluble hydrochlorides. Phenoxybenzamine[7] and theophylline were obtained from Merck Co. *l*-Epinephrine[8] and nicotinic acid were purchased locally. All solutions of amine derivatives were prepared fresh each day.

Oxycel purified ACTH, 100 U/mg, was purchased from Wilson Laboratories. Bovine growth hormone (NIH-GH-B3) was obtained from the Endocrinology Study Section of the National Institutes of Health. This preparation had a growth-promoting potency of 1 USP unit/mg and it contained 2 μU of TSH activity and 0.1 μU of ACTH per gram. All hormones or drugs were added at the start of the 4-hr incubation period unless otherwise stated.

In all the experiments a concentration of growth hormone (1 μg/ml) was used which is approximately ten to a hundred times greater than that needed to produce maximal stimulation of lipolysis in the presence of dexamethasone (1). Concentrations of ACTH and epinephrine were used in the various experiments which gave an acceleration of lipolysis comparable to that of growth hormone and dexamethasone.

[7] Dibenzyline®.

[8] Adrenalin®.

## RESULTS

Phenoxybenzamine is an alpha adrenergic blocking drug and would not be expected to block the receptors for catecholamines in adipose tissue since they appear to be beta receptors (11, 12). The addition of phenoxybenzamine to isolated fat cells at a concentration of 1 or 10 μg/ml had little effect on basal lipolysis and did not significantly inhibit the acceleration of lipolysis due to either ACTH or epinephrine (Table 1). However, there was no lipolytic effect of growth hormone plus dexamethasone in the presence of 10 μg per milliliter of phenoxybenzamine.

It has been reported (3, 4) that butoxamine inhibits the general metabolic effects of epinephrine *in vivo*. Butoxamine, at a concentration of 0.1 or 1.0 μg/ml, did not significantly affect basal lipolysis in isolated fat cells (Table 2). The lipolytic effect of growth hormone plus dexamethasone was reduced by about one-half in the presence of 1.0 μg/ml of butoxamine (Table 2).

The lipolytic action of ACTH was reduced by butoxamine at 1 μg/ml (Table 2). However, the effect of butoxamine on the activation of lipolysis by epinephrine was quite variable, and no significant effect of the drug could be detected at the concentrations used in the present experiments.

TABLE 2

*Effect of butoxamine on lipolysis in fat cells*

Free fat cells (10 mg per flask in Expt. 1 and 5 mg per flask in Expt. 2) were incubated for 4 hr in 2 ml of medium containing glucose (2.4 mM). The difference due to hormone addition is shown as the mean ± standard error of 5 paired replications in Expt. 2 and 10 in Expt. 1.

| Butoxamine (μg/ml) | Basal release | Increase due to dexamethasone, 0.016 μg/ml, + growth hormone, 1.0 μg/ml | Increase due to ACTH, 0.002 μg/ml | Increase due to epinephrine, 0.1 μg/ml |
|---|---|---|---|---|
| | | *Glycerol release (μmoles/g)* | | |
| Expt. 1 | | | | |
| 0 | 105 | +125 ± 15 | +110 ± 15 | +135 ± 25 |
| 1.0 μg/ml | 95 | +65 ± 20 | +40 ± 30 | +75 ± 50 |
| Expt. 2 | | | | |
| 0 | 180 | +180 ± 50 | +120 ± 30 | +160 ± 55 |
| 0.1 μg/ml | 185 | +110 ± 10 | +105 ± 95 | +155 ± 70 |
| | | *Fatty acid release (μmoles/g)* | | |
| Expt. 1 | | | | |
| 0 | 200 | +275 ± 35 | +255 ± 25 | +245 ± 30 |
| 1.0 μg/ml | 185 | +175 ± 50 | +95 ± 50 | +130 ± 90 |
| Expt. 2 | | | | |
| 0 | 375 | +365 ± 60 | +260 ± 65 | +325 ± 90 |
| 0.10 μg/ml | 390 | +275 ± 45 | +140 ± 50 | +280 ± 100 |

DCB is one of a group of halogenated analogs of epinephrine which are known to be beta adrenergic blocking drugs (11–14). These compounds also mimic the effects of catecholamines on beta adrenergic receptors. DCB stimulated lipolysis in isolated fat cells at a concentration of 1.0 but not 0.1 μg/ml (Table 3). In the presence of 0.1 μg/ml of DCB there was a greater stimulation of lipolysis by growth hormone and dexamethasone than in its absence. Although 0.1 μg/ml of DCB did not affect the

TABLE 3

*Effect of DCB on response of fat cells to ACTH, epinephrine, and dexamethasone plus growth hormone*

Free fat cells (8.5 mg per flask) were incubated for 4 hr in 2 ml of medium containing glucose (2.4 mM). The difference due to hormone addition is shown as the mean ± standard error of 6 paired replications.

| DCB (μg/ml) | Basal release | Increase due to dexamethasone, 0.016 μg/ml, + growth hormone, 1.0 μg/ml | Increase due to ACTH, 0.002 μg/ml | Increase due to epinephrine, 0.1 μg/ml |
|---|---|---|---|---|
| | | *Glycerol release (μmoles/g)* | | |
| 0 | 102 | +80 ± 18 | +75 ± 25 | +135 ± 40 |
| 0.1 | 113 | +145 ± 34 | +100 ± 40 | +125 ± 45 |
| 1.0 | 335 | +35 ± 12 | −10 ± 15 | −45 ± 25 |
| | | *Fatty acid release (μmoles/g)* | | |
| 0 | 200 | +200 ± 60 | +120 ± 50 | +340 ± 75 |
| 0.1 | 200 | +380 ± 85 | +235 ± 100 | +265 ± 90 |
| 1.0 | 700 | +140 ± 25 | −45 ± 35 | −170 ± 85 |

lipolytic action of epinephrine or ACTH, 1.0 μg/ml of DCB completely blocked the lipolytic effect of ACTH or epinephrine and lipolysis was actually less in the presence of the hormones and DCB than with DCB alone. However, growth hormone and dexamethasone still significantly stimulated lipolysis in the presence of 1.0 μg/ml of DCB. The reduced effect of growth hormone and dexamethasone in the presence of the high concentration of DCB is probably due to the fact that lipolysis was stimulated to near maximal values by DCB alone.

Because of the marked sympathomimetic effect of DCB the experiments were repeated using propranolol, which is a beta adrenergic blocking drug with very little sympathomimetic activity (15). The addition of propranolol, 5 μg/ml, completely blocked the lipolytic action of epinephrine, 0.1 μg/ml (Table 4). This concentration of propranolol is at least five times greater than that needed to completely block the lipolytic action of epinephrine, 0.1 μg/ml (unpublished results). However, propranolol, 5 μg/ml, had no inhibitory effect on the activation of lipolysis by either ACTH or growth hormone plus dexamethasone.

Nicotinic acid, which is apparently unrelated to any of the adrenergic blocking agents, has been reported to reduce lipolysis in incubated adipose tissue, from alloxan-diabetic rats (16). The experiments in Table 5 indicate that it markedly reduced basal lipolysis and virtually abolished the lipolytic effects of all the hormones tested.

Previously it has been shown that there is a lag in the onset of the lipolytic effect of growth hormone and dexamethasone since the increased lipolysis due to these hormones largely occurred during the second 2 hr of the 4-hr incubation period (1, 17). Insulin has been found to be equally effective in inhibiting the lipolytic action of these hormones whether added at the start of incubation period or 2 hr later (17). Nicotinic acid also blocked the lipolytic action of growth hormone and dexamethasone even when added 2 hr after these hormones (Table 5).

The lag in the onset of the lipolytic action of growth hormone and dexamethasone has been attributed to the involvement of RNA and protein synthesis for the following reasons. Puromycin ($10^{-4}$ M) inhibited the lipolytic effect of growth hormone and dexamethasone but not that of ACTH (1). This concentration of puromycin blocked incorporation of labeled amino acid into protein by isolated fat cells (1). The same concentration of the aminonucleoside of puromycin did not block the lipolytic action of growth hormone and dexamethasone (1). Actinomycin D ($10^{-7}$

TABLE 4

*Effect of propranolol on lipolytic action of hormones*

Free fat cells (20 mg per flask) were incubated for 4 hr in 2 ml of medium containing glucose (2.4 mM). The difference due to hormone addition is shown as the mean ± standard error of 7 paired replications.

| Propranolol (μg/ml) | Basal release | Increase due to dexamethasone, 0.016 μg/ml, + growth hormone, 1.0 μg/ml | Increase due to ACTH, 0.01 μg/ml | Increase due to epinephrine, 0.1 μg/ml |
|---|---|---|---|---|
| | | *Glycerol release (μmoles/g)* | | |
| 0 | 29 | +23 ± 5 | +49 ± 9 | +82 ± 11 |
| 5 | 27 | +16 ± 5 | +55 ± 10 | +4 ± 4 |
| | | *Fatty acid release (μmoles/g)* | | |
| 0 | 50 | +63 ± 6 | +110 ± 22 | +168 ± 20 |
| 5 | 37 | +53 ± 15 | +135 ± 24 | +7 ± 10 |

TABLE 5

*Effect of nicotinic acid on hormonal activation of lipolysis in fat cells*

Fat cells (14.5 mg per flask) were incubated for 4 hr in 2 ml of medium containing glucose (2.4 mM). The difference due to hormone addition is shown as the mean ± standard error of 4 paired replications.

| Nicotinic acid | Basal release | Dexamethasone, 0.016 μg/ml, + growth hormone, 1.0 μg/ml | ACTH, 0.002 μg/ml | Epinephrine, 0.1 μg/ml |
|---|---|---|---|---|
| | *Glycerol release (μmoles/g)* | | | |
| 0 | 30 | +75 ± 25 | +105 ± 25 | +110 ± 25 |
| 1.0 μg/ml added at 0 hr | 5 | +5 ± 5 | +15 ± 5 | +10 ± 2 |
| 1.0 μg/ml added at 2 hr | 30 | 0 ± 5 | — | — |
| | *Fatty acid release (μmoles/g)* | | | |
| 0 | 25 | +190 ± 50 | +250 ± 50 | +170 ± 35 |
| 1.0 μg/ml added at 0 hr | −45 | +3 ± 15 | −2 ± 10 | +10 ± 15 |
| 1.0 μg/ml added at 2 hr | −16 | +5 ± 5 | — | — |

M) inhibited the lipolytic action of growth hormone and dexamethasone and the incorporation of uridine into RNA, but not the lipolytic action of ACTH (1). The data in Table 6 indicate that actinomycin D ($2.5 \times 10^{-7}$ M) did not affect the lipolytic action of a very low concentration of epinephrine even though the actinomycin D was added 2 hr prior to addition of epinephrine. In the same experiment actinomycin D blocked the lipolytic action of growth hormone and dexamethasone in agreement with our earlier studies (1).

Methyl xanthine compounds such as theophylline or caffeine have been found to accelerate lipolysis in fat cells and potentiate the lipolytic action of catecholamines (18, 19) and cyclic 3′,5′-AMP (18). The experiments in Fig. 1 indicate that a low concentration of theophylline potentiated the lipolytic effect of growth hormone and dexamethasone in isolated fat cells incubated with glucose.

TABLE 6

*Effect of actinomycin D on lipolytic action of epinephrine*

Free fat cells (24 mg/flask) were incubated for 4 hr in 2 ml of medium containing glucose (2.4 mM). The difference due to epinephrine addition is shown as the mean ± standard error of 8 paired replications.

| Additions at start of 4 hr incubation period | Basal release | Increase due to epinephrine, 0.01 μg/ml, added at end of 2 hr incubation |
|---|---|---|
| | *Glycerol release (μmoles/g)* | |
| None | 17 | +27 ± 2 |
| Dexamethasone, 0.016 μg/ml, + growth hormone, 1.0 μg/ml | 30 | +35 ± 4 |
| Actinomycin, $2.5 \times 10^{-7}$ M | 13 | +26 ± 5 |
| Actinomycin + growth hormone and dexamethasone | 11 | +23 ± 5 |

The increase in lipolysis due to ACTH was also significantly greater in the presence of theophylline than in its absence (Fig. 2). The results in Fig. 2 confirm the earlier observation (1) that growth hormone and dexamethasone increased lipolysis in the absence of glucose and indicate

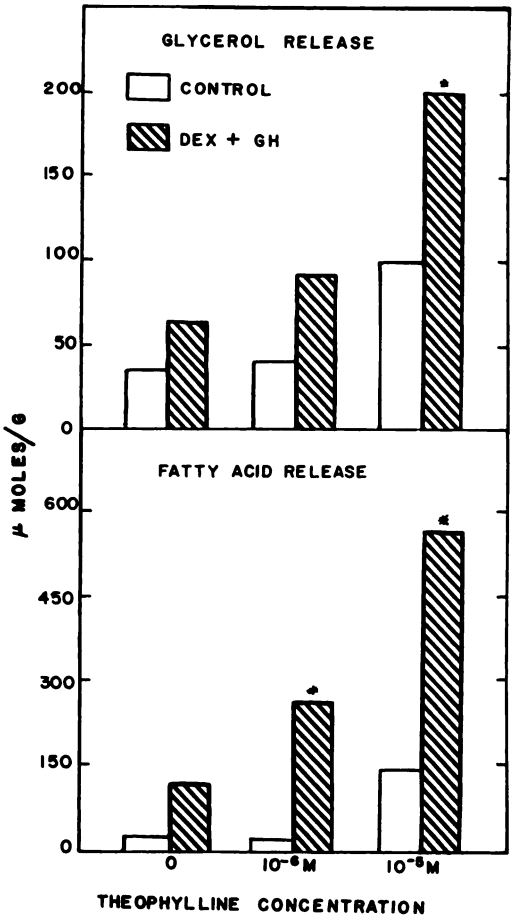


FIG. 1. *Fat cells (10 mg per flask) were incubated for 4 hr in 2 ml of medium containing glucose (2.4 mM)*

The values are shown as the means of three experiments. A statistically significant potentiation of the effects of *DEX + GH* (dexamethasone, 0.016 μg/ml and growth hormone, 1.0 μg/ml) by theophylline is indicated by an asterisk ($P < 0.05$ by paired comparisons). The comparisons are based on the paired differences between the absolute increase due to hormones in the absence and presence of theophylline.

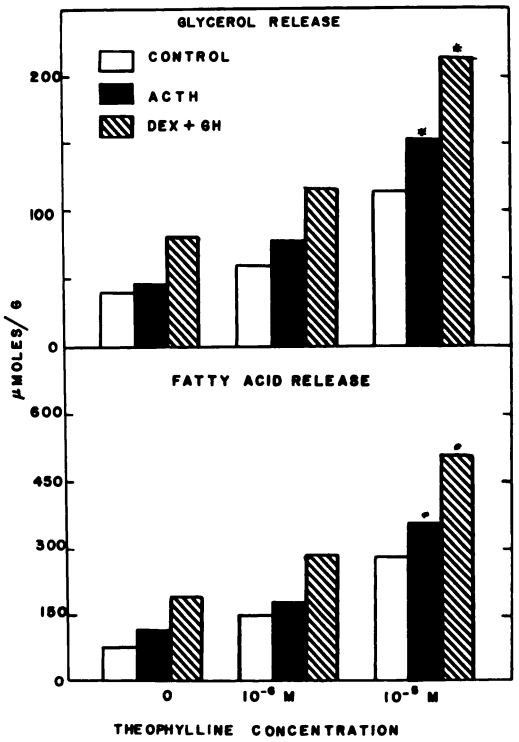


FIG. 2. *Free fat cells (12 mg per flask) were incubated for 4 hr in 2 ml of medium without added substrate*

The values are shown as the means of 4 experiments. A statistically significant potentiation of the effects of ACTH (0.001 μg/ml) or *DEX + GH* (dexamethasone, 0.016 μg/ml and growth hormone, 1.0 μg/ml) by theophylline is indicated by an asterisk ($P < 0.05$ by paired comparisons). The comparisons are based on the paired differences between the absolute increase due to the hormone in the absence and presence of theophylline.

that theophylline was equally effective in potentiating their action in the absence of glucose.

## DISCUSSION

The finding that phenoxybenzamine and butoxamine inhibited the lipolytic action of growth hormone and dexamethasone in isolated fat cells suggests that these compounds are not specific adrenergic blocking drugs. Phenoxybenzamine actually had no effect on the action of epinephrine or ACTH in isolated fat cells, which was expected since phenoxybenzamine has been found to be a relatively ineffective inhibitor of the effects of catecholamines on fatty acid mobilization *in vivo* (11, 20). Phenoxybenzamine is also an alkylating agent and probably reacts with other structures in addition to the alpha receptors for catecholamines. It has been postulated that the lipolytic action of growth hormone and

dexamethasone is mediated through a mechanism involving RNA and protein synthesis (1) and phenoxybenzamine may be interfering with these processes. Experiments are in progress with regard to this point.

Butoxamine has been reported to block the rise in plasma FFA concentration induced by catecholamines (3, 4). However, direct addition of butoxamine to incubated adipose tissue has not been found to inhibit the acceleration of lipolysis by catecholamines unless the pH of the buffer was below 7.0 and the concentration of butoxamine above 20 μg/ml (R. A. Salvador, personal communication). The present experiments suggest that the reduction in fatty acid mobilization in diabetic animals treated with phenoxybenzamine and butoxamine (3–5) was due to an inhibition of the lipolytic action of growth hormone and glucocorticoid.

These results provide further evidence that fatty acid mobilization during fasting and diabetes involves a nonadrenergic component. Several recent studies have presented data in agreement with this hypothesis. Stern and Maickel (21) found that chemical sympathectomy did not prevent mobilization of fatty acid during starvation but did block mobilization in cold-stressed animals. Stock and Westermann (22) have reported that various drugs which inhibited the acceleration of fatty acid mobilization by catecholamines did not reduce the concentration of plasma FFA in diabetic animals.

The failure to observe a lipolytic effect of epinephrine in the presence of 1.0 μg/ml of DCB confirms earlier studies with isolated fat cells (18), incubated adipose tissue (13, 14), and intact animals (12). The effect of growth hormone and dexamethasone in the presence of 0.1 μg/ml of DCB was almost twice what it was in the absence of DCB. However the increase due to growth hormone and dexamethasone in the presence of 1.0 μg/ml of DCB was less than in its absence, and this may be due to the fact that 1.0 μg/ml of DCB alone produced a large increase in lipolysis. Rodbell (23) has shown that when the primary binding sites for fatty acids on albumin are filled there is an inhibition of further lipolysis. In the presence of 1.0 μg/ml of DCB plus growth hormone and dexamethasone, the final medium FFA content was 5 μmoles per milliliter of 4% albumin. Since it takes a concentration of 6–9 μmoles of FFA per milliliter of 4% albumin to saturate the primary binding sites of the albumin (23) we were approaching the maximal binding capacity of the albumin.

Love *et al.* (13, 14) have reported that DCB did not accelerate lipolysis in incubated adipose tissue. The results in Table 3 demonstrated that DCB is a potent lipolytic agent in fat cells. The reason for the discrepancy is not known. However, it has been observed that other dichloroisoproterenol compounds mimic the action of the hormones whose function they block (11–14).

The present experiments indicate that propranolol is a potent adrenergic blocking agent with little sympathomimetic activity. Propranolol has also been found to be a competitive antagonist of the lipolytic action of epinephrine in isolated fat cells (24). Propranolol (15) and DCB (11–14) are beta adrenergic blocking drugs, and the inhibitory effect of these drugs on the lipolytic action of catecholamines suggests that the catecholamine receptor in fat cells is a beta adrenergic receptor. This was supported by the failure of the alpha blocking drug phenoxybenzamine to inhibit catecholamine-induced lipolysis. Very high concentrations of phentolamine, which is also an alpha adrenergic blocking drug, will inhibit the lipolytic action of epinephrine, 0.1 μg/ml, in fat cells from rats (24). However, propranolol is at least 300–400 times as potent an adrenergic blocking drug as phentolamine (on a molar basis) in blocking the lipolytic action of epinephrine in fat cells (24). Furthermore we have found that *dl*-isoproterenol is about 10 times as potent in stimulating lipolysis in fat cells obtained from rats as *l*-epinephrine, and *l*-norepinephrine about twice as potent as *l*-epinephrine (24). Rudman and associates have obtained similar differences between the potencies of these catecholamines in

stimulating lipolysis in slices of hamster adipose tissue (25). These observations are in accord with the suggestion by Mayer *et al.* (12) that the catecholamine-induced mobilization of free fatty acids is mediated via beta receptors.

The present experiments indicate that nicotinic acid is a relatively nonspecific inhibitor of lipolysis since it reduced basal lipolysis and blocked the lipolytic action of all the hormones. The effects of nicotinic acid are very similar to those of prostaglandin $E_1$ (24) and insulin (17), which are also nonspecific inhibitors of lipolysis. Both prostaglandin $E_1$ (24) and insulin (17) are competitive antagonists of the lipolytic action of epinephrine.

Nicotinic acid has been found to reduce the rate of fatty acid release by incubated adipose tissue from diabetic rats (16) and the plasma FFA concentration of diabetic dogs (26). However this indicates very little about the cause of the elevated lipolysis in diabetic animals since nicotinic acid is a nonspecific inhibitor of lipolysis. Stock and Westermann (22) have also noted that nicotinic acid blocked the acceleration of fatty acid mobilization *in vivo* by a wide variety of substances.

In the present experiments the reduction in fatty acid release seen in the presence of nicotinic acid was due to a direct inhibitory effect of this drug on lipolysis even though the cells were incubated in the presence of glucose (Table 5). Previously it was observed that insulin had a similar antilipolytic effect in cells incubated with glucose (17). A further similarity in the action of nicotinic acid (Table 5) and insulin (17) is the finding that both substances blocked the lipolytic action of growth hormone and dexamethasone whether added at the start of the 4-hr incubation period or 2 hr later. Actinomycin is another compound which inhibits the lipolytic action of growth hormone and dexamethasone, but actinomycin inhibits only if added at the start of the 4-hr incubation period and not if added 2 hr later (1).

The finding that the effects of phenoxybenzamine and of DCB on the lipolytic action of epinephrine and ACTH are different from those on that of growth hormone and dexamethasone demonstrates further differences between the action of these hormones. Previously it was found that the onset of the lipolytic action of ACTH and epinephrine is much faster than that of growth hormone and dexamethasone (1). The stimulation of lipolysis by growth hormone and dexamethasone is blocked by actinomycin or puromycin while that of ACTH (1) and epinephrine (Table 6) is unaffected by these compounds. The slow onset in the lipolytic action of growth hormone and dexamethasone and the inhibitory effect of actinomycin and puromycin on this process suggest that synthesis of RNA and protein molecules may be required before triglyceride hydrolysis is accelerated. In contrast, the activation of lipolysis by ACTH or epinephrine occurs rapidly and is not inhibited by actinomycin or puromycin, indicating that the mechanisms by which ACTH and epinephrine activate lipolysis are quite different from those for growth hormone and dexamethasone.

The fact that propranolol selectively inhibited the lipolytic action of epinephrine as compared to that of ACTH indicates that mechanisms by which these hormones produce a rapid acceleration of lipolysis are not identical. These results are in agreement with those of Stock and Westermann (22), who found that 1-methylphenoxy-3-isopropylaminopropanol-2 blocked the lipolytic effect of epinephrine at concentrations which had no effect on the lipolytic action of ACTH. Catecholamines appear to react with the beta adrenergic receptor in fat cells while ACTH reacts at a quite different receptor site. These results also rule out any mechanism for acceleration of lipolysis by ACTH which involves release of catecholamines stored in the fat cell.

There is at least one common feature in the mechanisms by which growth hormone and dexamethasone, epinephrine, and ACTH increase lipolysis. Theophylline potentiated the lipolytic action of ACTH (Fig. 2) and of growth hormone and dexamethasone (Figs. 1 and 2). A similar effect

of caffeine, which like theophylline is a methyl xanthine compound, on the lipolytic effect of catecholamines has been reported (18, 19). The methyl xanthines are potent inhibitors of the phosphodiesterase which inactivates cyclic 3′,5′-AMP (27), and Rizack postulated that catecholamines and ACTH activate a triglyceride lipase by increasing the conversion of ATP to cyclic AMP (28). Recently Butcher and associates (19) have shown that the concentration of cyclic 3′,5′-AMP in adipose tissue is increased by epinephrine and that caffeine potentiated the effect of epinephrine on both fatty acid release and accumulation of cyclic 3′,5′-AMP in adipose tissue. The addition of $N^6$-2′-*O*-dibutyryl cyclic 3′,5′-AMP, an analog of cyclic 3′,5′-AMP, increased fatty acid release by isolated fat cells (19). Their results strongly implicate 3′,5′ cyclic AMP in the lipolytic action of epinephrine. The present experiments indicate that if theophylline is acting solely by increasing the concentration of 3′,5′ cyclic AMP, then the lipolytic action of growth hormone and dexamethasone can also be potentiated by cyclic AMP.

Table 7 summarizes the effects of various drugs on the lipolytic action of the three hormones examined in the present study. The effects of the various drugs are based on the results of the present experiments except for the effect of actinomycin and puromycin on the lipolytic action of ACTH and growth hormone plus dexamethasone (1) and of puromycin on the lipolytic action of epinephrine (29). The selective effects of many of the drugs indicate quite distinct differences in the mechanisms by which these hormones accelerate lipolysis. On the basis of the available information we suggest that these hormones accelerate lipolysis by the following mechanisms: Catecholamines interact with a classical beta adrenergic receptor resulting in an increased formation of cyclic 3′,5′-AMP which rapidly activates a triglyceride lipase. ACTH may act similarly but does not react with the same receptor site as catecholamines. The stimulation of lipolysis by growth hormone and glucocorticoids occurs after a lag period of approximately 1–2 hr and appears to be mediated through a mechanism involving RNA and protein synthesis. The protein or proteins whose synthesis is stimulated by growth hormone and glucocorticoids accelerates lipolysis by a process which is potentiated by cyclic 3′,5′-AMP.

TABLE 7
*Summary of drug effects on the stimulation of lipolysis by hormones*

| Drug | Growth hormone + dexamethasone | Catecholamines | ACTH |
|---|---|---|---|
| Actinomycin | Yes[a] | No | No |
| Puromycin | Yes | No | No |
| Phenoxybenzamine | Yes | No | No |
| Nicotinic acid | Yes | Yes | Yes |
| Butoxamine | Yes | Yes? | Yes |
| DCB | No | Yes | Yes |
| Propranolol | No | Yes | No |
| Methyl xanthines | No[b] | No[b] | No[b] |

[a] An inhibitory effect of the drug on the lipolytic action of the hormones is indicated by a yes.
[b] The methyl xanthines actually potentiated the lipolytic action of all the above-mentioned hormones.

ACKNOWLEDGMENTS

The authors would like to thank Professor R. E. Parks, Jr., for his comments during the preparation of this manuscript and Miss Nancy Davis for her technical assistance in some of the experiments.

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