Inhibition of Activated Lipolysis by Acidosis

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SUMMARY

Rat epididymal adipose tissue was incubated in Krebs-Ringer phosphate medium with 5% albumin without glucose and with glucagon, ACTH, norepinephrine, or cyclic 3′,5′-AMP dibutyrate. pH of the medium was varied from 7.4 to 6.6. Glycerol release was measured and taken as the index of lipolytic activity. In a first series of experiments at pH 7.4, ACTH and glucagon-activated lipolysis was potentiated by increasing doses of theophylline. In a second series, with pH of the medium at 6.6, the lipolytic effects of these two hormones were significantly inhibited. When theophylline (10^{-2} M) was added in combination with optimal doses of the hormones, the rate of glycerol release was similar at normal and acid pH. These results were interpreted as indicating that H^+ might exert its inhibitory effect on a common mechanism which results in cyclic 3′,5′-AMP formation. This hypothesis was confirmed in a third series: when cyclic 3′,5′-AMP dibutyrate (10^{-3} M) was added to the medium, similar glycerol release was found at pH 7.4 and 6.6. When combined with norepinephrine, cyclic 3′,5′-AMP dibutyrate also reversed the inhibitory effects of acidosis. In a last series of experiments, it was shown that acidosis did not alter irreversibly the sites of action of the lipolytic agents. These results, when analyzed according to the drug-receptor theory, would indicate that acidosis might inhibit, at least in part, the different lipolytic drugs used in this study by hindering the formation of the drug-receptor complexes that activate lipolysis.

INTRODUCTION

The inhibition of catecholamine-induced lipolysis *in vitro* by acid pH (1) is also observed *in vivo* (2, 3). It was also shown, in both instances, that theophylline reversed this inhibition (1, 3). Such observations were interpreted as indicating that acid pH exerts its inhibitory effect primarily on the activating processes which lead to the formation of cyclic 3′,5′-AMP. The nature of this inhibitory effect remained, however, to be clarified. Such a clarification was the purpose of the present series of experiments, where the effect of acid pH on lipolysis activated by adrenocorticotropic hormone (ACTH) (4–7), glucagon (8), and cyclic 3′,5′-AMP dibutyrate (9) was studied.[2]

In a first series of experiments, the effect of acid pH on the lipolytic effect of ACTH and glucagon was studied. If the mechanism of action of lipolysis induced by these hormones is similar to that of norepinephrine, one could expect to observe the same inhibitory action of acid pH. Furthermore, theophylline should also reverse this inhibitory effect.

In a second series of experiments, the effect of acid pH on lipolysis activated by cyclic 3′,5′-AMP dibutyrate was studied. If acid pH acts primarily on the processes

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which produce cyclic 3′,5′-AMP formation, it should have little effect on the lipolytic action of this nucleotide.

All the results obtained were analyzed according to the method proposed by Ariëns (10) and by Furchgott (11) which made use of Stetten and Clark's model (12, 13) to describe the interaction between a drug and its receptor. The effect of acid pH on the activation of a "beta-type receptor" by lipolytic hormones was interpreted in the light of such analysis.

METHODS

Male Sherman rats (150–200 g) were allowed free access to food and water until decapitated. Both epididymal fat pads were cut into small pieces and mixed, and 100 mg of adipose tissue was incubated in Krebs-Ringer phosphate medium (final volume 2 ml). The medium contained half the recommended amount of Ca^{2+} (in order to avoid precipitation), 5% albumin, and no glucose. In most of the experiments performed, the adipose tissue was incubated for 1 hr at pH 7.4 with the drug or hormones under study. Glycerol release (in micromoles per gram of wet tissue) was determined at the end of the incubating period according to the colorimetric method of Lambert and Neish (14) as modified by Korn (15). In a first series of experiments, the net lipolytic effect of the drugs or of the combination of drugs was calculated by deducting the glycerol release from control tissue, taken from the same rats. Final pH was determined at the end of each incubation period. The pH fell usually by 0.2 pH unit during 60 min of incubation. In a second series of experiments, the effect of acidosis on the lipolytic effect of theophylline, ACTH, and glucagon was studied according to a protocol previously described (1). Portions (100 mg) of adipose tissue from the same rat were incubated at three different pH values (7.4, 7.0, and 6.6) for various periods of time—usually 30, 60, and 90 min.

In another series of experiments, adipose tissue was incubated first during 60 min at pH 7.4 and 6.6 without any addition of drug; the tissues were then removed from the tubes, washed twice in saline, and transferred to a fresh medium containing norepinephrine (10^{-5} M) at pH 7.4 for an additional 60-min period of incubation. Spontaneous lipolysis was measured in control samples, and glycerol was determined at the end of each period. All experiments were performed in room air in a shaker water bath at 37°. In some experiments, Ca^{2+}-free medium was used.

Bovine albumin fraction V used without further purification (free fatty acid content: 2.8 μg/g albumin) was purchased from the City Chemical Co., New York; norepinephrine bitartrate (Levophed) and isoproterenol from Winthrop Laboratories; epinephrine hydrochloride from Parke, Davis and Co.; theophylline base from Nutrition Biochemical Corp. Adrenocorticotropic hormone with an activity of 1 IU/mg was purchased from Armour Laboratories. Insulin-free glucagon was kindly supplied by Dr. W. R. Kirtley from Eli Lilly Co. (lots 60332 and 54493). For these two hormones the final concentrations of the solutions were calculated assuming a gram molecular weight of 4500 for ACTH and 3485 for glucagon. N^6-2′-*O*-Dibutyryl cyclic 3′,5′-AMP was prepared by Dr. Posternak (16). Before use the barium salt was first converted to the sodium salt. Freshly prepared stock solutions were used for all these studies.

Statistical analysis of the results was made according to the Student *t* test and *P* was evaluated for the 95% limit of confidence. The term "potentiation" in this study refers to any significant increase of glycerol production found when two drugs were combined over the addition of the two values attained when the two drugs were tested separately. Equations of the regression lines representing the rate of lipolysis (in micromoles per gram per minute) were calculated according to the method of least squares. Each line was tested for linearity (*F* test) and comparisons between the slopes were made according to standard methods (17).

As proposed by Stetten (13) and

modified by Ariëns (10) and Furchgott (11), the interaction between a drug and its receptors may be analyzed with the Michaelis-Menten equation. From this equation or its corresponding plot, three correlates were calculated: first, the maximal response (R_{max}); second, the reciprocal of the concentration of the drug that can produce half the maximal response ($\frac{1}{2} R_{max}$); and third, the apparent "affinity" (aK') of the receptor for the drug.

RESULTS

Effect of Ca^{2+}-Free Medium on Activated Lipolysis at Acid pH. Reversible Effect of Acidosis on Lipolysis

Norepinephrine activated lipolysis was the same in Ca^{2+}-free medium as in medium containing Ca^{2+}; and it was inhibited to the same extent by acidosis in Ca^{2+}-free medium and in medium containing Ca^{2+}.

Spontaneous lipolysis was not affected by acidosis, as already noted in previous experiments done in this laboratory. Furthermore, when adipose tissue first incubated for 2 hr at pH 6.6 was placed for the following hour in a medium at pH 7.4, norepinephrine-activated lipolysis proceeded at the same rate as it did in tissue incubated at pH 7.4 for 2 hr (Fig. 1). This observation indicates that the inhibition exerted by acidosis on lipolysis is reversible.

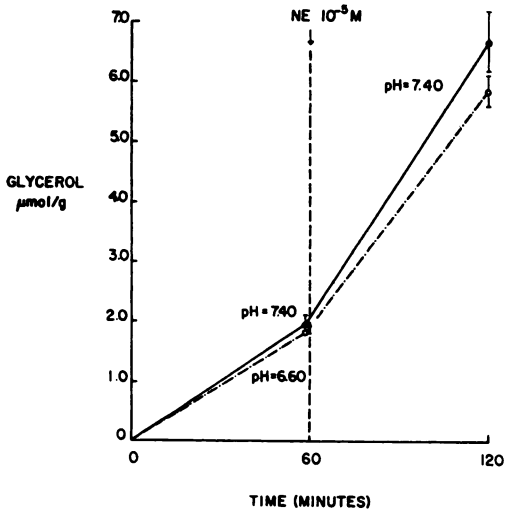

FIG. 1. *Reversible effects of acidosis on lipolysis*

Solid dots represent glycerol measurements when the fat pad was incubated at pH 7.40 for 2 hr. Open circles represent glycerol measurements when the fat pad was incubated at pH 6.6 during the first hour and at pH 7.40 during the second hour.

Effects of Acidosis and Theophylline on the Lipolytic Activity of Norepinephrine

The effect of acid pH on the log-dose response curves of norepinephrine is shown in Fig. 2. The general shape of the three curves remained similar at the different pH values studied and the autoinhibition observed with the highest concentrations of norepinephrine was not changed by acid pH. Table 1 shows the potentiation of the lipolytic activity of norepinephrine by theophylline when three different concentrations of both drugs were used at pH 7.4. The greatest potentiation occurred with the combination of the lowest concentrations of the two drugs which, when used separately, produced only small increases of glycerol release as compared with the controls. With the highest concentrations, the maximal response produced by theophylline (10^{-2} M) could not be surpassed by the addition of norepinephrine.

Effects of Norepinephrine, Theophylline, and Acidosis on the Lipolytic Activity of ACTH

The effects of ACTH on glycerol release are shown in Fig. 3. The R_{max} produced by ACTH was obtained with a dose of 10^{-1} U to 5×10^{-1} U of the preparation used in this study, which corresponds approximately to a concentration of 4.5×10^{-8} M to 9×10^{-8} M. The effects of three different concentrations of theophylline on increasing doses of ACTH are shown in Table 2. With the smallest concentration of theophylline (10^{-4} M), no difference was observed when compared to the effect of ACTH alone. With increasing doses of theophylline, a marked potentiation occurred, and there was a definite increase in the maximal response, which surpassed significantly the calculated response result-

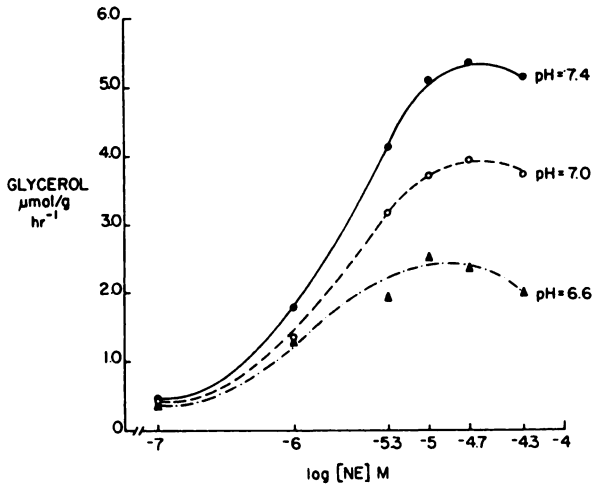

FIG. 2. *Relationship between the logarithm of the dose of norepinephrine and the lipolytic response of rat epididymal adipose tissue*

Tissue from the same rat was incubated with and without the drug. The response is expressed as increase in glycerol release produced by the drug at the end of incubation. The medium was Krebs-Ringer phosphate containing 5% albumin and no glucose titrated to the pH indicated. Each point represents the mean of 15 observations. Standard error of the mean (SEM) for each point ranged from ±0.2 to ±0.7.

ing from the addition of the effects of each drug used separately. In contrast, when ACTH and norepinephrine were combined (Table 3), the net release of glycerol was always lower than their calculated additive effect and did not surpass the maximal response reached by either drug used separately. The effects of acidosis on the rate of lipolysis induced by ACTH is shown in Fig. 4. At pH 6.6 (Fig. 4A), the lipolytic activity of this hormone was significantly inhibited (40%): 0.041 ± 0.018 μmole/g min^{-1}, as compared to the lipolytic rate at pH 7.4; 0.070 ± 0.012 μmole/g min^{-1} ($P < 0.001$). A similar inhibition was observed when theophylline was used

TABLE 1

Effect of theophylline and norepinephrine on the lipolytic response of rat adipose tissue, when the two compounds are used separately or in combination

The lipolytic response is expressed as net glycerol release (μmole/g/hr ± SEM) when the tissue was incubated with the drugs as indicated.

Concentration of drugs			Net glycerol release			
Theophylline	Norepinephrine	N[a]	Norepinephrine +theophylline combined	Total of norepinephrine +theophylline used separately	Δ[b]	P
10^{-4} M	10^{-6} M	6	3.6 ± 0.6	1.2 ± 0.3	+2.4	0.01
	10^{-5} M	6	5.4 ± 0.6	3.7 ± 0.5	+1.7	0.05
10^{-3} M	10^{-6} M	6	5.1 ± 0.5	2.6 ± 0.4	+2.5	0.01
	10^{-5} M	6	5.2 ± 0.5	4.8 ± 0.6	+0.4	—
10^{-2} M	10^{-6} M	6	5.4 ± 0.6	6.1 ± 0.7	−0.7	—
	10^{-5} M	6	5.2 ± 0.6	8.4 ± 1.0	−3.2	0.05

[a] N = Number of experiments.

[b] Difference in glycerol release of two preceding columns.

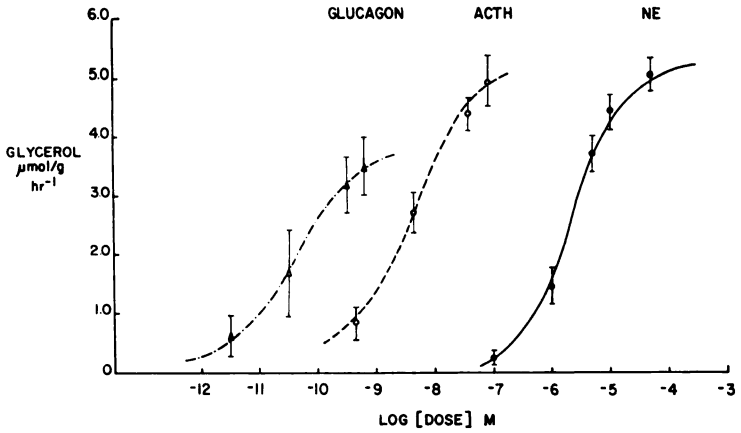

FIG. 3. *Comparison of the log-dose response curves of glucagon, ACTH, and norepinephrine at pH 7.4*
Conditions are identical to Fig. 1. Bars indicate ±SEM.

at acid pH (Fig. 4B). At pH 6.6 the rate of lipolysis induced by theophylline was 0.024 ± 0.014 μmole/g min^{-1} instead of 0.075 ± 0.012 μmole/g min^{-1} at pH 7.4. When ACTH and theophylline were combined at concentrations which resulted in a maximal response (Table 2), the lipolytic rates were the same at pH 7.4 and 6.6: 0.139 ± 0.020 μmole/g min^{-1} and 0.115 ± 0.015 μmole/g min^{-1}, respectively (Fig. 4C). Similar observations were made at pH 7.0, but in this case the inhibition was less pronounced (20%) than at pH 6.6. At pH 7.4, the lipolytic rate resulting from the combination of the two drugs was approximately equal to the addition of the rates for each individual drug, while at pH 6.6 the rate of lipolysis when the two drugs were combined, was almost twice the addition of the corresponding rates observed when either drug was used alone. When ACTH and norepinephrine were combined, the inhibitory effect of acidosis on lipolysis was not reversed: 0.058 ± 0.009 μmole/g min^{-1} at pH 7.4, and 0.019 ± 0.007 μmole/g min^{-1} at pH 6.6 (Fig. 4D).

TABLE 2
Effect of theophylline and ACTH on the lipolytic response of rat adipose tissue when the two compounds are used separately and in combination

The lipolytic response is expressed as net glycerol release (μmole/g/hr ± SEM) when the tissue was incubated with the drugs as indicated.

Concentration of drugs			Net glycerol release			
Theophylline	ACTH	N[a]	ACTH + theophylline combined	Total of ACTH + theophylline used separately	Δ[b]	P
10^{-4} M	4.5×10^{-9} M	6	1.9 ± 0.4	2.2 ± 0.7	−0.3	—
	4.5×10^{-8} M	6	4.1 ± 0.6	3.6 ± 0.7	+0.5	—
10^{-3} M	4.5×10^{-9} M	6	5.3 ± 0.7	1.9 ± 0.6	+3.4	0.02
	4.5×10^{-8} M	6	7.2 ± 0.7	3.6 ± 0.8	+3.6	0.01
10^{-2} M	4.5×10^{-9} M	6	7.4 ± 0.9	3.5 ± 0.8	+3.9	0.01
	4.5×10^{-8} M	6	8.5 ± 0.7	4.9 ± 0.8	+3.6	0.01

[a] N = Number of experiments.
[b] Difference in glycerol release of two preceding columns.

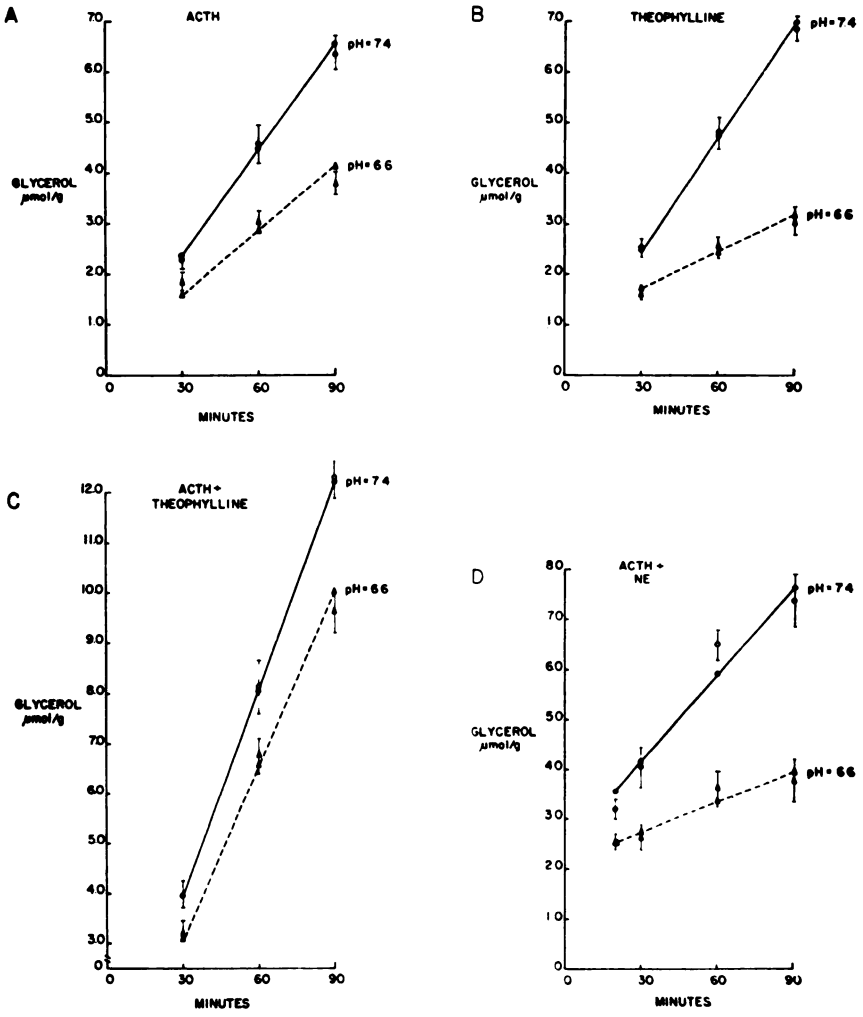

FIG. 4. *Effects of acidosis on the rate of glycerol release from rat adipose tissue incubated with ACTH, theophylline, norepinephrine (NE), or combinations of these drugs*

One sample of tissue was incubated for each period of time. The equations of the regression lines are as follows:

(A) For ACTH (4.5×10^{-8} M) ($n = 8$)
at pH 7.4, $y = 0.070x + 0.264$
at pH 6.6, $y = 0.041x + 0.431$

(B) For theophylline (10^{-2} M) ($n = 8$)
at pH 7.4, $y = 0.075x + 0.233$
at pH 6.6, $y = 0.024x + 1.028$

(C) For ACTH (4.5×10^{-9} M) plus theophylline (10^{-2} M) ($n = 8$)
at pH 7.4, $y = 0.139x - 0.200$
at pH 6.6, $y = 0.115x - 0.336$

(D) For ACTH (4.5×10^{-9} M) plus NE (10^{-5} M) ($n = 5$)
at pH 7.4, $y = 0.058x + 2.400$
at pH 6.6, $y = 0.019x + 2.190$

Solid points are calculated from the equations. Open points are the experimental points.

TABLE 3

Comparative effect of norepinephrine and ACTH on the lipolytic response of rat adipose tissue, when the two compounds are used separately or in combination

The lipolytic response is expressed as net glycerol release (μmole/g/hr ± SEM) when the tissue was incubated with the drugs as indicated.

Concentration of drugs			Net glycerol release			
Norepinephrine	ACTH	N^a	ACTH + norepinephrine combined	Total of ACTH + norepinephrine used separately	Δ^b	P
10^{-7} M	4.5×10^{-9} M	6	3.2 ± 0.3	3.5 ± 0.2	−0.3	—
	4.5×10^{-8} M	6	5.2 ± 0.3	5.2 ± 0.4	—	—
10^{-6} M	4.5×10^{-9} M	6	4.6 ± 0.4	6.7 ± 0.6	−2.1	0.01
	4.5×10^{-8} M	6	4.8 ± 0.3	8.6 ± 0.4	−3.8	0.01
10^{-5} M	4.5×10^{-9} M	6	5.7 ± 0.3	9.2 ± 0.8	−3.5	0.01
	4.5×10^{-8} M	6	5.6 ± 0.4	11.0 ± 0.7	−5.4	0.01

[a] N = Number of experiments.

[b] Difference in glycerol release of two preceding columns.

Effects of Theophylline, Norepinephrine, and Acidosis on the Lipolytic Effect of Glucagon

The log-dose response curve for glucagon is shown in Fig. 3. The maximal response was reached with a concentration of 1 μg/ml or approximately 3.5×10^{-9} M. No autoinhibition occurred when concentrations up to 2×10^{-8} M were used. The potentiation by theophylline of the lipolytic response of adipose tissue to glucagon is represented in Fig. 5. These results are

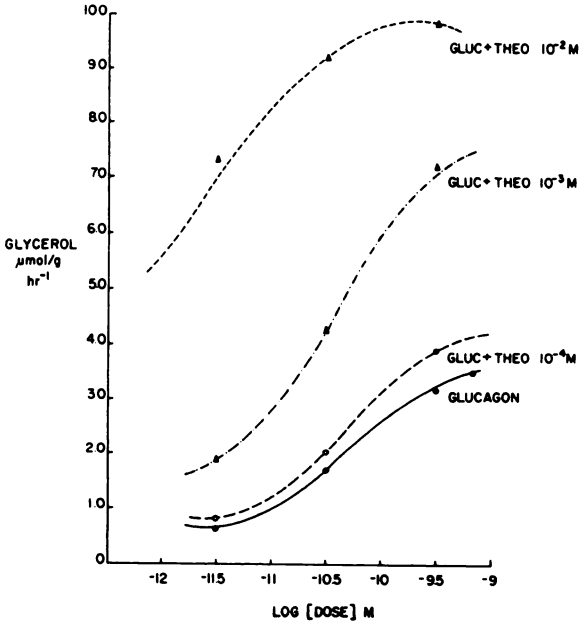

FIG. 5. *Comparison of the log-dose response curves of glucagon and glucagon combined with increasing doses of theophylline at pH 7.4*

Conditions are identical to Fig. 1.

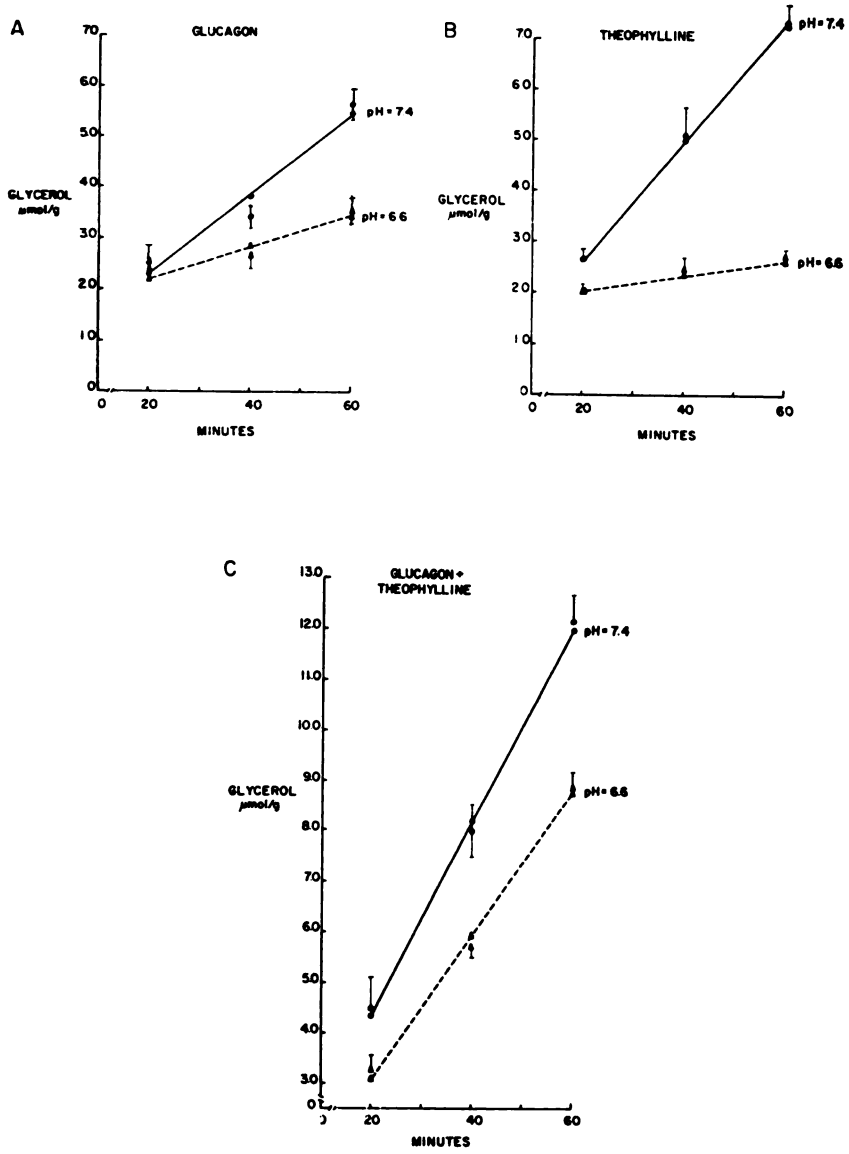

FIG. 6. *Effects of acidosis on the rate of glycerol release from rat adipose tissue incubated with glucagon, theophylline, or a combination of these drugs*

The conditions are the same as in Fig. 3. The equations of the regression lines are as follows:

(A) For glucagon (3.5×10^{-9} M) ($n = 9$)
at pH 7.4, $y = 0.080x + 0.612$
at pH 6.6, $y = 0.029x + 1.699$

(B) For theophylline (10^{-2} M) ($n = 9$)
at pH 7.4, $y = 0.116x + 0.356$
at pH 6.6, $y = 0.015x + 1.773$

(C) For glucagon (3.5×10^{-9} M) plus theophylline (10^{-2} M) ($n = 9$)
at pH 7.4, $y = 0.193x + 0.483$
at pH 6.6, $y = 0.142x + 0.267$

TABLE 4

Comparative effect of norepinephrine and glucagon on lipolytic response of rat adipose tissue when the two compounds are used separately or in combination

The lipolytic response is expressed as net glycerol release (μmole/g/hr ± SEM) when the tissue was incubated with the drugs as indicated.

Concentration of drugs			Net glycerol release			
Norepinephrine	Glucagon	N^a	Glucagon + norepinephrine combined	Total of glucagon + norepinephrine used separately	Δ^b	P
10^{-7} M	3.5×10^{-11} M	6	1.3 ± 0.3	1.5 ± 0.3	−0.2	—
	3.5×10^{-10} M	6	1.6 ± 0.2	2.4 ± 0.4	−0.8	—
10^{-6} M	3.5×10^{-11} M	6	2.7 ± 0.3	2.6 ± 0.5	+0.1	—
	3.5×10^{-10} M	6	2.7 ± 0.4	3.4 ± 0.5	−0.7	—
10^{-5} M	3.5×10^{-11} M	6	5.0 ± 0.3	6.2 ± 0.2	−1.2	0.01
	3.5×10^{-10} M	6	4.8 ± 0.4	7.0 ± 0.4	−2.2	0.01

[a] N = Number of experiments.

[b] Difference in glycerol release of two preceding columns.

comparable to those found with ACTH, and the lipolytic activity of glucagon was significantly potentiated by increasing doses of theophylline. The effects of the combination of glucagon and norepinephrine are shown in Table 4. With the lower concentration of norepinephrine, the effects were comparable to those occurring with glucagon alone. With 10^{-6} M norepinephrine, the response reached a plateau which represented approximately the additive effect of the two hormones. When the highest concentrations of the two drugs were combined, the plateau reached was equal to the response produced by norepinephrine alone.

The effects of acid pH on the lipolytic response to glucagon are reported in Fig. 6. When the pH of the incubation medium was 7.4, the rate of lipolysis was 0.080 ± 0.015 μmole of glycerol/g min^{-1} and 0.029 ± 0.012 μmole/g min^{-1} at pH 6.6, which represents an inhibition of approximately 60% ($P < 0.005$) (Fig. 6A). A similar inhibition of the lipolytic activity of theophylline occurred at pH 6.6 (Fig. 6B): 0.015 ± 0.005 μmole/g min^{-1} instead of 0.116 ± 0.016 μmole/g min^{-1} at pH 7.4, which represents an 80% inhibition. When both glucagon and theophylline were combined with concentrations which produce a maximal response (glucagon 3.5×10^{-9} M, and theophylline 10^{-2} M), the rates of lipolysis were 0.193 ± 0.025 μmole/g min^{-1} at pH 7.4 and 0.142 ± 0.015 μmole/g min^{-1} at pH 6.6 (Fig. 6C). These two rates are not significantly different. The rate of lipolysis at pH 6.6, when the two drugs are combined, is higher than a simple additive effect of the two drugs given separately.

Lipolytic Effects of Norepinephrine and Cyclic 3′,5′-AMP Dibutyrate at pH 7.4 and pH 6.6

When the sodium salt of cyclic 3′,5′-AMP was used, no definite lipolytic activity was observed. Subsequently, the dibutyryl derivative of cyclic 3′,5′-AMP (9) was tried. The dose response curve obtained with this derivative is shown in Fig. 7 and compared to the curve obtained for theophylline (pooled data found in these series and other experiments). In the range of concentrations used (10^{-4} M to 10^{-2} M), the two drugs showed similar lipolytic activities on rat adipose tissue. Table 5 summarizes the results, comparing the net glycerol release due to cyclic 3′,5′-AMP dibutyrate and norepinephrine at pH 7.4 and 6.6. Cyclic 3′,5′-AMP dibutyrate was not affected by acidosis and when it was

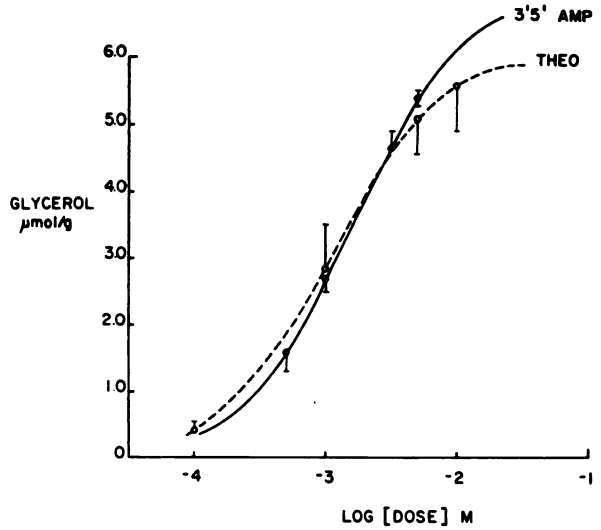

FIG. 7. *Comparison of the log-dose response curves of theophylline (n = 15) and cyclic 3',5'-AMP dibutyrate (n = 4)*

Conditons are identical to Fig. 1.

added to norepinephrine the inhibition which occurred at pH 6.6 was almost completely reversed.

The rates of lipolysis produced by these compounds fitted the different plots of the Michaelis-Menten equation. Figure 8 shows two examples which illustrate the lipolytic responses of cyclic 3',5'-AMP dibutyrate and glucagon. Table 6 summarizes the values of R_{max} and aK' constants obtained from the present data. For comparison, data obtained in this laboratory for isoproterenol and epinephrine have been added to the table.

TABLE 5

Effects of cyclic 3',5'-AMP dibutyrate, norepinephrine, and the combination of these compounds on net glycerol release at normal and acid pH

Net glycerol release is expressed in μmole/g/hr (n = 16).

	3',5'-AMP dibutyrate (10^{-3} M)	Norepinephrine (10^{-5} M)	Norepinephrine + 3',5'-AMP dibutyrate
pH 7.4	3.0 ± 0.4	5.3 ± 0.6	6.9 ± 0.8
pH 6.6	2.7 ± 0.4	3.0 ± 0.4	5.8 ± 0.7
P	NS[a]	<0.005	NS

[a] NS = not significant

DISCUSSION

In the present series of experiments, glycerol production rather than free fatty acid concentration was taken as the index of activated lipolysis (18–20). The estimation of glycerol release from rat adipose

TABLE 6

Calculated values for R_{max} and apparent affinities (aK') for the lipolytic effects of different compounds or hormones on rat adipose tissue

Compound or hormone	pH	R_{max}	aK' (×10^5)
Norepinephrine	7.4	6.10	3.8
	7.0	4.15	5.1
	6.6	2.50	8.2
Isoproterenol	7.4	5.40	38
Epinephrine	7.4	4.76	3.6
Glucagon	7.4	3.80	710,000
ACTH	7.4	5.15	4,500
Theophylline	7.4	5.88	0.005
	7.0	4.80	0.003
	6.6	3.45	0.002
Cyclic 3',5'-AMP dibutyrate	7.4	7.20	0.006

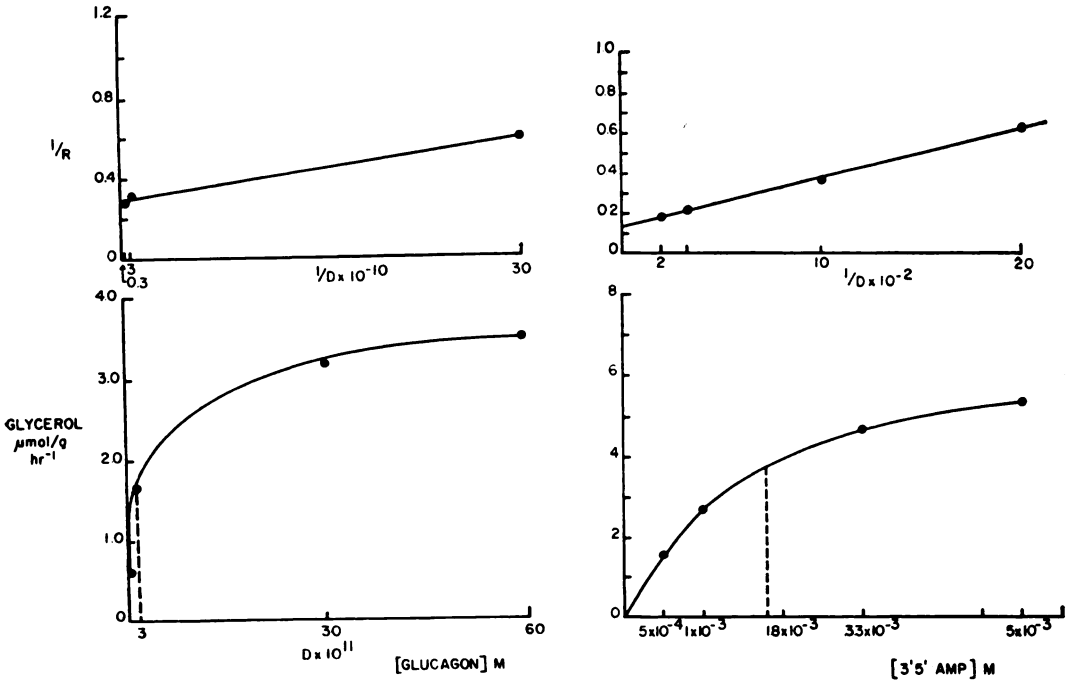

FIG. 8. *Two plots of dose response experiments on the action of glucagon and cyclic 3',5'-AMP dibutyrate* The data for glucagon are the same as in Fig. 2 and for cyclic 3',5'-AMP dibutyrate as in Fig. 6.

tissue *in vitro* (12) provides a simple and accurate model for the study of the actions of drugs, hormones, and acidosis on a "beta-type receptor" which has been postulated to trigger lipolysis through the adenyl cyclase-cyclic 3',5'-AMP system (21). All the drugs and hormones tested in this system fitted the different modifications of Stetten's model, indicating that they might act on that same system which results in glycerol release. But these agents might not necessarily act through a common "receptor." In fact, differences were found between the R_{max} and affinities of the catecholamines, ACTH, and glucagon. The R_{max} produced by ACTH is similar to that of catecholamines. Such a result, according to Ariëns and others (10, 12) may indicate that this hormone combines with the same number of receptors as catecholamines, the resulting complexes being equally active. By contrast, the lipolytic effect of glucagon, which has a lower R_{max}, appears to be different from that of ACTH. However, even the most purified glucagon preparations are not completely insulin free, and this might explain the reduced R_{max} without assuming any different mechanism of action or a different "receptor."

The similarity between the slopes of the midportion of the log-dose response of cyclic 3',5'-AMP dibutyrate and theophylline (Fig. 7) and their similar "affinity" may suggest a similar mechanism of action in the end results of these two drugs, observations which confirm other chemical and biological observations (9, 21).

The potentiation exerted by theophylline on the lipolytic actions of the three hormones studied (Tables 1 and 2, Fig. 5) confirms the concept of a two-enzyme system controlling the activation of lipolysis in rat adipose tissue (20, 22). A large body of experimental evidence supports the concept proposed by Sutherland *et al.* (21) that cyclic 3',5'-AMP plays a key role in this activation. The hormones used here are thought to activate its production through the adenyl cyclase system, while theophylline inhibits diphosphodiesterase.

If the different hormones tested activate the adenyl cyclase system directly, a similar potentiation of their effect should be expected when combined with theophylline (22). However, differences in the potentiation exerted by theophylline on the lipolytic effects of these hormones were observed. Small concentrations of theophylline potentiated norepinephrine-activated lipolysis maximally, but did not affect that of ACTH or glucagon. A marked autoinhibition occurred when norepinephrine was present at higher concentrations. By contrast, the combination of ACTH or glucagon with increasing doses of theophylline resulted in a higher R_{max} than the addition of the two separate maximal responses. This would suggest that a rate-limiting factor is present with high norepinephrine concentrations, but not with ACTH or glucagon. It has been suggested that the adenyl cyclase system might be the beta-receptor (23). However, results from this and other laboratories have pointed out the differences between the actions of known lipolytic and beta-reacting agents, either in the same or different species (6, 24–27). The present data would support the concept that different "receptors" or mechanisms may lead to the activation of the adenyl cyclase system (26).

In contrast, the effect of acid pH on the lipolytic activating system is more uniform: the lipolytic actions of these three hormones are inhibited by acidosis. The reversal of this inhibition by theophylline as previously described for norepinephrine, both *in vivo* (3) and *in vitro* (1), is also present for ACTH and glucagon. These results could suggest that a common mechanism is involved in this inhibition. The explanation first proposed for this phenomenon (1) was that acidosis inhibited the processes responsible for the production of cyclic 3′,5′-AMP: when theophylline is present, the small amount of cyclic 3′,5′-AMP still produced by norepinephrine might accumulate and lead to a normal activation of the lipase. This interpretation is substantiated by the present data and, furthermore, by the experiments in which an active derivative of cyclic 3′,5′-AMP (16), a compound which is not affected by acid pH, duplicated the lipolytic effect of theophylline. The reversal by theophylline of the inhibition produced by acidosis had already been interpreted as indicating that the hormone-sensitive lipase responsible for the triglyceride breakdown was not directly altered by this degree of extracellular acidosis (3).

This inhibitory effect of acid pH could be explained in part if acid pH hinders the formation of a "drug-receptor" complex as a result of changes in the ionization of the active sites of the receptor (28). However, the exact mechanism of the inhibitory effect of acid pH on activated lipolysis will require considerably more study.

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