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Forskolin as an Activator of Cyclic AMP Accumulation and Lipolysis in Rat Adipocytes

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SUMMARY

Forskolin increased cyclic AMP accumulation in isolated adipocytes and markedly potentiated the elevation of cyclic AMP due to isoproterenol. In adipocyte membranes, forskolin stimulated adenylate cyclase activity at concentrations of 0.1 μM or greater. Forskolin did not affect the EC_{50} for activation of adenylate cyclase but did increase the maximal effect of isoproterenol. Neither the soluble nor particulate low-K_m cyclic AMP phosphodiesterase activity was affected by forskolin. Low concentrations of forskolin (0.1–1.0 μM), which significantly elevated cyclic AMP levels, did not increase lipolysis, whereas similar increases in cyclic AMP levels due to isoproterenol elevated lipolysis. Forskolin did not inhibit the activation of triacylglycerol lipase by cyclic AMP-dependent protein kinase or the subsequent hydrolysis of triacylglycerol. Higher concentrations of forskolin (10–100 μM) did increase lipolysis. Both the increased cyclic AMP production and lipolysis due to forskolin were inhibited by the antilipolytic agents insulin and N^6-(phenylisopropyl)adenosine. Hypothyroidism reduced the ability of forskolin to stimulate cyclic AMP production and lipolysis. These results indicate that forskolin increases cyclic AMP production in adipocytes through an activation of adenylate cyclase. Lipolysis is activated by forskolin but at higher concentrations of total cyclic AMP than for catecholamines.

INTRODUCTION

Hormonal activation of adipocyte lipolysis is associated with phosphorylation of triacylglycerol lipase catalyzed by a cyclic AMP-stimulated protein kinase (1, 2). Adipocytes from hypothyroid rats exhibit a reduced cyclic AMP and lipolytic response upon stimulation with hormone (3) that appears to be due to an impaired coupling of activated hormone-receptor complexes to adenylate cyclase (4, 5). Forskolin, a diterpene derivative found in the Indian plant *Coleus forskohli*, activates adenylate cyclase in both intact cells and plasma membranes (6). The present studies were designed to determine whether forskolin activates adenylate cyclase and lipolysis in intact adipocytes and whether the defect in adenylate cyclase activation in hypothyroidism is also observed with forskolin.

MATERIALS AND METHODS

Adipocytes were isolated by collagenase treatment, according to the method of Rodbell (7), from the parametrial, omental, and epididymal adipose tissue of 175- to 225-g male or female Sprague-Dawley rats (CD strain; Charles River Breeding Laboratories, Wilmington, Mass.). Hypothyroidism was induced by maintaining rats on an iodine-deficient diet (United States Biochemical Corporation, Cleveland, Ohio) and drinking water containing propylthiouracil (6.25 mg/dl) (ICN Nutritional Biochemicals, Cleveland, Ohio) for 30 days (3). Animals had access to water and food ad libitum.

Adipocytes were incubated in Krebs-Ringer phosphate buffer containing 120 mM NaCl, 1.4 mM $CaCl_2$, 5.2 mM KCl, 1.4 mM $MgSO_4$, 10 mM Na_2HPO_4 (pH 7.4), 3% albumin, and the indicated additions. Glycerol release was determined as previously described (8). Cyclic AMP accumulation was determined by a modification of the protein kinase binding procedure (9). The free cyclic AMP was separated from the bound cyclic AMP by charcoal adsorption (10). Cyclic AMP release to the medium was determined by removing an aliquot of the medium from beneath the floating fat cells after an incubation for 4 or 30 min in a shaking water bath. The medium was added to HCl and the remainder of the sample (cells plus medium) was acidified with HCl for the determination of total cyclic AMP.

Adipocyte plasma membrane preparations were obtained from the microsomal pellet by centrifugation on a sucrose-density gradient at 900,000 × *g* min (11). Adenylate cyclase activities of these membranes were determined by a modification of the method of Cooper *et al.* (12) in a mixture containing 0.2 mM [^{32}P]ATP (50 cpm/

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pmole), 30 mM Tris-HCl (pH 7.5), 4 mM $MgCl_2$, 0.4 mM cyclic AMP, 0.1% bovine serum albumin, 10 mM creatine phosphate, creatine phosphokinase (10 units/ml), the indicated effectors, and membrane protein (5–20 μg) in a final volume of 100 μl. The assay was initiated by adding membrane protein and was carried out at 30° for 30 min. The reaction was terminated by the addition of 1 ml of 1% sodium dodecyl sulfate. ^{32}P-Labeled cyclic AMP was purified by sequential chromatography on Dowex and alumina as described by Salomon (13). ^{3}H-Labeled cyclic AMP was added as a recovery standard.

Cyclic AMP phosphodiesterase activity was determined by the procedure of Shepherd *et al.* (14). Fat cells were washed twice, resuspended in 0.25 M sucrose-10 mM Tris-HCl (pH 7.4), and disrupted in a Polytron homogenizer (Brinkmann Instruments, Westbury, N. Y.) for 5 sec at Setting 5. The preparation was centrifuged at 15,000 × *g* for 1 min. The pellet was discarded and the supernatant was centrifuged at 30,000 × *g* for 30 min at 4°. The fat cake was removed. The supernatant was used for studies on soluble phosphodiesterase activity, and the pellet was resuspended in 0.4 ml of the homogenization medium for use in studies on the particulate phosphodiesterase activity. The phosphodiesterase activity was assayed in the presence of 0.125 μM cyclic AMP by a modification of the procedure of Thompson *et al.* (15). The pH 5.2 triacylglycerol lipase was isolated from rat fatpads by the procedure of Khoo *et al.* (16) and assayed as described by Shepherd *et al.* (14). Protein was determined by the dye-binding procedure of Bio-Rad Laboratories (Richmond, Calif.).

(−)-Isoproterenol, snake venom (*Ophiophagus hannah*), and triolein were obtained from Sigma Chemical Company (St. Louis, Mo.), insulin from Eli Lilly (Indianapolis, Ind.), PIA[1] from Boehringer Mannheim (Indianapolis, Ind.), forskolin (7β-acetoxy-8,13 epoxy-1α,6β,9α-trihydroxy-labd-14-en-11-one) from Calbiochem, crude collagenase (*Clostridium histolyticum*, Lot 4177 CLS11 40C190) from Worthington Biochemicals (Freehold, N. J.), bovine serum albumin (Fraction V, Lot T 13705) from Armour Pharmaceutical Company (Chicago, Ill.), and enzymes for glycerol determinations from Boehringer Mannheim. Carboxyl-labeled [^{14}C]triolein, [α-^{32}P]ATP, and ^{3}H-labeled cyclic AMP were obtained from New England Nuclear Corporation (Boston, Mass.). Dowex AG 50-WX4 (200–400 mesh) and neutral alumina AG7 (100–200 mesh) for adenylate cyclase assay were obtained from Bio-Rad Laboratories. Dowex 1-×8 (200–400 mesh) for cyclic AMP phosphodiesterase assay was obtained from Sigma Chemical Company. Forskolin was dissolved in 95% ethanol at a concentration of 15 mM. This solution was diluted with water to give the desired concentration. Controls contained the same amount of added alcohol.

RESULTS

Forskolin activation of cyclic AMP accumulation and lipolysis. Forskolin increases cyclic AMP production by rat cerebral cortical slices (6). In isolated rat adipocytes, the addition of 1 μM forskolin doubled cyclic AMP accumulation after only 1 min (Fig. 1). The time course for forskolin stimulation appeared similar to that for 0.1 μM isoproterenol with an initial rise at 1 min followed by a slight decrease from the maximal cyclic AMP response over a 30-min incubation. The elevation of cyclic AMP due to isoproterenol (0.1 μM) was potentiated 10-fold by forskolin.

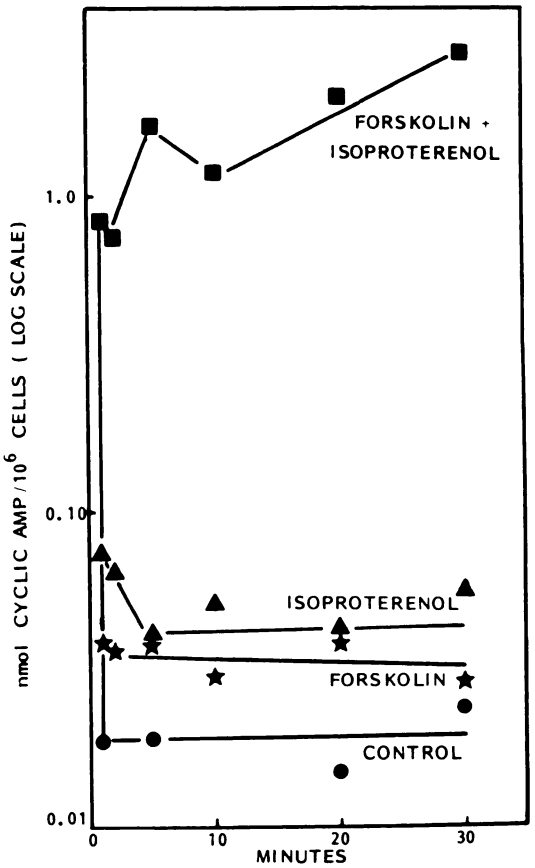

FIG. 1. *Time course for stimulation of cyclic AMP accumulation by forskolin*

Adipocytes (2.4 × 10^5 cells) were incubated for the indicated times without (●) or with 1 μM forskolin (★), and 0.1 μM isoproterenol (▲) or isoproterenol plus forskolin (■). The values are means of six experiments. The increases in cyclic AMP due to isoproterenol were significant at all time points, as were the increases due to forskolin alone at all points except 30 min ($p = 0.05$ by paired comparisons).

As shown in Fig. 2a, forskolin had two major effects on the dose-response curve for isoproterenol stimulation of cyclic AMP accumulation measured at 3 min. Forskolin markedly increased the amount of cyclic AMP accumulated in response to a given isoproterenol concentration. In addition, forskolin enhanced the potency of isoproterenol. Forskolin alone produced only a slight increase in cyclic AMP production in this experiment. However, in other experiments, 10 μM forskolin was a much more potent stimulator of cyclic AMP accumulation (Fig. 3).

Maximal cyclic AMP accumulation due to hormones is obtained in the presence of adenosine deaminase and methylxanthine. Adenosine deaminase degrades adenosine, which is secreted by adipocytes and which is an inhibitor of adenylate cyclase. Methylxanthines block adenosine interaction with adenylate cyclase and also inhibit cyclic AMP phosphodiesterase (1). The results in Fig. 2b demonstrate that the increases in cyclic AMP

[1] The abbreviation used is: PIA, N^6-(phenylisopropyl)adenosine.

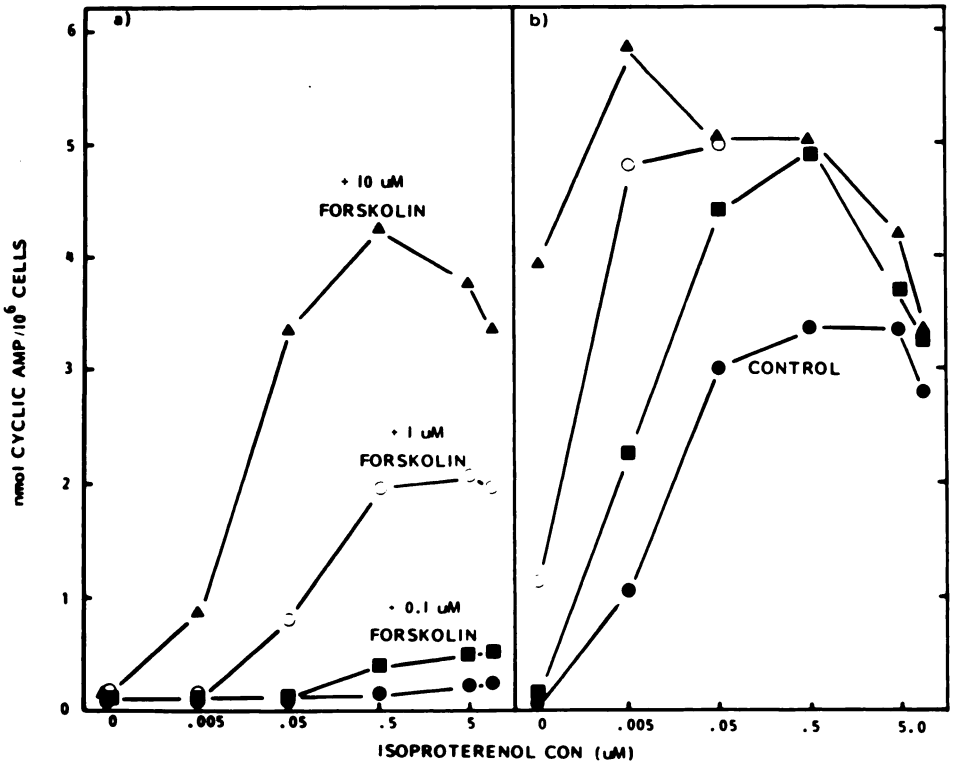

FIG. 2. *Effect of forskolin on the dose-dependent stimulation of cyclic AMP accumulation by isoproterenol*

Adipocytes (2.4×10^5) were incubated for 3 min with the indicated isoproterenol concentrations without (●) plus 0.1 μM forskolin (■), 1 μM forskolin (○), or 10 μM forskolin (▲). a, Adipocytes incubated without adenosine deaminase and theophylline; b, adipocytes incubated with adenosine deaminase (1 μg/ml) and 100 μM theophylline.

accumulation due to isoproterenol and forskolin were much greater in the presence of adenosine deaminase and theophylline. Forskolin potentiated the effects of low concentrations of isoproterenol under all conditions. However, at isoproterenol concentrations of 5 or 10 μM, cyclic AMP production was actually less in the presence of 10 μM forskolin (Fig. 2).

Lipolysis is stimulated by agents which elevate cyclic AMP (1). The dose-response curve for forskolin (1–100 μM) activation of cyclic AMP accumulation and lipolysis in adipocytes is shown in Fig. 3. Although 1 μM forskolin increased cyclic AMP accumulation more than 4-fold in these experiments at 30 min, it had little effect on lipolysis. In contrast, 0.1 μM isoproterenol, while having the same effect on cyclic AMP accumulation, gave a maximal activation of lipolysis. However, an activation of lipolysis as well as a large increase in cyclic AMP was obtained with higher forskolin concentrations (10–100 μM).

The relationship between cyclic AMP accumulation and lipolysis was further examined in the studies shown in Table 1 with measurements of cyclic AMP after 3 and 30 min of incubation and of lipolysis after 30 min. A low concentration of isoproterenol (0.033 μM) activated lipolysis without appreciably affecting total cyclic AMP accumulation at either 3 or 30 min. In contrast, 1 μM forskolin increased cyclic AMP accumulation at 30 min but did not affect lipolysis (Table 1).

Inhibition by antilipolytic agents of forskolin action. The effect of antilipolytic agents on the regulation of cyclic AMP accumulation and lipolysis by forskolin was examined. In adipocytes, adenosine (12, 17) and PIA are potent inhibitors of cyclic AMP production with PIA being considerably more potent than adenosine (18, 19). As shown in Table 2, PIA did not reduce basal cyclic AMP or glycerol release but did inhibit the rise in cyclic AMP due to 10 μM forskolin (73% inhibition), theophylline plus forskolin (78% inhibition), or 1.0 μM isoproterenol (91% inhibition). Glycerol release was also inhibited by PIA. However, an inhibitory effect of PIA on cyclic AMP accumulation greater than that on glycerol release was observed in most cases. Thus, the glycerol release due to 10 μM forskolin was inhibited by 40%, theophylline plus forskolin (92% inhibition), and 0.1 μM isoproterenol (50% inhibition) by PIA. No inhibition of glycerol release was observed in the presence of 0.1 μM isoproterenol plus forskolin. Insulin had effects similar to those of the adenosine analogue on both cyclic AMP and glycerol release (Table 2).

Effect of hypothyroidism on forskolin action. In hypothyroid rats, an impaired interaction of the guanyl nucleotide regulatory protein with the receptor and/or adenylate cyclase (3–5) appears to result in a reduced cyclic AMP production and glycerol release in response to isoproterenol (1, 3). As shown in Fig. 4, the degree of forskolin stimulation and forskolin potentiation of isoproterenol action was also reduced in adipocytes from hypothyroid rats. These results indicate that, although forskolin stimulates adenylate cyclase and potentiates the effects of isoproterenol on cyclase activity in both hypothyroid and euthyroid states, forskolin does not restore the catalytic activity of adenylate cyclase in the hypothyroid state to values observed with euthyroid controls.

Glycerol release due to 0.1 μM isoproterenol or 10 μM

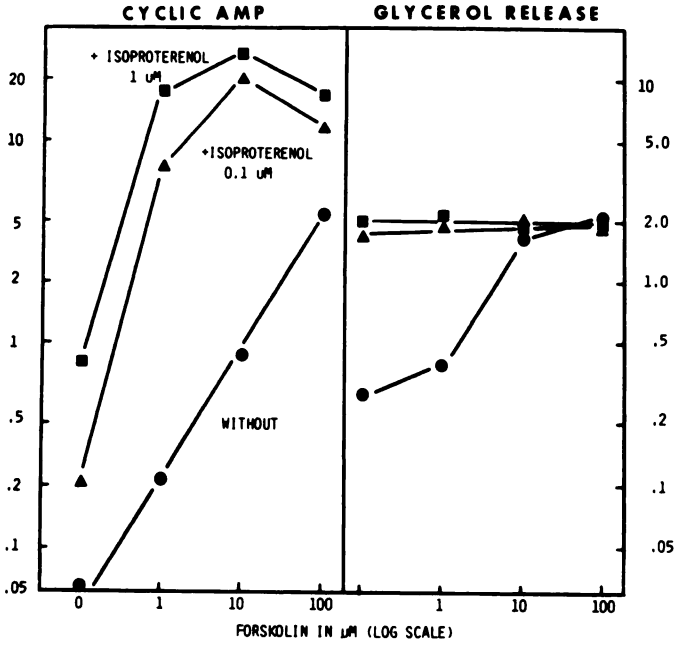

FIG. 3. *Stimulation of lipolysis and cyclic AMP accumulation by forskolin in adipocytes*

Adipocytes (2.5×10^5) were incubated for 30 min without (●) plus 0.1 μM isoproterenol (▲) or with 1 μM isoproterenol (■) plus the indicated concentrations of forskolin. The data are the average of two experiments. The values for cyclic AMP are in nanomoles per 10^6 cells and for glycerol release in micromoles per 10^6 cells, plotted on a logarithmic scale.

forskolin was maximally activated in adipocytes from euthyroid rats (Fig. 4). In contrast, the stimulation of glycerol release due to forskolin or isoproterenol was reduced in adipocytes from hypothyroid rats. The combination of 1 μM isoproterenol and forskolin (1 or 10 μM) restored the glycerol response to values observed in the euthyroid state, indicating, as has been observed before (3), that the maximal lipolytic response is not affected by hypothyroidism. Further elevations in cyclic AMP levels by higher isoproterenol and forskolin concentrations, although reduced in hypothyroidism, do not appear to be involved in the regulation of lipolysis.

Effect of forskolin on triacylglycerol lipase activity. The inability of forskolin to activate lipolysis under conditions where cyclic AMP production appeared adequate suggested that forskolin might inhibit the basal triacylglycerol lipase activity or its activation by a cyclic AMP-dependent protein kinase. The triacylglycerol lipase activity of the pH 5.2-precipitable fraction is a partially purified triacylglycerol lipase which can be activated by the addition of cyclic AMP or ATP (2). Forskolin (10 μM) had no significant effect on either basal or cyclic AMP-activated triacylglycerol lipase activity (3 ± 4% inhibition of basal activity and 8 ± 14% inhibition of the activated enzyme as the mean ± standard error of three experiments). In these experiments, forskolin was present only during the period in which triacylglycerol lipase activity was assayed. In another series of experiments, the effect of forskolin on cyclic AMP-dependent activation of triacylglycerol lipase activity was examined. Forskolin at 1, 10, or 20 μM had no effect on the activation of triacylglycerol lipase by cyclic AMP in the presence of ATP (Table 3). These results indicate that neither the activity of the triacylglycerol lipase nor its activation by the cascade reaction involving the cyclic AMP-dependent kinase and phosphorylation of lipase is inhibited by forskolin.

TABLE 1

Forskolin stimulation of cyclic AMP accumulation at 3 and 30 min

Adipocytes (2.3×10^5 cells) were incubated for 3 or 30 min with the additions indicated. The values are the means of five paired replications. The significance of changes from the controls was determined by the Student's *t*-test on the logarithms of the paired differences.

Addition	Forskolin concentration				
	0	0.033 μM	0.1 μM	1.0 μM	10 μM
	Cyclic AMP at 3 min				
	pmoles/10^6 cells				
None	34	36	27	33	118[a]
Isoproterenol, 0.01 μM	32	40	37	118[b]	728[b]
Isoproterenol, 0.033 μM	35	36	54[a]	333[b]	—
Isoproterenol, 0.10 μM	93[b]	120[b]	183[b]	851[b]	—
	Cyclic AMP at 30 min				
	pmoles/10^6 cells				
None	25	35	24	58[a]	743[b]
Isoproterenol, 0.01 μM	28	31	30	169[b]	1625[b]
Isoproterenol, 0.033 μM	37	49[a]	58	729[b]	—
Isoproterenol, 0.10 μM	54[b]	126[b]	289[b]	1982[b]	—
	Glycerol release at 30 min				
	μmoles/10^6 cells				
None	0.06	0.01	0.03	0.09	0.63[b]
Isoproterenol, 0.01 μM	0.10	0.15	0.26[a]	0.49[a]	0.63[b]
Isoproterenol, 0.033 μM	0.28[a]	0.39[a]	0.46[a]	0.62[b]	—
Isoproterenol, 0.10 μM	0.47[a]	0.56[b]	0.61[b]	0.66[b]	—

[a] $p = 0.05$.
[b] $p = 0.01$.

TABLE 2

Inhibition of forskolin action by insulin and PIA

Adipocytes (2.0×10^5) were incubated for 30 min without PIA, with 1 μM PIA, or with insulin (240 μU/ml) and the indicated additions. The values are the means of five paired replications.

Addition	Cyclic AMP			Glycerol release		
	Control	PIA	Insulin	Control	PIA	Insulin
	pmoles/10^6 cells			*μmoles/10^6 cells*		
None	24	32	28	0.04	0.04	0.03
Forskolin, 1 μM	48	54	39	0.05	0.06	0.07
Forskolin, 10 μM	2220	590	275	0.32	0.19	0.26
Theophylline, 100 μM	26	37	21	0.11	0.01	0.05
Theophylline, 100 μM, + forskolin, 1 μM	193	42	135	0.63	0.05	0.36
Isoproterenol, 0.1 μM	77	36	35	0.44	0.22	0.32
Isoproterenol, 1 μM	1055	86	65	0.60	0.47	0.64
Isoproterenol, 0.1 μM, + forskolin, 1 μM	2320	430	830	0.60	0.69	0.65

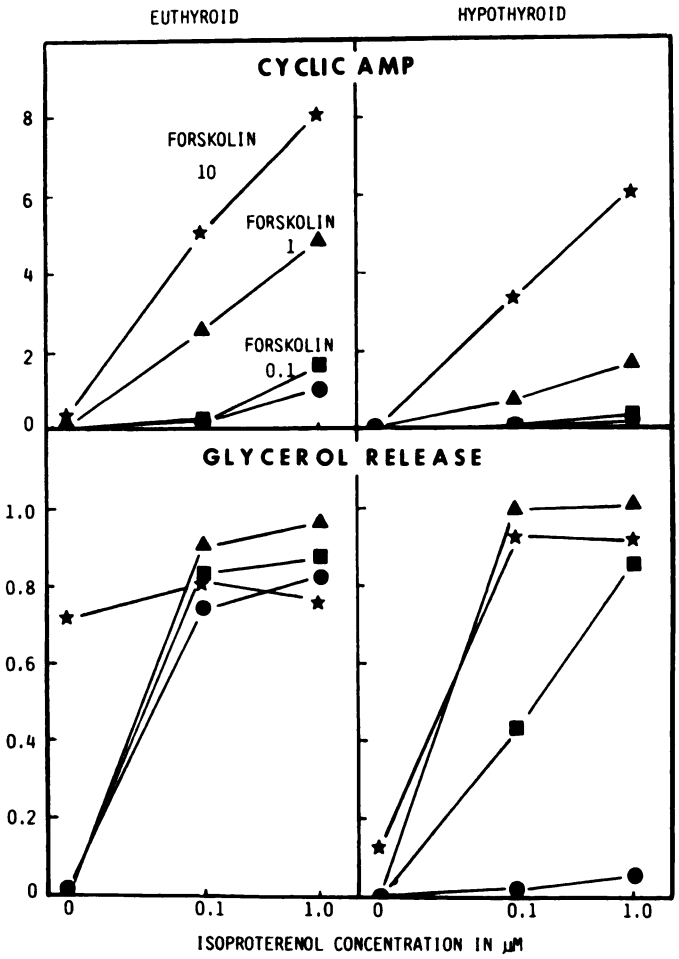

FIG. 4. *Forskolin effects on lipolysis and cyclic AMP accumulation in adipocytes from euthyroid and hypothyroid rats*

Adipocytes from euthyroid (1.9×10^5 cells) or hypothyroid rats (2.0×10^5) were incubated for 30 min without or with the indicated concentrations of isoproterenol. The values are the means of the values of three experiments using adipocytes from hypothyroid rats and five experiments using adipocytes from euthyroid rats. Cyclic AMP accumulation is expressed in nanomoles per 10^6 cells and that in the absence of forskolin is depicted by (●). Values for cyclic AMP accumulation in the presence of 0.1 μM forskolin (■) and the absence of isoproterenol were 0.08 and 0.02 nmole/10^6 cells, respectively, in adipocytes from euthyroid and hypothyroid rats. Values for cyclic AMP accumulation due to 1 μM forskolin (▲) were 0.09 and 0.02 nmole/10^6 cells; those in the presence of 10 μM forskolin (★) were 0.36 and 0.12 nmole/10^6 cells, respectively, in adipocytes from euthyroid and hypothyroid rats incubated in the absence of isoproterenol. Glycerol release is shown in micromoles per 10^6 cells.

Studies on cyclic AMP efflux. The efflux of cyclic AMP from adipocytes was examined to determine whether the forskolin stimulated secretion of cyclic AMP into the medium. After a 4-min incubation in the presence of 100 μM theophylline and adenosine deaminase (0.5 μg/ml) to maximize cyclic AMP production, there was a large accumulation of cyclic AMP (1500 pmoles/10^6 cells) in response to stimulation by 0.1–10 μM isoproterenol, 10–100 μM forskolin, or the combination of forskolin plus isoproterenol. Approximately 9% of the total cyclic AMP was released to the medium under these conditions. After 30 min, at least 57% of the cyclic AMP had been secreted into the medium. In no instance did forskolin enhance the movement of cyclic AMP into the extracellular compartment.

TABLE 3

Failure of forskolin to affect triacylglycerol lipase activation by cyclic AMP

The pH 5.2 precipitate from rat parametrial adipose tissue was incubated for 10 min without or with 1, 10, or 20 μM forskolin in the presence of 0.5 mM ATP and the indicated concentrations of cyclic AMP in 200 μl of activation buffer (5 mM $MgCl_2$, 50 mM Tris-HCl, 1 mM dithiothreitol, 0.5 mM ethylene glycol bis (β-aminoethyl ether)*N,N,N′,N′*-tetraacetic acid, and 1 mM theophylline). The activation buffer was diluted to 0.9 ml with a substrate mixture containing 0.07 μmole of [^{14}C]triolein (99.7 mCi/mmole) plus 0.1 μmole of albumin and was incubated for 30 min. The results are the means of four paired replications using separate preparations of the enzyme. The increases in lipase activity due to cyclic AMP are shown as means ± standard error of the paired differences.

Forskolin concentration	Basal activity	Concentration of cyclic AMP			
		0.33 μM	1 μM	3.3 μM	10 μM
μM	*nmoles/mg*	*% increase in lipase activation*			
0	24	30 ± 10	45 ± 8	36 ± 8	46 ± 14
1	28	17 ± 10	30 ± 6	29 ± 9	44 ± 21
10	25	21 ± 4	23 ± 3	30 ± 5	30 ± 2
20	24	45 ± 15	42 ± 8	46 ± 6	60 ± 28

Forskolin effects on cyclic AMP phosphodiesterase activity. The marked potentiation of the catecholamine response in adipocytes by forskolin might involve cyclic AMP phosphodiesterase inhibition, since the effects of forskolin appear to be similar to those of methylxanthines. However, the activity of the low-K_m cyclic AMP phosphodiesterase was unaffected by forskolin (Table 4).

TABLE 4

Cyclic AMP phosphodiesterase activity and forskolin

Adipocytes were homogenized, and the supernatant obtained after low-speed centrifugation (15,000 × *g* for 1 min) was centrifuged at 30,000 × *g* for 30 min. The cyclic AMP phosphodiesterase activity of the supernatant (soluble) and the pellet (particulate) from this centrifugation was determined by incubation of approximately 30 μg of protein in each preparation for 10 min in the presence of 0.125 μM cyclic AMP with the indicated additions. The values are means ± standard error of three experiments. The particulate preparation hydrolyzed approximately 37% of the added cyclic AMP, and the soluble preparation (supernatant) hydrolyzed 52% of the added cyclic AMP in the absence of any additions.

Addition	Cyclic AMP phosphodiesterase activity	
	Soluble enzyme	Particulate enzyme
	% inhibition by added agents	
Theophylline, 3.3 μM	5.4 ± 1.0	7.5 ± 1.4
Theophylline, 10 μM	9.9 ± 1.8	10.3 ± 0.8
Theophylline, 100 μM	38.5 ± 3.8	46.4 ± 3.2
Theophylline, 250 μM	59.0 ± 19.7	67.0 ± 2.3
Theophylline, 100 μM + forskolin, 1 μM	40.8 ± 2.1	48.6 ± 1.1
Theophylline, 100 μM + forskolin, 10 μM	39.3 ± 2.9	49.4 ± 2.3
Forskolin, 0.1 μM	3.3 ± 1.2	2.4 ± 2.6
Forskolin, 1 μM	3.4 ± 1.1	4.5 ± 3.4
Forskolin, 10 μM	4.2 ± 2.1	5.6 ± 3.2
Forskolin, 100 μM	3.2 ± 1.1	1.5 ± 2.4

In contrast, theophylline, a phosphodiesterase inhibitor, produced a concentration-dependent inhibition of both the soluble and particulate low-K_m phosphodiesterase activity. Forskolin, in combination with theophylline, also did not inhibit phosphodiesterase activity. These results indicated that the stimulation of cyclic AMP accumulation by forskolin in intact cells was not due to an inhibition of cyclic AMP degradation.

Forskolin stimulation of adenylate cyclase. Forskolin directly activated adipocyte adenylate cyclase (Fig. 5; Table 5). Forskolin (0.1–10 μM) increased adenylate cyclase activity of adipocyte plasma membranes in the absence or presence of 1 μM GTP (Table 5). Even in the presence of saturating levels of guanine nucleotides [100 μM guanosine 5′-*O*-(3-thiotriphosphate)], forskolin produced a further stimulation of adenylate cyclase (Fig. 5). The addition of 100 μM forskolin in the experiments shown in Fig. 5 increased cyclase activity to a level comparable to the combination of 10 μM forskolin and 1 μM isoproterenol. The EC_{50} for activation of cyclase by forskolin was only 1 μM in adipocyte membranes in the presence of 100 μM guanosine 5′-*O*-(3-thiotriphosphate).

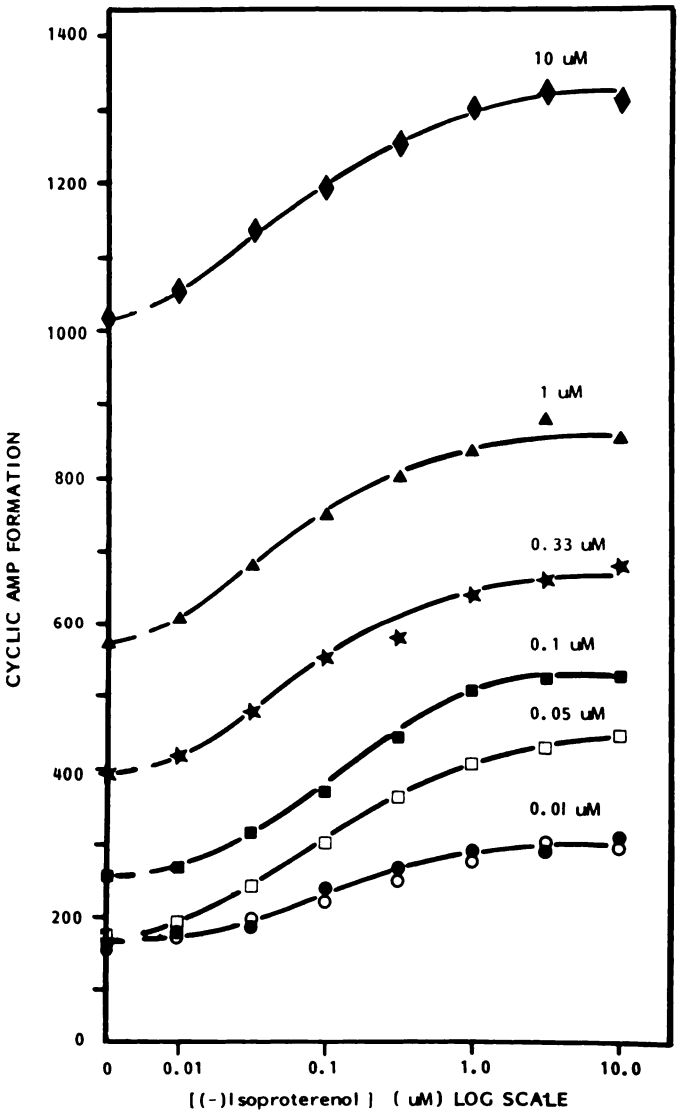

FIG. 5. *Forskolin activation of adipocyte adenylate cyclase*

Fat cell membranes were assayed for adenylate cyclase activity in the presence of 100 μM guanosine 5′-*O*-(3-thiotriphosphate) and the indicated concentrations of (−)-isoproterenol alone (○) or forskolin: 0.01 μM (●), 0.05 μM (□), 0.10 μM (■), 0.33 μM (★), 1 μM (▲), or 10 μM (♦). The data are the means of three determinations and are expressed as picomoles of cyclic AMP formed per milligram of protein per minute. The standard deviations in each experiment are not shown since they were smaller than the symbols. The experiment was replicated three times on separate membrane preparations with similar results.

TABLE 5

Effects of forskolin on (−)-isoproterenol and GTP activation of adipocyte membrane adenylate cyclase

Fat cell membranes were incubated for 30 min with the indicated additions. The data are means ± standard deviation of three determintions and are expressed as picomoles of cyclic AMP formed per milligram of protein per minute.

Isoproterenol concentration	GTP concentration	−Forskolin	+Forskolin		
			0.1 μM	1 μM	10 μM
μM	μM	*pmoles cyclic AMP/mg/min*			
0	0	12 ± 1	32 ± 1	110 ± 2	328 ± 3
0.1	0	13 ± 1	38 ± 3	124 ± 1	356 ± 1
1.0	0	16 ± 0	45 ± 1	143 ± 1	374 ± 3
0	1	15 ± 1	38 ± 1	119 ± 1	312 ± 2
0.1	1	23 ± 1	60 ± 3	159 ± 4	354 ± 6
1.0	1	41 ± 1	100 ± 1	226 ± 6	426 ± 3
0	100	6 ± 1	13 ± 1	34 ± 1	86 ± 1
0.1	100	8 ± 1	16 ± 1	43 ± 1	96 ± 2
1.0	100	12 ± 1	24 ± 1	60 ± 2	118 ± 2

GTP has a biphasic effect on adipocyte adenylate cyclase with stimulation of cyclase at low GTP concentrations and inhibition at high GTP concentrations (12). Despite the inhibition of adenylate cyclase activity by high GTP concentrations (100 μM), forskolin still activated adenylate cyclase (Table 5).

In contrast to the results seen on cyclic AMP accumulation by intact adipocytes (Table 1), forskolin did not affect the EC_{50} for activation of adenylate cyclase by isoproterenol (Fig. 5). However, a low concentration of forskolin (0.05 μM), which had no effect on basal adenylate cyclase activity, increased the maximal activation due to isoproterenol (Table 5). These data indicate that forskolin is a direct activator of adenylate cyclase which does not require the presence of hormone or added guanine nucleotides. In membranes, forskolin augments the maximal effect of isoproterenol, whereas in intact adipocytes forskolin increases the maximal response to hormone and also increases the potency of the hormone.

DISCUSSION

Forskolin produced a rapid increase in cyclic AMP production in adipocytes within 1 min. This effect of forskolin is in sharp contrast to cholera toxin, which activates cyclic AMP production in adipocytes after 1–2 hr (20, 21). The stimulated cyclic AMP production appears to be through a direct activation of adenylate cyclase and not through an effect on cyclic AMP degradation or egress of cyclic AMP to the medium.

It has been proposed that forskolin directly activates adenylate cyclase and facilitates the activation of adenylate cyclase activity by the guanyl nucleotide regulatory protein (6). In intact adipocytes, forskolin stimulated

cyclic AMP production and augmented the increases due to isoproterenol. Forskolin increased the potency and the magnitude of the response to isoproterenol (Fig. 2). In adipocyte membranes, 0.05 μM forskolin increased the maximal stimulation of adenylate cyclase due to isoproterenol without producing an increase in basal activity. Higher forskolin concentrations increased both the basal activity and the response to a maximal stimulation by isoproterenol. However, in isolated membranes, no enhancement of the EC_{50} for isoproterenol activation of adenylate cyclase was observed.

The direct stimulation of adenylate cyclase by forskolin is similar to the effects obtained with NaF and guanine nucleotides. However, no increase in cyclic AMP accumulation in intact cells is produced by these agents, probably because of the low permeability of intact cells to these compounds. In contrast, forskolin readily increases cyclic AMP accumulation of intact cells, suggesting that forskolin may readily penetrate the membrane.

Since forskolin potentiated isoproterenol stimulation of cyclic AMP accumulation in intact adipocytes, it may also bind to a site involved in the interaction of the hormone-receptor complex with catalytic cyclase. The synergism between forskolin and isoproterenol stimulation of cyclic AMP accumulation is striking. Furthermore, the interaction of forskolin with adipocytes appeared to be relatively selective, since a concentration as low as 0.033 μM forskolin potentiated the action of an equivalent concentration of isoproterenol (Table 3). This may indicate that forskolin interacts with those proteins involved in the activation of adenylate cyclase that are affected by the catecholamine-receptor complex.

In adipocytes, forskolin stimulation of both cyclic AMP and lipolysis was reduced by the antilipolytic agents adenosine and insulin, indicating that the action of forskolin is modulated by known regulators of adenylate cyclase and lipolysis. Forskolin action was potentiated by adenosine deaminase and theophylline (Fig. 2). The inability of forskolin to restore the cyclic AMP response of adipocytes from hypothyroid rats to euthyroid values is consistent with current views that hypothyroidism modulates adenylate cyclase activity through a regulatory component in the membrane (5). Although a major effect of forskolin on intact adipocytes may be facilitation of interaction of the hormone-receptor complex with the guanyl nucleotide complex and adenylate cyclase, forskolin cannot correct the impaired coupling between the guanyl nucleotide and cyclase that is thought to be present in hypothyroidism.

The present findings suggest that lipolysis may be determined by small local changes in total cyclic AMP. Recent studies on heart cells indicate that the action of hormones may be a consequence of a selective activation of cyclic AMP-dependent protein kinases. Hayes *et al.* (22) found that prostaglandin E_1 elevated cyclic AMP without inducing contraction, whereas isoproterenol increased cardiac contractibility as well as cyclic AMP. Our results with forskolin are comparable, since forskolin elevates total cyclic AMP levels without stimulating lipolysis while the converse is true with isoproterenol.

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REFERENCES

1. Fain, J. N. Hormonal regulation of lipid mobilization from adipose tissue, in *Biochemical Actions of Hormones* (G. Litwack, ed.), Vol. 7. Academic Press, New York, 119–204 (1980).
2. Corbin, J. D., and E. G. Krebs. A cyclic AMP stimulated protein kinase in adipose tissue. *Biochem. Biophys. Res. Commun.* **36**:328–336 (1969).
3. Malbon, C. C., F. J. Moreno, R. J. Cabelli, and J. N. Fain. Fat cell adenylate cyclase and β-adrenergic receptors in altered thyroid states. *J. Biol. Chem.* **253**:671–678 (1978).
4. Malbon, C. C. The effects of thyroid status on the modulation of fat cell β-adrenergic receptor agonist affinity by guanine nucleotides. *Mol. Pharmacol.* **18**:193–198 (1980).
5. Fain, J. N. Catecholamine-thyroid hormone interactions in liver and adipose tissue. *Life Sci.* **28**:1745–1754 (1981).
6. Seamon, K. B., W. Padgett, and J. W. Daly. Forskolin: a unique diterpene activator of adenylate cyclase in membranes and intact cells. *Proc. Natl. Acad. Sci. U. S. A.* **78**:3363–3367 (1981).
7. Rodbell, M. Metabolism of isolated fat cells. I. Effects of hormones on glucose metabolism and lipolysis. *J. Biol. Chem.* **239**:375–380 (1964).
8. Fain, J. N., M. P. Czech, and R. Saperstein. Lipolysis, in *Methods in Investigative and Diagnostic Endocrinology, Vol. 2A: Peptide Hormones* (S. A. Berson and R. S. Yalow, eds.). North-Holland, Amsterdam, 267–273 (1973).
9. Gilman, A. G. Protein binding assay for adenosine 3′:5′-cyclic monophosphate. *Proc. Natl. Acad. Sci. U. S. A.* **67**:305–312 (1970).
10. Brown, B. L., J. D. Albano, R. P. Ekins, A. M. Sgherzi, and W. Tampion. A simple sensitive saturation assay method for the measurement of adenosine 3′:5′-cyclic monophosphate. *Biochem. J.* **121**:561–562 (1971).
11. McKeel, D. W., and L. Jarrett. Preparation and characterization of a plasma membrane fraction from isolated fat cells. *J. Cell Biol.* **44**:417–432 (1970).
12. Cooper, D. M. F., W. Schlegel, M. C. Lin, and M. Rodbell. The fat cell adenylate cyclase system: characterization and manipulation of its bimodal regulation by GTP. *J. Biol. Chem.* **254**:8927–8931 (1979).
13. Salomon, Y. Adenylate cyclase assay. *Adv. Cyclic Nucleotide Res.* **10**:35–55 (1979).
14. Shepherd, R. E., C. C. Malbon, C. Smith, and J. N. Fain. Lipolysis and adenosine 3′:5′-monophosphate metabolism in isolated white fat cells from genetically obese hyperglycemic mice (ob/ob). *J. Biol. Chem.* **252**:7243–7248 (1977).
15. Thompson, W. J., W. L. Terasaki, P. M. Epstein, and S. J. Strada. Assay of cyclic nucleotide phosphodiesterase and resolution of multiple molecular forms of the enzyme. *Adv. Cyclic Nucleotide Res.* **10**:69–92 (1979).
16. Khoo, J. C., D. Steinberg, J. J. Huang, and P. R. Vagelos. Triglyceride, diglyceride, monoglyceride, and cholesterol ester hydrolases in chicken adipose tissue activated by adenosine 3′:5′-monophosphate-dependent protein kinase. *J. Biol. Chem.* **251**:2882–2890 (1976).
17. Fain, J. N., R. H. Pointer, and W. F. Ward. Effects of adenosine nucleosides on adenylate cyclase phosphodiesterase, cyclic AMP accumulation and lipolysis in fat cells. *J. Biol. Chem.* **247**:6866–6872 (1972).
18. Fain, J. N., and C. C. Malbon. Regulation of adenylate cyclase by adenosine. *Mol. Cell. Biochem.* **25**:146–169 (1979).
19. Fain, J. N. Inhibition of adenosine cyclic 3′:5′-monophosphate accumulation in fat cells by adenosine, N^6-(phenylisopropyl)adenosine and related compounds. *Mol. Pharmacol.* **9**:595–604 (1973).
20. Vaughan, M., N. F. Pierce, and W. B. Greenough, III. Stimulation of glycerol production in fat cells by cholera toxin. *Nature (Lond.)* **226**:658–659 (1970).
21. Manganiello, V. C., C. J. Lovell-Smith, and M. Vaughan. Effects of choleragen on hormonal responsiveness of adenylate cyclase in human fibroblasts and rat fat cells. *Biochim. Biophys. Acta* **451**:62–71 (1976).
22. Hayes, J. S., L. L. Brunton, and S. E. Mayer. Selective activation of particulate cAMP-dependent protein kinase by isoproterenol and prostaglandin E_1. *J. Biol. Chem.* **255**:5113–5119 (1980).

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