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ROLE OF ALBUMIN IN HORMONE-STIMULATED LIPOLYSIS

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Abstract—The addition of epinephrine (10^{-6} M) to isolated fat cells resulted in increased lipolytic activity. In the presence of 1, 2 and 4% bovine serum albumin (BSA), lipolytic rates were linear for a 60 min period of time. Rates of lipolysis were increased by increasing the BSA concentration. In other experiments, the effect of glucose (10 mg/ml) was tested on basal, epinephrine-stimulated, and theophylline-stimulated lipolytic activity. In the absence of BSA, glucose resulted in a nearly 3-fold increase in lipolytic rates under all three conditions. In the presence of BSA (4%), the addition of glucose resulted in a further increase in lipolytic activity. The time course of cyclic AMP accumulation following the addition of epinephrine (3×10^{-6} M) was determined in the absence and presence of BSA (4%). In the presence of BSA, accumulation of the cyclic nucleotide continued for a longer period of time and reached a value nearly twice that in the absence of BSA. In another experiment, cyclic AMP accumulation at 5 min following the addition of epinephrine was increased by the addition of 1% BSA. An additional increase was observed by the addition of 2% BSA. When the BSA concentration was increased to 4%, no additional increase was observed. BSA (4%) was also shown to increase the lipolytic response to dibutytyl cyclic AMP. It was concluded that the presence of albumin promoted lipolysis by preventing a negative feedback effect of free fatty acids on cyclic AMP accumulations at some point distal to the production of cyclic AMP. Additionally, it was suggested that albumin facilitates the lipolytic process by some mechanism in addition to reducing intracellular free fatty acid levels.

A number of hormones and pharmacological agents are known to increase lipolytic activity in adipose tissue [1–3]. There is strong evidence that those agents stimulate the breakdown of triglyceride through a sequence of reactions involving an increase in cyclic 3′, 5′-adenosine monophosphate (cyclic AMP), an activation of protein kinase, and an activation of the triglyceride lipase [4]. The addition of lipolytic agents to adipose tissue also results in the appearance of materials which inhibit the lipolytic process [5, 6].

The complete hydrolysis of one molecule of triglyceride results in the production of one molecule of glycerol and three molecules of free fatty acids. The resulting free fatty acids are known to exert an inhibitory influence on the lipolytic process [7, 8]. The addition of albumin to the incubation medium prevents this inhibitory influence, presumably by physically binding the free fatty acid molecules [7, 9].

In addition to free fatty acids, other materials have been found to exert an inhibitory influence on the lipolytic process. Adenosine is a potent inhibitor of lipolysis, but no evidence exists for its increased production in the presence of lipolytic agents [10, 11]. Ho and Sutherland [5] have reported the existence of an antagonist to the lipolytic process which increases after exposure to a lipolytic stimulus.

The present studies were conducted to investigate the feedback regulation of the lipolytic process and to examine how the presence of albumin alters the reactions.

MATERIALS AND METHODS

Experiments were carried out on fed, Sprague–Dawley rats weighing 180–200 g. Fat pads were removed, and isolated fat cells were prepared by the method of Lech and Calvert [12]. Rates of lipolysis were determined by measuring the production of glycerol as described by Chernick [13]. The incubation chamber contained between 0.1 and 0.3 mg protein/ml of medium.

Cyclic AMP levels were determined as described by Allen and Beck [14]. Fat cells were incubated in Krebs Ringer bicarbonate buffer (pH 7.4) containing 1 mM theophylline in an atmosphere of 95% O_2 and 5% CO_2. The final volume was 2.0 ml. Incubation was terminated by the addition of 1 ml of 15% trichloroacetic acid and the samples were treated as described elsewhere [14]. Cyclic AMP was determined by the competitive protein binding assay of Gilman [15] and the dry weight of the tissue was determined as described by Butcher *et al.* [16].

All results are expressed as means ± the standard error of the mean. Significance was calculated using Student's *t*-test for paired comparisons; $P < 0.05$ was considered significant. Bovine serum albumin (fraction V), l-epinephrine and dibutyryl cyclic AMP were purchased from the Sigma Chemical Company (St. Louis, MO). ACTH was purchased from Park, Davis, & Co. (Detroit, MI).

RESULTS

The accumulation of glycerol after 30 and 60 min of incubation with epinephrine (1 μM) was determined in the presence of 0.5, 1.0, 2.0 and 4.0% BSA (Fig. 1). In the presence of 0.5% BSA, a small decrease in lipolytic rates was observed in the second 30-min incubation period. At the other three concentrations of BSA, lipolytic rates were linear for the entire incubation period.

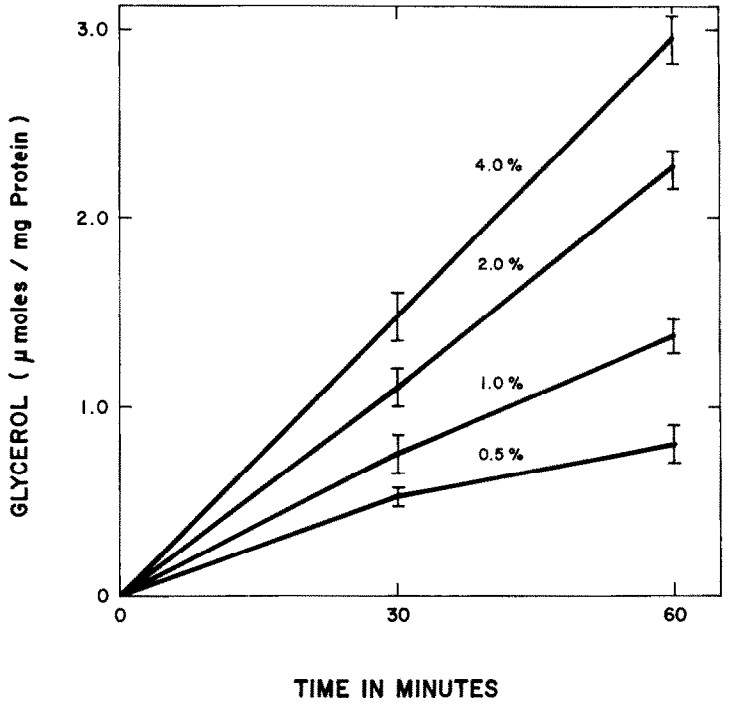

Fig. 1. Effects of BSA on the lipolytic response to epinephrine. Fat cells were incubated for 30 or 60 min in the presence of epinephrine (1 μM) and various concentrations of BSA. Results are from six experiments and are expressed as means ± S. E. M. The numbers above each line refer to the concentrations of BSA (w/v) used in those samples.

Differences in lipolytic rates were observed in the presence of the different concentrations of BSA. Each step increase in BSA concentration resulted in a significant elevation of lipolytic activity. Significant increases were seen at both 30 and 60 min for all concentrations of BSA tested.

In a series of five experiments, the effect of glucose (10 mg/ml) was tested on basal, epinephrine-stimulated, and theophylline-stimulated lipolytic activity (Table 1). This effect of glucose was examined in the absence of BSA and in the presence of 4% BSA. In the absence of BSA, glucose resulted in a nearly 3-fold increase in basal, epinephrine-stimulated, and theophylline-stimulated lipolytic activity. Lipolytic rates in the absence of glucose and in the presence of 4% BSA were higher than the corresponding rates in the absence of BSA. The addition of glucose (10 mg/ml) resulted in a further increase in lipolytic activity under basal and epinephrine-stimulated conditions. The addition of glucose to the theophylline-stimulated cells did not result in a significant increase in lipolytic rates.

The time course of cyclic AMP accumulation following the addition of epinephrine (3 μM) was determined in the absence and presence of BSA (4%) (Fig. 2). Initial rates of accumulation appear to be similar under the two conditions. However, in the absence of BSA, accumulation of cyclic AMP tends to fall off, reaching a maximum by 3 min and remaining at that level for at least an additional 7 min. In contrast, accumulation of cyclic AMP in the presence of BSA continued for approximately 5 min, after which time no further accumulation was observed.

In another series of experiments, basal and epinephrine-stimulated cyclic AMP levels were determined with increasing concentrations of BSA (Fig. 3). All samples were incubated for 5 min. BSA had no effect on

Table 1. Effect of glucose and BSA on basal, epinephrine-stimulated, and theophylline-stimulated lipolysis *

	Glycerol release (μmoles/mg protein/hr)					
	No BSA			4% BSA		
Glucose	Basal	Epinephrine	Theophylline	Basal	Epinephrine	Theophylline
0	0.24 ± 0.10	0.52 ± 0.09	1.19 ± 0.28	0.41 ± 0.14†	6.25 ± 1.01†	9.54 ± 1.44†
10 mg/ml	0.96 ± 0.14	1.55 ± 0.26‡	4.04 ± 0.72‡	1.14 ± 0.25†,‡	11.62 ± 2.08†,‡	14.33 ± 2.60†

* Results are from four experiments and are expressed as the mean ± S. E. M. The final concentration of epinephrine was 1μM and of theophylline was 0.1 mM.
†$P < 0.05$, as compared to corresponding value in the absence of BSA.
‡$P < 0.05$, as compared to corresponding value in the absence of glucose.

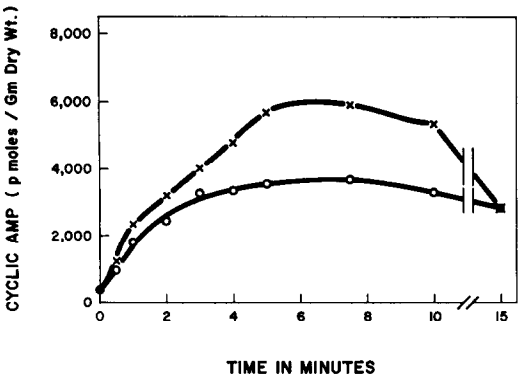

Fig. 2. Time-course of the accumulation of cyclic AMP with and without BSA. Fat cells were incubated for various times with epinephrine (3 μM) and 0 or 4% BSA. The results are the means of four experiments. The open circles are samples with no BSA, and the X's are samples containing 4% (w/v) BSA. Theophylline (0.1 mM) was present in all samples.

the basal level of cyclic AMP in those cells. The addition of 1% or 2% BSA resulted in greater accumulations of cyclic AMP in the epinephrine-stimulated cells. Increasing the concentration to 4% gave no greater effect than that observed with 2% BSA.

The lipolytic response to increasing concentrations of dibutyryl cyclic AMP was determined in the absence and presence of BSA (4%) (Fig. 4). In the absence of BSA a small but significant response to this analog of cyclic AMP was observed at 10^{-3} M and 3.3×10^{-3} M. In the presence of BSA, concentrations of 10^{-4} M and greater resulted in significant lipolytic responses, all of which were much greater in magnitude than those observed in the absence of BSA.

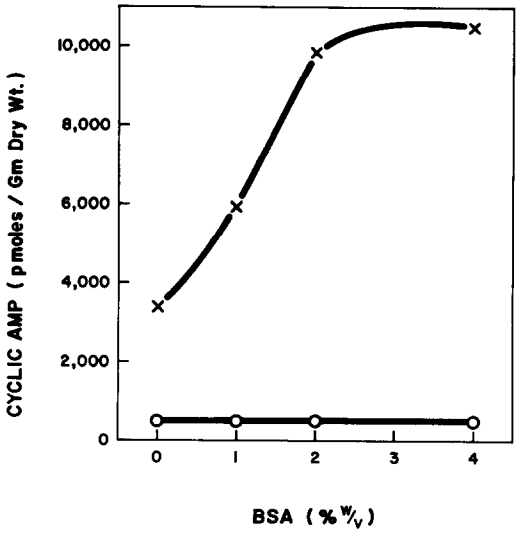

Fig. 3. Effect of BSA on cyclic AMP accumulation. Fat cells were incubated with various concentrations of BSA for 5 min after which cyclic AMP levels were determined. The open circles represent basal levels. The X's represent epinephrine (5 μM) stimulated levels. The results are the means of eight experiments. Theophylline (0.1 mM) was present in all samples.

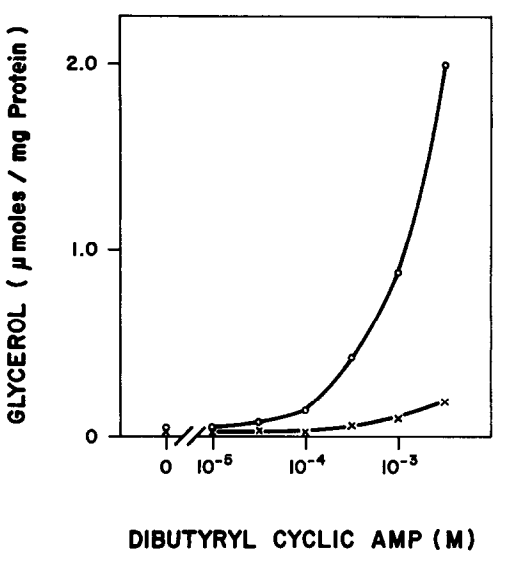

Fig. 4. Effects of BSA on lipolytic response to dibutyryl cyclic AMP. Various concentrations of dibutyryl cyclic AMP were added to fat cells in the presence and absence of 4% (w/v) BSA and incubated 1 hr. Open circles represent values in the presence of BSA and X's represent the absence of BSA. All values are the means of three experiments.

DISCUSSION

Substantial evidence exists that free fatty acids exert a negative feedback effect on the lipolytic process [7, 8]. Additionally, it has been demonstrated that the presence of albumin in the incubation medium results in a much larger stimulation of lipolysis by various hormones [7, 17]. These two facts formed the basis for the assumption that albumin acts as a trap for the released free fatty acids and thereby prevents them from exerting a negative effect on the lipolytic process. Although this conclusion would appear to be correct, it does not explain fully the role of albumin in facilitating hormone-stimulated lipolysis. Evidence presented here suggests that albumin functions in some additional way to allow the lipolytic response to reach a higher level. If albumin acted only as a trap for free fatty acids, it would be expected that initial rates of lipolysis (prior to appreciable accumulations of free fatty acids) would be the same at all albumin concentrations. As free fatty acids begin to accumulate intracellularly, they should inhibit the lipolytic process, and lipolytic rates should be reduced and no longer linear with time. The lower the concentration of albumin, the faster the free fatty acid level in the cells should rise and the sooner should lipolytic rates deviate from linearity. Angel *et al.* [17] using 5% albumin have shown that intracellular levels of free fatty acids reach a maximum in 15 min following the addition of hormonal stimulant. Using lower concentrations of albumin, as reported here, should result in a more rapid accumulation of intracellular free fatty acids and a more rapid inhibition. However, this is not the case. With as little as 1% BSA rates of lipolysis remain linear over a 60-min incubation period. Increasing the concentration of BSA to either 2% or 4% results in higher lipolytic rates that were linear over the 60-min

period of time. If albumin were acting only as a sink for accumulated free fatty acids, lipolytic rates should deviate from linearity at lower concentrations of albumin as the fatty acids saturate the binding sites on the albumin molecules. Increasing the concentration of BSA and thus providing more binding sites for the free fatty acids would alleviate the inhibition and restore linearity. The fact that lipolytic rates were linear for 1 hr in the presence of 1% BSA and that increasing the BSA concentration to 2% or 4% increased the rate of lipolysis suggests that the BSA was acting by some mechanism in addition to sequestering the free fatty acids. This same line of reasoning rules out the possibility that albumin prevented the accumulation of some other inhibitory substance, such as adenosine.

The accumulation of cyclic AMP in the presence of albumin more closely approached what would be predicted from a negative feedback action of the accumulated free fatty acids. Initial rates of accumulation of cyclic AMP are approximately the same in the presence and absence of BSA. However, in the absence of BSA, maximum accumulation of cyclic AMP was achieved in 3 min. However, in the presence of BSA, cyclic AMP continued to accumulate in the fat cells for an additional 2 min, reaching a level approximately twice that seen in the absence of BSA. These data are consistent with the findings of Fain and Shepherd [8, 18] that free fatty acids exert an inhibitory effect on adenylate cyclase and cyclic AMP accumulation in isolated fat cells. The addition of albumin to the incubation medium prevented the accumulation of free fatty acids within the cells and thereby prevented the inhibitory effect of these agents on the adenylate cyclase. This led to an increased rate of production of cyclic AMP and to a prolonged period of accumulation.

The hypothesis that albumin acts by some mechanism other than sequestration of free fatty acids is supported by the experiments with glucose added to the incubation medium. Angel *et al.* [19] have demonstrated that the addition of glucose prevents the intracellular accumulation of free fatty acids in fat cells, presumably by supplying alpha-glycerol phosphate and thereby promoting re-esterification of the fatty acids. In the present study, the addition of 10 mg/ml of glucose to albumin-free incubation medium significantly increased lipolytic rates under basal, epinephrine-stimulated, and theophylline-stimulated conditions. The addition of 4% BSA to the glucose-containing incubation medium resulted in an additional increase in lipolytic activity. Assuming that glucose prevented the accumulation of intracellular free fatty acids, the ability of BSA to further promote lipolytic activity must be the result of some action other than the sequestration of free fatty acids.

Other evidence suggests that albumin facilitates lipolysis at some point in addition to adenylate cyclase. The addition of 1% and 2% BSA to the incubation medium allowed for a concentration-dependent increase in the accumulation of cyclic AMP levels; however, a further increase in BSA concentration to 4% resulted in no further increase in the accumulation of cyclic AMP. The effects of BSA on lipolytic activities showed a different pattern. Increasing the concentration of albumin from 1% to 2% resulted in a substantial increase in lipolytic activity and a further increase to 4% BSA resulted in an additional increase in lipolytic rates. It would appear, therefore, that the effects of higher concentrations of BSA on the lipolytic process cannot be explained solely on the basic of an increased accumulation of cyclic AMP. Conceivably, albumin prevented an inhibition of some step distal to the production of cyclic AMP. Malgieri *et al.* [20] have suggested an inhibitory action of free fatty acids on triglyceride lipase which could be prevented by the presence of albumin.

This conclusion is also supported by the observation that BSA greatly increased the lipolytic response to dibutryl cyclic AMP which presumably stimulated lipolysis without an increase in endogenous cyclic AMP levels. These results suggest that the presence of bovine serum albumin results in the allevation of some inhibitory influence distal to the production of cyclic AMP.

In conclusion, it would appear that the ability of albumin to promote hormone-stimulated lipolysis is due in part to an absorption of free fatty acids. This in turn prevents the free fatty acids from exerting a negative-feedback effect at the level of cyclic AMP accumulation and at some point distal to the production of cyclic AMP.

Additionally, evidence is presented which suggests that albumin facilitates the lipolytic process by some mechanism in addition to reducing intracellular free fatty acid levels.

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