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Control of the Synthesis of Fatty-Acid Synthetase in Rat Liver by Insulin, Glucagon, and Adenosine 3′:5′ Cyclic Monophosphate

(fat-free diet/diabetes)

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ABSTRACT **The usual increase in the activity of liver fatty-acid synthetase that occurs on refeeding of a fat-free diet to previously fasted rats is abolished in diabetic animals. Insulin specifically restores this increase by enhancement of the rate of synthesis of fatty-acid synthetase. However, glucagon and cyclic AMP inhibit the increase in the activity of fatty-acid synthetase. Therefore, the concentration of fatty-acid synthetase in rat liver is under the control of the relative concentrations of insulin and glucagon.**

Nutritional factors are known to regulate the activities of several lipogenic enzymes in mammalian liver (1). For example, feeding a high carbohydrate, fat-free diet to previously fasted rats causes an increase in the quantity of the liver enzymes involved in fatty-acid synthesis (1). This increase has been shown to be due to an increase in the rate of synthesis of acetyl-CoA carboxylase (2), fatty acid synthetase (3, 4), citrate cleavage enzyme, malic enzyme (5), glucose-6-phosphate dehydrogenase (6), and 6-phosphogluconate dehydrogenase (7). Evidence suggests that activities of certain lipogenic enzymes of liver are also under hormonal control (1).

We have been particularly interested recently in the regulatory processes that are involved in the induction* of supranormal activities of hepatic fatty-acid synthetase by feeding a high carbohydrate, fat-free diet to previously starved rats. Insulin has been implicated as a possible regulator of the concentration of fatty-acid synthetase in mammalian liver (3, 8), but no conclusive evidence has yet been provided in support of a causal relationship between the insulin concentration in the animal and the induction of fatty-acid synthetase.

It will be demonstrated in this paper that insulin is indeed involved in the induction of hepatic fatty-acid synthetase to a supranormal activity during high carbohydrate, fat-free diet feeding of rats previously fasted for 48 hr. It will also be shown that glucagon antagonizes induction of the synthetase by insulin. Furthermore, dibutyryl cyclic AMP mimics the action of glucagon.

MATERIALS AND METHODS

Insulin (N.P.H. Iletin) and glucagon were obtained from Eli Lilly & Co.; dibutyryl cyclic AMP and theophylline, from Sigma Chemical Co.; L-[*U*-^{14}C]leucine (250 Ci/mol), from New England Nuclear; fat-free diet, from Nutritional Biochemical Corp. Streptozotocin and glucagon-free insulin were gifts from Dr. W. E. Dulin, Upjohn Co., and Dr. R. E. Chance, Eli Lilly & Co., respectively. Other chemicals were of reagent grade.

Treatment of Animals. Male albino rats (140–160 g) of the Holtzman strain were housed in individual metabolism cages in order to collect urine for glucose analysis. Rats to be made diabetic were initially fasted for 24 hr, injected with streptozotocin (65 mg/kg) into the tail vein (9), then fasted for another 24 hr. At the end of this period of fasting the animals were diabetic (as tested by blood glucose: >300 mg/100 ml; urine glucose: 0.5% or more). Insulin and glucagon were administered subcutaneously, whereas dibutyryl cyclic AMP and theophylline were administered intraperitoneally.

Rat-Liver Supernatant Solutions Were Prepared and Assayed for Fatty-Acid Synthetase Activity as described by Craig *et al.* (4).

Preparation of Purified Fatty-Acid Synthetase. Fatty-acid synthetase was purified from the rat-liver supernatant solution as reported by Burton *et al.* (3) and modified by Craig *et al.* (4). Sucrose density gradient centrifugation of the final product was performed as reported by Craig *et al.* (4).

Analysis of Radioactivity. Radioactivity in purified fatty-acid synthetase was determined on glass-fiber filters after precipitation with trichloroacetic acid. Samples were assayed in a toluene-based scintillation fluid with a Packard liquid scintillation spectrometer.

RESULTS

The change in activity of liver fatty-acid synthetase as a function of dose of insulin is reported in Fig. 1. It is obvious that fatty-acid synthetase activity is negligible in diabetic rats, even though these animals ate well during the 48-hr refeeding period. In contrast, the diabetic rats that received insulin showed a dramatic increase in fatty-acid synthetase activity. There was a linear increase in the activity of the enzyme as a function of insulin dose up to 1.0 unit. A slower rate of increase occurred up to 3 units, where enzyme activity reached a plateau. Pure insulin gave a response identical to commercial insulin (N.P.H. Iletin) in the induction of fatty-acid synthetase when tested at a dose of 3 units.

* The term "induction" is used to mean a selective stimulation of the rate of enzyme synthesis; no genetic or other mechanism is implied.

The increase in fatty-acid synthetase activity as a function of time after insulin administration is shown in Fig. 2. It is apparent that the increase in activity of the fatty-acid synthetase is completely abolished in diabetic rats throughout the refeeding period. Strikingly, the insulin-treated diabetic rats showed a progressive linear increase in enzyme activity with time, after an initial lag period of 6 hr. The activities at 24 and 48 hr of refeeding were comparable to the values obtained for normal rats that were subjected to an identical dietary regimen.

In order to prove that the net increase in enzyme activity in diabetic rats treated with insulin is due to an adaptive increase in enzyme synthesis, fatty-acid synthetase was isolated and purified from diabetic rats treated or not treated with insulin. Enzyme was isolated after 0 and 48 hr of refeeding the fat-free diet.

Rats were pulse-labeled with L-[*U*-^{14}C]leucine for 60 min, and then radioactivity was determined in the purified fatty-acid synthetase. Diabetic rats had a very low concentration of fatty-acid synthetase at 0 time that failed to increase significantly even after 48 hr of refeeding (Table 1). However, there was a 20-fold increase in the enzyme activity isolated from liver after the administration of insulin to diabetic rats. Further support for the conclusion that insulin affects the rate of synthesis of fatty-acid synthetase is obtained from a comparison of the rate of synthesis of this enzyme relative to total protein synthesis. Thus, the relative rate of synthesis of fatty-acid synthetase for diabetic rats treated with insulin was about 10 times greater than that for untreated diabetic rats.

Glucagon is the physiological antagonist of insulin and it is known to increase the amount of cyclic AMP (10). It was therefore relevant to determine the effect of glucagon and cyclic AMP on the dietary induction of the fatty-acid synthetase. It is obvious (Fig. 3) that glucagon has a marked inhibitory effect on induction of the fatty-acid synthetase. Furthermore, the inhibition is linear with increasing concentrations of glucagon. It was further found that glucagon inhibits this induction process by 41% at 24 hr, and by 59% at 48 hr of refeeding (Table 2). Significantly, dibutyryl cyclic AMP inhibited the induction of fatty-acid synthetase by

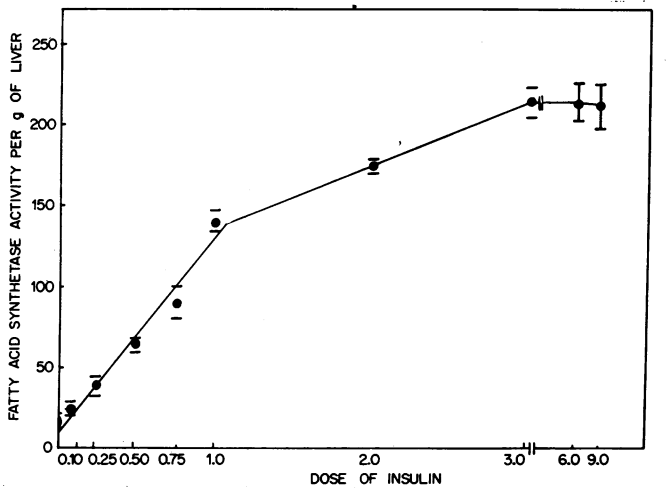

FIG. 1. Effect of insulin dose on liver fatty-acid synthetase activity in refed diabetic rats. Diabetic rats fasted for 48 hr (see *Methods*) were refed the fat-free diet for 48 hr. From the beginning of the refeeding period insulin was administered to each rat at the indicated dose in units (IU)/100 g of body weight. However, each dose of insulin per day was divided into two equal dosages and administered at 12-hr intervals. Each point represents the mean (±SE) of values obtained from two or three rats, except at the dosages of 1 and 3 units where four and seven rats were used, respectively. Enzyme units are expressed as nmol of palmitate formed per min at 30°.

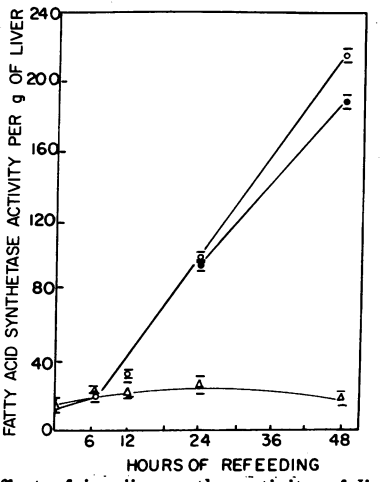

FIG. 2. Effect of insulin on the activity of liver fatty-acid synthetase as a function of time of refeeding of diabetic rats. Conditions were the same as in Fig. 1, except that each diabetic rat in the insulin-treated group received 3 units of insulin per 100 g of body weight, and each group of animals was killed at the indicated time interval of refeeding. O——O, insulin-treated diabetic rats; Δ——Δ, diabetic rats; ●——●, normal control rats.

TABLE 1. *Effect of insulin on fatty-acid synthetase content of liver in refed diabetic rats**

Treatment of diabetic rats†	Fatty-acid synthetase‡ (mg per liver)	Total radioactivity in fatty-acid synthetase (A)§ (dpm per liver)	Total radioactivity in soluble protein (B)¶ (dpm per liver × 10^{-3})	Relative rate of synthesis of fatty-acid synthetase (A/B × 10^{3})
Refed at 0 hr	0.5	172	109	1.6
Refed at 48 hr	0.5	272	68	4.0
Insulin-treated and refed at 48 hr	9.9	7900	209	37.8

* Each value represents the average obtained from two rats.

† Rats were fasted 48 hr and then refed a fat-free diet for the specified time.

‡ These values represent amounts of pure enzyme obtained after purification by sucrose density gradient centrifugation (4).

§ Rats received a single intraperitoneal injection of L-[*U*-^{14}C]-leucine (2 μCi/100 g of body weight) and were then killed 60 min after the injection. Liver fatty-acid synthetase was purified by sucrose density gradient centrifugation as reported (4).

¶ These values represent the radioactivity incorporated into the 105,000 × *g* supernatant solution.

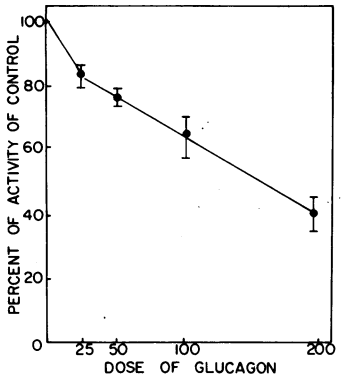

FIG. 3. Effect of glucagon on the increase of liver fatty-acid synthetase in refed fasted rats. Normal control rats were fasted for 48 hr and then refed a fat-free diet for 48 hr. From the time of refeeding glucagon was administered to each rat at the indicated dose (μg/8 hr per 100 g of body weight). Each point represents the mean value obtained for two rats, except for the group that received 200 μg of glucagon. Five rats were used in this group and the value represents the mean (±SE).

64% when administered in conjunction with theophylline (Table 2). Theophylline, which has no effect on the induction process (Table 2), was administered to reduce the degradation of cyclic AMP.

It is pertinent to point out that each of the groups of rats that received hormonal or other treatment had good appetites and that each gained weight over 48 hr of refeeding the fat-free diet (Table 2). However, rats in the diabetic, glucagon-, or cyclic AMP-treated groups did not gain as much as those in the normal or in diabetic groups treated with insulin. Hence, it is concluded that the changes in enzyme activity that occurred from one group to another are due to an effect of the hormone and not due to starvation.

DISCUSSION

It has been established (4) that the increase in the activity of hepatic fatty-acid synthetase that occurs on refeeding fasted rats a high carbohydrate, fat-free diet is due to an increase in the rate of synthesis of this enzyme and not due to a decrease in the rate of its degradation. Furthermore, the activity of liver fatty-acid synthetase, as well as the rate of synthesis of this enzyme, in the fasted state is very low (4). Therefore, even if insulin were to completely block the degradation of fatty-acid synthetase, the observed 20-fold rise in the activity of this enzyme would not have occurred within 48 hr of refeeding. Consequently, insulin must increase the rate of synthesis of fatty-acid synthetase. Thus, the involvement of a specific hormone, insulin, in the control of the rate of synthesis of hepatic fatty-acid synthetase has been demonstrated for the first time.

Rats are known to normally secrete 2–3 units of insulin per day (11). Hence, it is significant that the maximum effect of insulin on the induction of fatty-acid synthetase occurs at a concentration of 3 units. It is concluded, therefore, that the specific effect of insulin on the induction of fatty-acid synthetase is a physiological response. A similar response has been reported for tyrosine aminotransferase, except that normal rather than diabetic rats were used in that study (12). On the contrary, no response was observed in the induction of liver glucose-6-phosphate dehydrogenase (6) or 6-phosphogluconate dehydrogenase (7) at concentrations up to 4 units of insulin. Responses were obtained, however, at concentrations of 8–12 units of insulin. These investigators suggest that the increased intake of high carbohydrate diet that occurs at these high doses of insulin is responsible for the induction of the two enzymes, and that the response is not due to insulin *per se*.

Streptozotocin, an antibiotic produced by *Streptomyces acromogenes*, has been shown to be a highly specific cytotoxic

TABLE 2. *Effect of insulin, glucagon, and cyclic AMP on the amount of liver fatty-acid synthetase*

Duration of refeeding (hr)	Condition*	Treatment	Enzyme activity† (units/g of liver)	% Inhibition‡	% Increase in body weight§
0	Diabetic	None	15.1 ± 4.3	92	0
24	Diabetic	None	26.8 ± 6.2	72	4.5 ± 0.5
24	Diabetic	+ Insulin¶	102.1 ± 4.2	—	14.5 ± 0.5
24	Normal	None	96.5 ± 3.5	0	14.0 ± 1.0
24	Normal	+ Glucagon‖	57.0 ± 1.0	41	9.5 ± 1.0
48	Diabetic	None	17.8 ± 4.3	91	4.8 ± 1.5
48	Diabetic	+ Insulin¶	216.3 ± 9.0	—	20.0 ± 2.0
48	Normal	None	189.0 ± 7.6	0	21.3 ± 2.6
48	Normal	+ Glucagon‖	76.5 ± 8.5	59	12.5 ± 1.5
48	Normal	+ Cyclic AMP**	47.1 ± 5.2	64	13.5 ± 1.5
48	Normal	+ Theophylline††	192.0 ± 4.2	0	20.0 ± 2.0

* All animals were fasted for 48 hr, and then refed a fat-free diet for 24 or 48 hr.

† Enzyme activity is expressed as nmol of palmitate formed per min at 30°; each point represents the mean (±SE) of values obtained from at least three rats.

‡ Based on the activity for control animals at either 24 or 48 hr of refeeding.

§ The increase in body weight that occurred during the refeeding period.

¶ Insulin was administered at a dose of 3 units/day per 100 g of body weight.

‖ Glucagon was administered at a dose of 200 μg/8 hr per 100 g of body weight.

** Dibutyryl cyclic AMP was administered at a dose of 5 mg/8 hr per 100 g of body weight. In addition, the rats received theophylline (2 mg/8 hr per 100 g of body weight).

†† Theophylline was administered at a dose of 2 mg/8 hr per 100 g of body weight.

drug that causes the necrosis of β-cells of the pancreas, thereby producing insulin deficiency in rats within 24 hr of administration (9, 13). Furthermore, it has little side effect, if any, due to the marked difference in the diabetogenic (65 mg/kg) and LD_{50} doses (130 mg/kg). Streptozotocin-induced diabetic rats ate well during the 48-hr period of refeeding. However, they did not gain weight to the same extent as the normal or the diabetic rats receiving insulin (Table 2). Therefore, the lack of induction of the fatty-acid synthetase in diabetic rats could not be due to the lack of food intake. Hence, it is evident that insulin and not the high carbohydrate, fat-free diet is vital in the induction of the synthesis of the fatty-acid synthetase of rat liver. Furthermore, it is probable that the high carbohydrate, fat-free diet stimulates the secretion of insulin (10).

A 20-fold increase in the actual amount of the purified fatty-acid synthetase isolated from diabetic rats treated with insulin, as compared to untreated rats (Table 1), clearly shows that insulin brings about an adaptive increase in the rate of synthesis of this enzyme and not merely an activation of previously existing enzyme. This is further demonstrated by a marked stimulation in the rate of synthesis of fatty-acid synthetase relative to total protein synthesis in diabetic rats treated with insulin, as compared to untreated diabetic rats. The relative rates of synthesis of fatty-acid synthetase were determined in order to evaluate the specific stimulation of the synthesis of this enzyme irrespective of changes in amino-acid uptake or pool size in the diabetic rats treated or not treated with insulin. Thus, it is evident that insulin induces an increase in the synthesis of fatty-acid synthetase irrespective of whether the comparisons are based on the absolute amount of enzyme or on the relative rates of synthesis of the enzyme.

The inhibitory effect of glucagon on the induction of the fatty-acid synthetase (Fig. 3 and Table 2) supports the role of glucagon as an antagonist of insulin. Significantly, the rats that received glucagon had good appetites and they gained weight during the refeeding period (Table 2). Thus, the observed inhibitory effect of glucagon on the induction of fatty-acid synthetase is a true effect. This result is in agreement with the effect of glucagon on the activity of glucose-6-phosphate dehydrogenase in rat liver (14). In contrast, glucagon stimulates the rate of formation of rat-liver tyrosine transaminase (12).

The marked inhibitory effect of dibutyryl cyclic AMP on the induction of the fatty-acid synthetase suggests that the intracellular concentration of this cyclic nucleotide might be the critical factor in the control of the induction of this enzyme. Glucagon is known to increase the amount of cyclic AMP (10), whereas insulin decreases it (15). Cyclic AMP has also been shown to induce the synthesis of several enzymes that are involved in gluconeogenesis and amino-acid catabolism (16), whereas it blocks the induction of lipogenic enzymes [(14) and present investigation] and glucokinase (17, 18). These results are compatible with the physiological actions of insulin and glucagon (10). Hence, it seems likely that the relative concentrations of insulin and glucagon may be vital in determination of the actual concentration of cyclic AMP. Cyclic AMP would then regulate the activity of the fatty-acid synthetase.

It has been established that cyclic AMP exerts its control in bacterial systems by binding with a cyclic AMP-receptor protein (19, 20). It has also been proposed that the multiple effects of cyclic AMP are mediated through its interaction with regulatory proteins (21). Hence, it is conceivable that cyclic AMP may regulate the activity of fatty-acid synthetase in rat liver by interacting with a regulatory protein.

Many of the lipogenic enzymes respond to various nutritional states similar to fatty-acid synthetase (1). Furthermore, the induction of at least two lipogenic enzymes, fatty-acid synthetase (present investigation) and glucose-6-phosphate dehydrogenase (14), are known to be inhibited by cyclic AMP. Therefore, it may well be that these lipogenic enzymes are coordinately controlled by cyclic AMP.

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