Role of the pituitary and the adrenal in the mobilization of free fatty acids and lipoproteins*

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SUMMARY

Treatment of normal rats with epinephrine in oil resulted in a rapid rise in plasma free fatty acid (FFA) levels. The return of FFA levels to normal coincided with the steeply increasing blood glucose concentration. The plasma FFA response to epinephrine was abolished by hypophysectomy or by adrenalectomy. The *in vitro* rate of release of FFA from the epididymal fat bodies of operated animals was only about one-half that from fat bodies of normal animals. The *in vitro* rate of release of FFA from fat bodies removed 30 minutes after injection of epinephrine was two to three times as high as that in fat bodies taken from noninjected animals. The fat bodies taken from hypophysectomized and adrenalectomized animals showed stimulation by epinephrine, but the absolute rates of release were lower than those observed in fat bodies taken from intact rats. Normal rats receiving epinephrine showed highly significant elevations of serum cholesterol and phospholipid levels but no rise in triglyceride levels. The cholesterol and phospholipid responses to epinephrine were also abolished by hypophysectomy and substantially reduced by adrenalectomy. Attempts to mimic the action of epinephrine and induce an elevation of plasma lipoprotein levels by infusing sodium oleate intravenously were negative; the amounts which could be safely infused, however, may have been inadequate. It was concluded that the pituitary and adrenal glands play an important role in the response to the lipid-mobilizing action of epinephrine, both in terms of FFA and lipoprotein responses.

In a previous paper the dual effect of epinephrine in mobilizing both free fatty acids (FFA)[1] and lipoproteins in dogs was described (1). It was shown that after subcutaneous injection of epinephrine in oil, the plasma FFA levels reached peak values in 1 to 3 hours and returned toward normal as the blood glucose levels rose toward a maximum. When hyperglycemia was produced by feeding glucose prior to the injection of epinephrine or when insulin was given along with epinephrine, the plasma FFA levels failed to rise, indicating that the rate of glucose utilization was a key factor in controlling the FFA response. Blocking the FFA response in either of these two ways reduced the magnitude of the subsequent rise in the other serum lipids (cholesterol and phospholipids) but did not completely prevent it. These experiments did not establish to what extent the lipoprotein elevation might be dependent, directly or indirectly, upon FFA mobilization from adipose tissue.

The present studies in rats were undertaken to explore further the mechanism of action of epinephrine in mobilizing lipids and to identify some of the factors controlling these lipid responses. Since the adrenal cortex has been implicated in the control of lipoprotein levels (2, 3), and since epinephrine has been reported to stimulate pituitary and adrenal function (4, 5, 6), the effects of adrenalectomy and of hypophysectomy

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[1] FFA designates long-chain fatty acids not found chemically in covalent linkage.

were investigated. Comparisons were made of the effect of epinephrine on the *in vitro* rate of release of FFA from adipose tissue taken from normal and operated animals. Finally, attempts were made to affect rat plasma lipoprotein levels by maintaining high plasma FFA concentrations by constant intravenous infusion of sodium oleate.

METHODS

Normal, adrenalectomized, and hypophysectomized male rats of the Charles River strain were purchased from the Charles River Breeding Laboratories. A few studies were also done with normal and adrenalectomized Sprague-Dawley rats from the NIH colony. The control lipid levels and the responses observed were similar enough in the two groups to permit pooling of the data for final statistical analysis.[2] The adrenalectomized rats were offered saline, but no supportive hormonal treatment was given to either set of operated animals. They were given the following purified low-fat diet ad lib.: 37% casein, 33% sucrose, 23% corn starch, 1% cod liver oil, 0.1% choline, 5% salt mixture (7), and vitamin supplements. The experiments with the hypophysectomized animals were begun between 2 and 3 weeks after operation. The adequacy of the hypophysectomy was indicated by the absence of any weight gain measured during the week prior to the experimental period. The adrenalectomized rats were studied 7 to 10 days after operation. The completeness of adrenalectomy was confirmed after sacrifice. The weight of control animals ranged from 130 to 170 g, while that of operated animals ranged from 100 to 150 g. After intraperitoneal injection of sodium pentobarbital, blood samples were drawn into heparinized syringes from the aorta and transferred to tubes containing sodium fluoride.

Cholesterol, phospholipids, triglycerides, FFA, and glucose were determined as previously described (1). Epinephrine in oil,[3] 2 mg per kg, was injected subcutaneously in the neck region.

Tissue FFA release was studied *in vitro* with epididymal fat bodies removed under pentobarbital anesthesia. After rinsing three times in 0.85% NaCl, they were incubated at 37°, and pH 7.4 in 4% bovine serum albumin, dissolved in 0.85% NaCl or 0.15 M tris (hydroxymethyl) aminomethane buffer. The FFA

increment in the medium was measured at the end of 1 to 3 hours of incubation and expressed as μeq released per gram wet weight of tissue per hour.

To prepare rats for FFA infusion, the deep femoral vein was exposed under ether anesthesia and a soft vinyl catheter (0.7 mm outside diameter, 0.4 mm inside diameter) was inserted and threaded up into the inferior *vena cava*. The animal was placed in a restraining cage and allowed to recover from the anesthesia. A solution containing 40 μeq per ml of sodium oleate[4] and 2 mg per ml of bovine serum albumin in physiological saline was then infused at a steady rate, using a Braun pump (motor-driven plunger in a calibrated glass syringe). Blood samples for plasma FFA determination in these experiments were taken from the tail vein.

RESULTS

Epinephrine-Induced Changes in Plasma Cholesterol, Phospholipids, and Triglycerides. As shown in Table 1, two daily injections of epinephrine in oil caused significant elevations of plasma cholesterol and phospholipid levels, determined 24 hours after the last injection. Hematocrits measured in five control and five epinephrine-treated rats did not differ significantly, so the observed lipid elevations cannot be attributed to hemoconcentration. The animals were fed ad lib. during the study but fasted overnight prior to sampling. The increase in phospholipids (34.4%) was somewhat smaller than that of cholesterol (54.6%), so the cholesterol-phospholipid ratio after treatment was significantly higher. This is in accord with the pattern of response reported previously in dogs (1) where fractionation of the serum lipoproteins showed that the largest relative increase after epinephrine was in the cholesterol-rich d = 1.019 to 1.063 lipoprotein fraction. There was no rise in triglyceride levels, implying little change in the lower density lipoprotein fractions, again in accord with the results in dogs (1).

Hypophysectomized rats showed no significant elevation in any of the serum lipid fractions after epinephrine treatment (Table 1). However, the higher initial levels of cholesterol and phospholipids and the lower levels of triglycerides in these operated animals should be noted. This elevation of basal lipid levels, possibly related to loss of thyroid function, may have masked any effect of epinephrine on lipoprotein metabolism.

TABLE 1. Changes in Rat Plasma Lipids After Two Daily Injections of Epinephrine in Oil (2 mg/kg)

	No. of Animals	Treatment	Plasma Lipid Levels (mg/100 ml) (Mean ± Standard Deviation)			
			Total Cholesterol	Free Cholesterol	Phospholipids	Triglycerides
Normal	9	Control	51.1 ± 6.3	12.8 ± 2.4	93.3 ± 9.9	46.9 ± 17.2
		Epinephrine	79.0 * ± 5.8	21.2 * ± 7.6	125.4 * ± 19.5	46.7 † ± 11.5
Hypophysectomized	12	Control	98.5 ± 14.9	32.2 ± 4.6	122.0 ± 16.3	28.1 ± 14.5
		Epinephrine	106.6 † ± 17.2	34.3 † ± 4.1	123.2 † ± 11.2	22.4 † ± 8.2
Adrenalectomized	9	Control	53.9 ± 7.9	17.0 ± 4.3	96.9 ± 12.8	38.3 ± 12.1
		Epinephrine	70.1 ‡ ± 7.3	19.6 † ± 4.3	100.0 † ± 8.8	36.1 † ± 12.9

* Difference from control value significant at p < 0.01 level.
† Difference from control value not significant.
‡ Difference from control value significant at p < 0.05 level.

In the adrenalectomized animals, epinephrine failed to cause any significant change in free cholesterol, phospholipid, or triglyceride levels (Table 1). There was a barely significant rise in total cholesterol, but the elevation was smaller than that observed in intact animals (30.0% as against 54.6%). The results indicate that adrenal function is required to obtain the full effect of epinephrine on lipoprotein mobilization.

Epinephrine-Induced Changes in Plasma FFA, Blood Glucose, and in Vitro FFA Release. The time course of the plasma FFA and blood glucose response to epinephrine was obtained by sacrificing groups of animals at different time intervals. The variability encountered when comparing different animals necessitated the use of rather large numbers in each group. The results are summarized in the scattergrams of Figure 1. Curves are drawn through the mean values at each time point.

In the normal animals the mean plasma FFA elevation was maximal at 15 to 45 minutes, and normal values were reached again after 1 hour. The response in rats at a dose of 2 mg epinephrine per kg is thus more rapid, briefer in duration, and of lesser magnitude than that observed in dogs at a dose of 1 mg per kg (1). The increase in blood glucose level was also more rapid, the peak of hyperglycemia being reached by 1 hour. As in the dog studies, the FFA level tended to return toward normal when the glucose level rose

toward its maximum, implying a possible homeostatic interrelationship between these two responses to epinephrine.

Of particular interest was the inability of the adrenalectomized and hypophysectomized animals to react after epinephrine with the expected elevation of plasma FFA (Fig. 1). There was, on the other hand, a glucose response comparable to that in intact animals, although it was somewhat delayed.

The observed rise in FFA levels in normal animals most probably reflects an increased rate of release of FFA from depot fat. The apparent lack of FFA elevation in the absence of pituitary or adrenal function could be due to (a) a decreased effect of epinephrine on FFA production, either due to reduced tissue stimulation or to a reduction in available fat stores, (b) an increased epinephrine effect on the rate of FFA removal and utilization, or (c) a combination of the two. In order to test the first possibility, the epididymal fat bodies were taken from normal, adrenalectomized, and hypophysectomized rats at different time intervals after injection of epinephrine, and the rate of FFA release was measured *in vitro*. The results are shown in the lower frames of Figure 1. (Plasma FFA and blood glucose levels in these animals are included in the same figure.) Before epinephrine treatment (zero time values), the rate of *in vitro* release from adipose tissue taken from adrenalectomized or hypophysec-
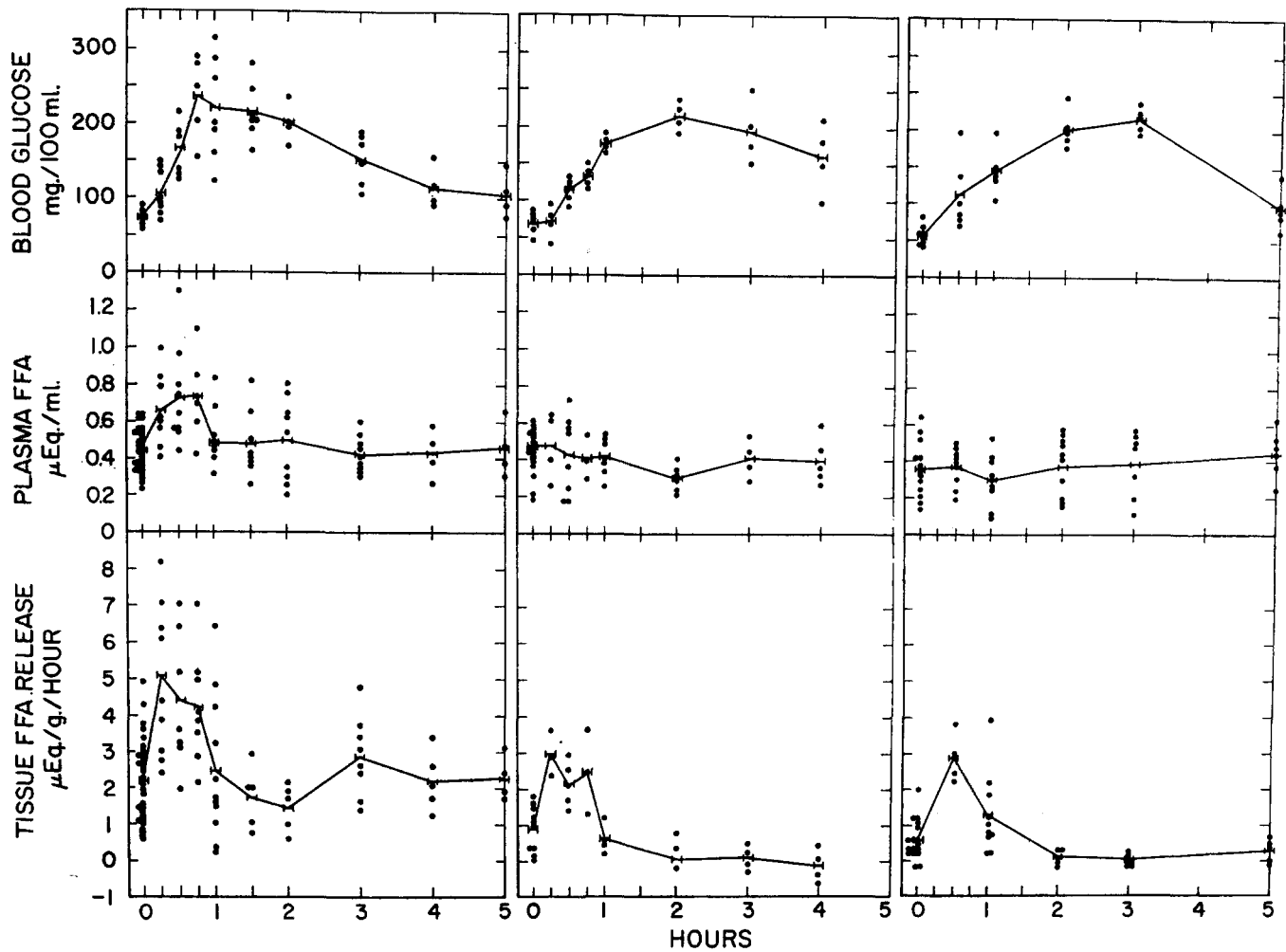
FIG. 1. Time course of responses to epinephrine in oil (2 mg/kg) injected subcutaneously. Groups of rats were sacrificed at various time intervals and each point represents the result for an individual rat. Top: changes in blood glucose with time. Middle: changes in plasma FFA with time. Bottom: changes in rate of release of FFA from epididymal fat body removed at time of sacrifice and then incubated *in vitro* (see Methods). Left: normal rats. Center: hypophysectomized rats. Right: adrenalectomized rats.

tomized animals was only about one-half that from normal rats' adipose tissue. This suggests that rats deprived of pituitary or adrenal function mobilize fat at a lower than normal rate.

There was an increase in the rate of *in vitro* FFA release after epinephrine in all groups, but the absolute rate of release in both adrenalectomized and hypophysectomized animals after stimulation was less than that in intact animals. In another series of experiments, when, instead of injecting the animals with epinephrine prior to removal of tissues, the hormone was added *in vitro* to tissues taken from adrenalectomized or hypophysectomized rats, the degree of stimulation was again less than that obtained with similarly treated tissues from normal rats. Thus the absence of a rise in plasma FFA levels appears to be due, at least in part, to a failure of the adipose tissue to respond adequately to epinephrine. Whether there is also a greater relative increase in rate of removal remains to be determined.

The rate of FFA release from adipose tissue of normal rats measured *in vitro* at different times after epinephrine correlated inversely with the blood glucose level at the time the tissues were removed. The *in vitro* rat of FFA release showed an initial prompt rise, paralleling the initial rise in plasma FFA levels. Later, as the blood glucose rose to substantial levels, the release rate began to fall off and then remained low

during the hyperglycemic phase. Then as the blood glucose returned toward normal, there appeared to be a rebound in the rate of FFA release.

FFA Infusion Studies. As discussed in a previous paper (1), there are a number of instances in which elevated FFA levels and elevated lipoprotein levels are associated. In the present studies the lipoprotein response and the FFA response were both abolished or markedly reduced by adrenalectomy or by hypophysectomy, again raising the question of a cause-and-effect relation between the early FFA response on the one hand and the later lipoprotein response on the other. Therefore an attempt was made to simulate the epinephrine effect by maintaining an isolated plasma FFA elevation and avoiding any of the concomitant metabolic effects of epinephrine. To achieve this, a procedure for intravenous FFA infusion was elaborated, recognizing the experimental problem posed by the hemolytic activity of fatty acids. Satisfactory results were obtained when the tip of the catheter was placed in the upper half of the inferior *vena cava*, where good mixing tended to prevent the formation of high local concentrations of the infused solution. Sodium oleate was chosen because of its solubility. Its hemolytic action was reduced by including a small amount of albumin in the solution. In most experiments relatively rapid rates of infusion were tolerated without gross hemolysis, as evidenced by absence of hemoglobinuria. As shown in Table 2, infusions at

rates as high as 0.5 to 1 μeq per minute could be maintained for up to 30 hours. Significant elevations of plasma FFA were achieved for prolonged periods of time in all cases, but there were no significant changes in plasma cholesterol, phospholipids, or triglycerides.

DISCUSSION

The present studies show that rats respond to epinephrine in oil with a prompt but transient elevation of plasma FFA levels. The return of FFA levels toward normal coincides in time with the rise of glucose levels toward a maximum. This correlation is in agreement with the results of similar studies in dogs (1) and leads to the suggestion that the epinephrine-induced hyperglycemia directly or indirectly limits the FFA response to the hormone. Gordon and Cherkes (8) have demonstrated a direct suppressive effect of glucose on FFA release *in vitro*.

Increased glucose mobilization does not appear to be the first mechanism by which the organism meets demands for oxidizable substrate during sympathetic discharge, usually a prominent feature associated with stress reactions. The time relations observed in the present studies with rats and in the previous studies with dogs (1, 9) indicate that actually the earliest response is an increased mobilization of fat in the form of FFA. Therefore the adipose tissue fat, sometimes considered to be a relatively nonlabile store of substrate, appears under emergency conditions to be more rapidly available as a source of calories than carbohydrate.

The characteristic FFA response to epinephrine was abolished by either adrenalectomy or hypophysectomy. In the hypophysectomized animals, 2 to 3 weeks elapsed between the time of operation and the initiation of the experiment, and most probably at that time there remained very little adrenocortical function in these animals. The results in the hypophysectomized animals may therefore be attributable exclusively to secondary adrenocortical insufficiency. This interpretation is supported by previous studies in dogs (9), showing that cortisone restores the responsiveness in both adrenalectomized and hypophysectomized animals. On the other hand, Goodman and Knobil (10) report that hypophysectomized monkeys, unable to respond to epinephrine with a rise in FFA, recover their responsiveness when treated with thyroid-stimulating hormone. Furthermore, Engel *et al.* (11) report that growth hormone can elevate plasma FFA levels in hypophysectomized rats, and studies in this labora-

TABLE 2. EFFECT OF INTRAVENOUS INFUSION OF SODIUM OLEATE * ON PLASMA FFA LEVELS

Rat	Time of Infusion	Total Fatty Acid Infused	Plasma FFA Level
	Hrs	*μeq*	*μeq/ml*
1	0	0	0.70
	3.3	108.5	1.20
	30.4	1125.5	1.38
2	0	0	1.20
	18.7	524	1.77
3	0	0	0.63
	3.5	132	0.86
	20.8	756.8	0.94
4	0	0	0.75
	19.5	935	2.84

* 40 μeq/ml physiological saline containing 2 mg bovine serum albumin per ml.

tory demonstrate a similar action of growth hormone in dogs.[5] Thus the possibility that loss of other pituitary factors involved in lipid mobilization may play some role cannot be ruled out.

The lipoprotein responses to epinephrine in the operated animals were also either completely abolished (hypophysectomy) or very substantially reduced (adrenalectomy). We have previously reported similar results in dogs (9), and in those studies demonstrated that administration of cortisone restored the lipoprotein response to epinephrine both in adrenalectomized and hypophysectomized animals. Normal adrenocortical function appears to be essential, therefore, for both responses to epinephrine, that is, elevation of FFA levels and of lipoprotein levels. As mentioned above, the possible importance of pituitary factors other than adrenocorticotropin cannot be decided. The fact that adrenalectomized animals did show some elevation of cholesterol ester levels may indicate that pituitary factors are in fact operative.

The absolute *in vitro* rate of release of FFA from the epididymal fat body prior to epinephrine injection was lower in hypophysectomized and adrenalectomized rats than in intact rats. Furthermore, the peak rate of release attained after epinephrine injection was lower in fat bodies taken from the operated animals. Similar results have been obtained in adrenalectomized rats by Reshef and Shapiro (12), using adipose tissue of the mesentery. This decreased sensitivity to epinephrine may well be enough to account for the absence of any rise in plasma FFA levels in these animals after the hormone was given. On the other hand, the relatively smaller than normal amount of carcass fat in the operated animals (13), by limiting the total amount of substrate available for mobilization, may be a contributing factor. This defect in lipid mobilization from adipose tissue may clarify a number of earlier observations on the inability of hypophysectomized and adrenalectomized animals to respond to conditions that lead to extensive shifts of body lipid in normal animals (14 to 17). It would also provide an explanation for the tendency of hypophysectomized animals to show a higher respiratory quotient than normal animals during extended fasting (18).

It should be noted that the plasma FFA levels of the operated rats were similar to those of the intact rats despite the apparently lower rate of FFA release indicated by the *in vitro* studies. This suggests a decrease in the rate of utilization of plasma FFA in the absence of pituitary or adrenal function, or both.

Adrenalectomy and hypophysectomy abolished or drastically reduced both the FFA and the lipoprotein elevation after epinephrine, raising the questions whether the two responses are causally related, whether they both stem from a single primary action of epinephrine, or whether they represent two independent actions of the hormone. Since the FFA mobilization after epinephrine precedes the lipoprotein response, the possibility must be considered that the lipoprotein elevation is induced by the transfer of significant amounts of FFA from adipose tissue to the liver. Earlier investigations have shown that such lipid transfer does indeed occur during epinephrine treatment in rats (17). Recent studies in dogs by Feigelson *et al.* (19) show that continuous intravenous infusion of epinephrine or norepinephrine leads to striking increases in the triglyceride content of the liver. These triglyceride fatty acids might provide substrate to support or stimulate an increased rate of lipoprotein synthesis. Failure to elicit hyperlipoproteinemia in rats in the present studies by direct infusion of sodium oleate does not rule out this possibility. The total amounts of FFA infused, even at the maximum rate that could be maintained without untoward effects, probably fell short of the amount of FFA delivered to the circulation from the total body fat stores in a much shorter period after stimulation with epinephrine. Moreover, the infusion of exogenous FFA appears to reduce normal endogenous FFA production,[6] so that the amount of FFA entering the circulation and being deposited in the liver in these studies may be insufficient to initiate increased lipoprotein formation. Finally, there is the possibility that toxicity of the fatty acid infused at high concentration may have masked an effect on lipoprotein mobilization. Better techniques for FFA administration will be helpful in elucidating the problem of their role as precursors in the synthesis of the lipid moiety of lipoproteins.

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[6] See footnote 5.

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