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Free Fatty Acids Activate the Hypothalamic-Pituitary-Adrenocortical Axis in Rats*

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ABSTRACT

Intravenous administration of Intralipid 10% increases blood levels of essential free fatty acids. In rats and man, this is associated with an inhibition of GH secretion from the anterior pituitary. Because GH is lipolytic, the inhibition of its secretion may represent a negative feedback action of the fats on pituitary sensitivity to GH-releasing hormone. Since corticosterone, the final secretory product of the rat hypothalamic-pituitary-adrenocortical (HPA) axis, is also lipolytic, we tested the hypothesis that FFA would inhibit the HPA axis. Rats were cannulated via the jugular vein and infused with different doses of heparin-Intralipid 10% or heparin-saline; sequential blood samples were obtained and analyzed for ACTH, corticosterone, FFA, and glucose. Intralipid at 2.85 ml/kg increased plasma FFA to over 3 meq/liter by 15 min, with a return to baseline by 60–90 min. There was no effect of the infusion on plasma osmolarity or pH. At 60 min, plasma ACTH levels were significantly elevated to over 1500 pg/ml in Intralipid-infused rats, but were unchanged in saline controls. This dose of Intralipid increased corticosterone levels by nearly 20-fold at 120 min. At 180 min, corticosterone levels were still significantly greater than those in saline controls. Lower doses of Intralipid also significantly elevated both FFA and corticosterone levels, but by 180 min, levels of both were similar to those in controls. The effects of Intralipid on corticosterone secretion could not be attributed to the presence of glycerol in the suspension, since glycerol infusions had no significant effect on steroid levels compared to those in saline controls. In dexamethasone-pretreated rats, there was no significant rise in plasma corticosterone after either of two Intralipid doses, suggesting that the action of Intralipid was at a site within the HPA axis above the adrenal gland. This finding also suggested that the high steroid levels after Intralipid treatment were not due to interference with the corticosterone RIA. This was verified by the finding that there was no increase in plasma immunoreactive corticosterone after Intralipid infusion into adrenalectomized rats. Intralipid also caused an increase in plasma glucose levels that was first significant at 60 min and declined to baseline by 180 min, possibly reflecting increased autonomic activity or peripheral insensitivity to insulin. The results suggest that high circulating FFA levels activate, rather than inhibit, the HPA axis in rats. Since stress activates glucocorticoid production and increases FFA levels due to lipolysis, it is possible that FFA and the HPA axis constitute a previously unrecognized positive feedback loop. Finally, the widespread use of Intralipid to ameliorate clinical disorders of metabolism should be considered in light of its potential effects on adrenal steroid secretion. (*Endocrinology* **131:** 2313–2318, 1992)

THE CONCEPT of feedback in endocrinology is a complex one that involves factors other than end-product hormones (1). For example, it is becoming increasingly clear that products of metabolism, such as glucose (2), pyruvate (3), and FFA (4, 5), may all be part of feedback loops that exert control over continued hormone secretion.

One well studied interaction between a hormone axis and metabolic feedback controllers is that of GH and FFA. In man and in animal models, elevation of FFA leads to an inhibition of plasma GH levels (4–9). The site(s) and mechanism of action of FFA are presently uncertain. However, the effects of FFA have been shown to be attenuated in rats by infusion of antiserum to somatostatin, suggesting a hypothalamic action (4). On the other hand, FFA have been shown to directly inhibit the action of GH-releasing hormone on pituitary secretion of GH *in vitro* (10) or in rats with lesions of the medial basal hypothalamus (5). Thus, FFA appear to exert inhibitory control at more than one site in this system, suggesting that they may play an important role in the acute control of GH release. This is especially interesting from a feedback perspective, since one of the actions of GH in all mammals thus far studied is activation of lipolysis, which leads to elevated FFA levels in blood.

The hypothalamic-pituitary-adrenocortical (HPA) axis is another endocrine pathway that, in addition to its numerous other actions, promotes lipolysis and elevated FFA levels in blood. Since the secretion of GH and that of glucocorticoids are at times under similar control (*e.g.* during hypoglycemia in man), we hypothesized that FFA would also exert negative feedback control over the HPA axis. To test this hypothesis, we infused rats with Intralipid 10%, a suspension of fats and glycerol used clinically to relieve the symptoms of essential FFA deficiency and other metabolic disorders. We have found that, contrary to the GH-releasing hormone/GH pathway, FFA actually stimulate the HPA axis in rats, suggesting the possibility of a previously unrecognized positive feedback interaction.

Materials and Methods

Animals

All experiments described in this paper were approved by the Boston University Institute Animal Care and Use Committee. Adult male Sprague-Dawley rats (~250 g) were purchased from Holtzman (Madison, WI) and housed in a 12-h light, 12-h dark photoperiod, with food and water *ad libitum*. A Silastic-tipped polyethylene cannula was inserted into the right jugular vein of rats anesthetized with choral hydrate-Nembutal or, in later experiments, with ketamine-xylazine. The cannula was fed into the right atrium for infusions and for sampling of mixed venous blood. The cannula was flushed at least once each day with

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heparin-saline to maintain patency. Experiments were performed 2 days after surgery. All experiments were performed in the same room that housed the rats during the 2-day recovery period to minimize nonspecific stress associated with moving the cages. One group of rats also received a left carotid cannula in addition to the right jugular cannula. These rats were used to simultaneously measure FFA levels in arterial blood while infusing heparin (100 U/ml)-Intralipid 10% into the venous circulation. This study was performed in urethane-anesthetized rats that were cannulated 1 h before the infusion of Intralipid. The acute preparation and use of anesthesia during the infusion eliminated the difficulties associated with maintaining patent carotid cannulae over several days.

Intralipid infusions

All experiments were begun within 2–3 h of the onset of light. After a single baseline sample was obtained, heparin-Intralipid 10% (Kabi Vitrum, Inc., Alameda, CA; 0.285–2.850 ml/kg BW) or an equivalent volume of heparin-saline (37 C) was infused iv over 2 min into awake, freely moving rats. The infusate was adjusted with heparin-saline to maintain a constant volume in all control and experimental rats. Further blood samples (0.3–0.5 ml) were then obtained at various intervals over the next several hours. All blood samples were collected into heparinized tubes containing EDTA to a final concentration of 2 mM to prevent clotting and proteolysis. Blood was centrifuged immediately at 4 C, and plasma was frozen in aliquots at −25 C. Volume was replaced with heparin-saline. On any given day, one saline control and at least three Intralipid infusions at different doses were simultaneously infused into four or five rats, so that any variability due to ambient conditions could be controlled for among treatment groups.

In some experiments, rats were pretreated with two sc injections of 100 μg dexamethasone 4 and 2 h before saline or Intralipid infusion. In another set of experiments, previously cannulated rats were bilaterally adrenalectomized under ether anesthesia 1 day before infusion and returned to their home cages with food and 0.5% saline water. Infusion of the highest dose of Intralipid (2.85 ml/kg) was begun after a single baseline sample was obtained, and then three further samples were collected at 30-min intervals.

To determine FFA levels in diabetic rats, streptozotocin (65 mg/kg) was injected iv into adult male rats via an indwelling right atrial cannula. Rats were killed by decapitation 3 days after injection, and trunk blood was stored for future determination of plasma glucose and FFA levels.

Miscellaneous

Intralipid 10% is a suspension of soybean oil and glycerol. In plasma, the major FFA generated from this suspension in decreasing order are: linoleic acid (50%), oleic acid (26%), palmitic acid (10%), linolenic acid (9%), and others (5%). ACTH was determined in 50 μl plasma by RIA using the IgG Corp. (Nashville, TN) antibody, as previously described (11). Corticosterone was determined with one of two commercial kits [Ventrex Laboratories, Inc. (Portland, ME), and ICN Biomedicals, Inc. (Costa Mesa, CA); the second source was used when the first source ceased supplying the kits]. Glucose was measured using the glucose oxidase method (Trinder kit, Sigma, St. Louis, MO). Nonesterified FFA were determined using a colorimetric assay kit provided by Eiken Chemical Co. Ltd. (Tokyo, Japan). Osmolarity was measured in 50 μl plasma using a μOsmette microOsmometer (Precision Systems, Natick, MA). Data were analyzed first by one- or two-way analysis of variance (ANOVA), followed by *post-hoc* tests (paired or unpaired *t* test) for individual comparisons.

Results

Infusion of 0.57–2.85 ml/kg Intralipid into urethane-anesthetized rats elevated plasma FFA in a dose-dependent manner up to 6-fold above basal values at 15 min, with a return to basal values (preinfusion) by 90 min for all doses (Fig. 1). There was no effect of saline infusion on FFA levels under similar conditions. In a sample of nine diabetic rats,

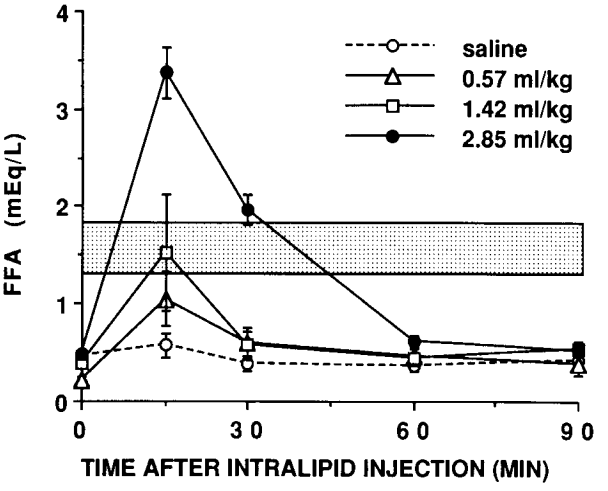

FIG. 1. Effect of Intralipid 10% on plasma levels of FFA. Saline (2.85 ml/kg) or Intralipid 10% (0.57, 1.42, or 2.85 ml/kg) was infused iv after collection of a baseline arterial blood sample in anesthetized rats. Subsequent blood samples were removed via the arterial cannula and were immediately centrifuged at 4 C. FFA were determined using a colorimetric assay kit, with absorbance at 555 nm. Each *point* is the mean and SE for five animals. The *hatched bar* represents the mean and SE of FFA levels determined in nine diabetic rats.

the mean plasma FFA level was 1641 ± 191 μeq/liter (Fig. 1, *hatched area*). The mean plasma glucose level in these rats was 370 ± 9 mg/dl (not shown).

In separate experiments, Intralipid infusion at 2.85 ml/kg had no effect on plasma osmolarity or pH 15 min after infusion (saline, 297 ± 0.9 mosmol/liter, pH 7.42 ± 0.02; Intralipid, 294 ± 1.5 mosmol/liter, pH 7.44 ± 0.02; n = 6).

Intralipid at each of three doses significantly elevated plasma corticosterone in awake, freely moving rats (Fig. 2). This was true whether the comparison was made within a group over time (one-way ANOVA) or between treatment groups (Intralipid *vs.* saline; two-way ANOVA). There was no significant increase in plasma corticosterone after saline or 0.28 ml/kg Intralipid infusion, although these two groups were significantly different from each other by two-way ANOVA. The first significant difference between Intralipid and saline groups was at 60 min. Only in the 2.85 ml/kg group did a significant difference in corticosterone levels remain at 180 min. It appeared that the data were best analyzed by comparing the percent change from baseline, since the baseline steroid levels were variable. Absolute values for each dose and time are presented in Table 1.

Plasma glucose levels were unchanged after saline infusion, but increased significantly from 60–120 min after 2.85 ml/kg Intralipid infusion (Fig. 3). These values were determined in blood from the same rats shown in Fig. 2.

In a separate group of conscious rats, plasma ACTH was measured 60 min after saline or 2.85 ml/kg Intralipid infusion. Intralipid significantly elevated plasma ACTH levels to more than 1500 pg/ml, while saline infusion had no effect (Fig. 4). Plasma corticosterone levels were also significantly elevated in this experiment after Intralipid injection (55 ± 19 *vs.* 225 ± 21 ng/ml; $P < 0.05$), but not after saline injection (21 ± 1 *vs.* 67 ± 24 ng/ml).

Dexamethasone pretreatment completely inhibited the

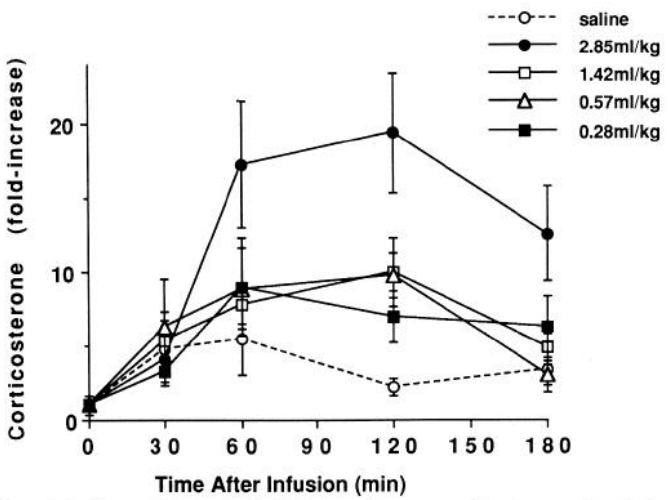

FIG. 2. Effect of Intralipid 10% on plasma corticosterone levels in conscious rats. Different doses of Intralipid 10% were infused over 2 min immediately after a baseline blood sample (time zero). Sequential blood samples were analyzed for corticosterone by RIA. Each *point* is the mean and SE of five to seven animals. The baseline values were 15 ± 2, 67 ± 42, 30 ± 8, 19 ± 4, and 19 ± 5 for saline and 0.28, 0.57, 1.42, and 2.85 ml/kg Intralipid, respectively. Indicators of significance have been eliminated for clarity of presentation. At the $P < 0.05$ level, all Intralipid groups were significantly different from saline-infused controls by two-way ANOVA. By one-way ANOVA, the three highest dose Intralipid groups showed a significant change over time, whereas the changes in saline- and 0.28 ml/kg Intralipid-infused rats were not significant. *Post-hoc* analyses revealed that the first significant difference *vs.* saline controls was at 60 min for all groups, and this difference continued at 120 min. Only the 2.85 ml/kg group was still significantly different from saline controls at 180 min.

TABLE 1. Absolute values for corticosterone (nanograms per ml) for the data shown in Fig. 2

Time (min)	Saline	Intralipid 10%			
		0.28 ml/kg	0.57 ml/kg	1.42 ml/kg	2.85 ml/kg
0	15 ± 2	67 ± 42	30 ± 8	19 ± 4	19 ± 5
30	58 ± 24	168 ± 77	128 ± 44	105 ± 32	56 ± 10
60	75 ± 33	316 ± 91	242 ± 82	133 ± 22	243 ± 51
120	30 ± 6	279 ± 89	292 ± 99	169 ± 33	289 ± 66
180	42 ± 18	290 ± 104	119 ± 66	85 ± 19	235 ± 78

Rats were infused with saline or intralipid at one of the four doses shown.

corticosterone responses to 1.42 and 2.85 ml/kg Intralipid in two separate experiments, one of which is depicted in Table 2.

Corticosterone levels in conscious adrenalectomized rats were near the limit of detection of the assay (<5 ng/ml); this value was not significantly changed during the 90-min period following Intralipid administration (Fig. 5).

Infusion of glycerol at a rate that matched that achieved with the 2.85 ml/kg dose of Intralipid significantly elevated plasma corticosterone at 60 min when evaluated using paired Student's *t* test ($P < 0.05$). However, corticosterone levels were not different at either time point between saline and glycerol-infused rats (Table 3).

Discussion

In rats, man, and other mammals, elevation of plasma FFA by infusion of Intralipid results in pronounced inhibition of

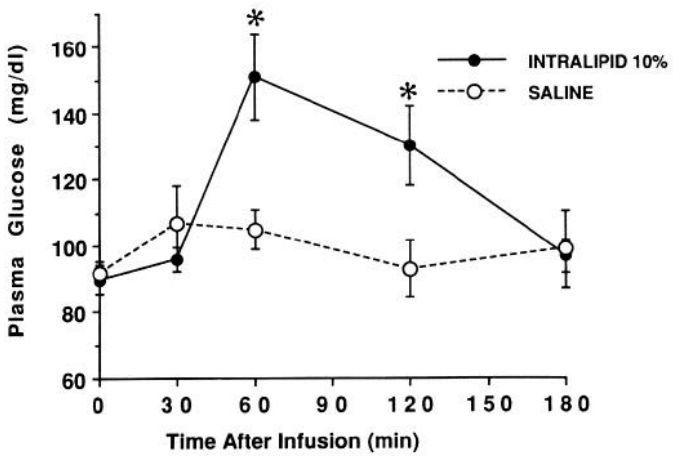

FIG. 3. Plasma glucose levels in conscious rats after Intralipid 10% infusion. Plasma from the saline- and 2.85 ml/kg Intralipid 10%-infused rats in Fig. 2 was further analyzed for glucose levels using the glucose oxidase method. *, At least $P < 0.05$ *vs.* zero time.

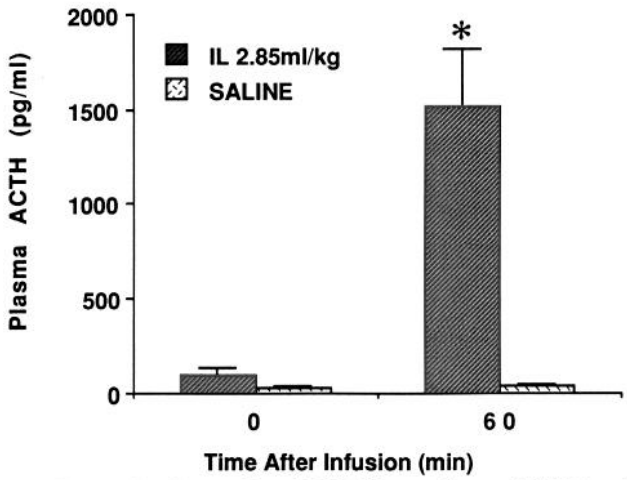

FIG. 4. Effect of saline or Intralipid 10% on plasma ACTH levels. Conscious cannulated rats were infused iv with either saline or Intralipid 10% (2.85 ml/kg) after collection of a baseline blood sample. A second sample was obtained 60 min after Intralipid 10% or saline infusion. ACTH was determined by RIA. Each *bar* is the mean and SE for three (saline) or six (Intralipid 10%) rats. *, At least $P < 0.05$ *vs.* respective baseline.

GH secretion (4–10, 12, 13). Since one of the actions of GH in mammals is lipolysis, it follows that FFA inhibition of GH represents a type of negative feedback, which may be called metabolic feedback (1). We postulated that since glucocorticoids, like GH, are lipolytic, FFA would also inhibit the HPA axis. This was tested by mimicking some of the protocols used in the GH literature, but applying them to the pituitary-adrenal system with two modifications. First, experiments were performed in conscious rats, unlike previous studies that have used anesthetized animals (4, 5, 7). This was done to eliminate potential effects of anesthesia on the HPA axis and to more closely approximate a normal physiological response. Second, the present studies employed lower doses of Intralipid than in previous reports. Again, this was done in an effort to test the effects of FFA elevations that might be similar to those produced under normal or pathological

TABLE 2. Effect of saline or Intralipid infusion on plasma corticosterone in dexamethasone-pretreated rats

Treatment	Time after infusion (min)			
	0	30	60	90
Saline	12 ± 4	10 ± 1	16 ± 2	17 ± 5
2.85 ml/kg Intralipid	16 ± 4	14 ± 3	14 ± 4	22 ± 6

Rats were injected with 100 μg dexamethasone 4 and 2 h before blood sampling. After an initial sample (time zero), saline or intralipid (2.85 ml/kg) was infused for 2 min; further blood samples were obtained at the indicated times. In a second identical experiment, except with an Intralipid dose of 1.42 ml/kg (or saline), all values were below the detection limit of the assay and did not increase after infusion (not shown). Values are expressed as nanograms per ml, and represent the mean and SE for four or five animals. There was no significant effect of Intralipid on plasma corticosterone levels with respect to time or compared to saline controls.

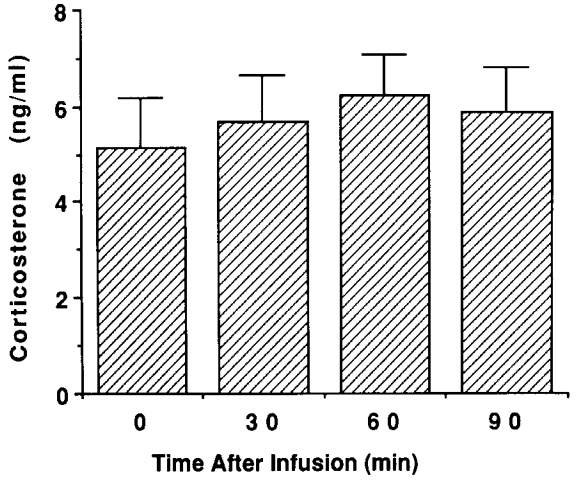

FIG. 5. Effect of Intralipid 10% in adrenalectomized rats. Previously cannulated male rats were bilaterally adrenalectomized under ether anesthesia and allowed 1 day to recover while drinking 0.5% saline water. At this time, a baseline blood sample was obtained, followed by the infusion of 2.85 ml/kg Intralipid 10%. Three more blood samples were obtained at 30-min intervals, and corticosterone was determined by RIA. Each *bar* is the mean and SE for six animals. Corticosterone was near the detection limit of the assay in all adrenalectomized animals and did not increase after Intralipid 10% was infused. The plasma ACTH level in these rats rose from 251 ± 102 pg/ml to 2794 ± 1005 pg/ml at 90 min (not shown).

TABLE 3. Effect of glycerol infusions on plasma corticosterone (nanograms per ml) in rats

Treatment	Time after infusion (min)	
	0	60
Saline	40 ± 13	139 ± 51
Glycerol	48 ± 21	164 ± 52[a]

Saline or glycerol was infused iv over 2 min in an equal volume to match the procedure for Intralipid infusion. The amount of glycerol in the infusate was equivalent to that in the 2.85 ml/kg Intralipid infusion. The infusion began immediately after collection of a baseline sample (time zero). A single sample was then obtained via the venous cannula 60 min after the infusion. Each value is the mean and SE for 10 or 11 animals.

[a] $P < 0.05$ *vs.* respective zero time value.

situations. Moreover, we noted in preliminary studies, using a dose of Intralipid equivalent to that used in many GH studies (~5 ml/kg), that rats became lethargic and occasionally failed to survive repeated blood sampling. It is unclear why the rats used in the present study seemed unable to cope with the higher doses of Intralipid employed by others; perhaps the use of anesthesia confers some protective mechanism against the combined stress of elevated FFA and blood withdrawal. At the highest dose used in the present work, rats occasionally appeared transiently lethargic, but showed no long term ill effects of the infusion (*e.g.* growth, water intake) (Rosen, K., and E. P. Widmaier, unpublished observations). Therefore, we focussed our efforts on these lower doses of Intralipid that were closer to or within the physiological range. In the present studies Intralipid elevated FFA to levels seen in normal stressed rats (14) and patients with metabolic disorders, such as fasting hypoglycemia (15). Higher doses of Intralipid increased FFA to levels comparable to those observed in the uncontrolled streptozotocin-diabetic rat. However, it should be noted that the levels of FFA achieved in the anesthetized rat are only an estimate of those reached in the blood of awake animals using identical procedures.

Contrary to our original hypothesis, elevations in FFA resulted in activation of the HPA axis, as evidenced by significantly increased plasma levels of ACTH and corticosterone. However, it appeared that the sampling protocol itself was mildly stressful, since saline infusion tended to result in elevated corticosterone levels as well, although the response was not significant. It is not known what effect, if any, this superimposed mild stress may have on the HPA axis response to Intralipid infusion. Although we did not observe clear evidence of a dose response, the highest dose of Intralipid produced the greatest increase in corticosterone levels. However, even at 20% of that dose, plasma corticosterone levels were significantly elevated compared to those in saline-infused controls, although the duration of the response was shorter than that at 2.85 ml/kg. Thus, it seems unlikely that the effects of Intralipid were due to some uncharacterized nonspecific stress associated with high levels of infused fats. For example, the effects of Intralipid were unrelated to alterations in blood pH and osmolarity, which remained unchanged when FFA concentrations were maximal.

Plasma glucose levels were elevated 60 min after Intralipid. This may be secondary to sympathomedullary activation, which usually occurs in tandem with HPA axis activation during stress, but was not quantified in this study. However, the increase in glucose could also have been secondary to FFA attenuation of glucose utilization, a long recognized phenomenon in mammals (16, 17). It is interesting to note, however, that euglycemia may be maintained after Intralipid infusion in man, although hepatic gluconeogenesis is apparently increased (18).

The site of action of Intralipid in inhibiting GH secretion in rats and man has been suggested to be both the hypothalamus and the pituitary (4–10). From the present results, it would appear that the action of the fats on the HPA axis of rats is at or above the level of the pituitary, since Intralipid infusion elevated ACTH, and no corticosterone response was observed after dexamethasone pretreatment. Thus, FFA do

not act by directly stimulating steroidogenesis from the adrenal cortex, in agreement with *in vitro* findings in bovine adrenal cells (19). Moreover, the elevated levels of corticosterone were not the result of an assay artifact resulting from interference in the RIA, since there was no rise in plasma immunoreactive corticosterone in Intralipid-infused adrenalectomized rats (or, again, in the dexamethasone-suppressed rats). Moreover, levels of both ACTH and corticosterone were elevated after the FFA had been cleared from the circulation and reduced to control values. FFA have been shown to have direct electrophysiological effects on cells of the central nervous system and to be taken up by cells in the brain (20–22). Thus, although the brain does not use FFA as a source of fuel, it is conceivable that the fats exert their effects on the HPA (and possibly GH) by directly activating an as yet unidentified "FFA-stat" in the central nervous system. This process is presumably selective, since FFA have been shown to have little or no effect on the secretion of several other hormones in man (18, 23), sheep (12), and rats (10).

The steroid and glucose responses to Intralipid could be due to the actions of a metabolite of one of the products of Intralipid. This could also partly explain why the effects of Intralipid on corticosterone secretion persist after the FFA are cleared from the blood. One of the major products of Intralipid is linoleic acid, which is an essential fatty acid and cannot be synthesized by the body. Linoleic acid is also the precursor for the formation of arachidonate, which, in turn, is metabolized intracellularly to prostaglandins and thromboxanes. Both of the latter substances could influence the HPA axis by a variety of pathways. For example, the vasodilatory effects of prostaglandins could be a sufficient stimulus to activate hindbrain afferents that innervate CRH-containing cells in the hypothalamus. However, rapid infusion of Intralipid has typically been found to be associated with moderate hypertension (24). Another possible explanation for the observed responses is that glycerol, which is present in the Intralipid suspension, might account for the stimulation seen after Intralipid infusion. However, glycerol infusions into rats under conditions identical to those used in the Intralipid experiments had no significant effect on corticosterone secretion compared to that in saline-matched controls. Unfortunately, there was considerable variability in resting and postinfusion steroid levels in both groups of rats; thus, it is possible that a small specific effect of glycerol may have been masked by the procedure.

Finally, acute elevation of FFA could be associated with physical sensations, such as nausea or other gastrointestinal symptoms, which might conceivably account in part for HPA activation. However, the effects of Intralipid on GH occur even in the anesthetized rat, and subjective feelings of physical distress have not been reported by Intralipid-infused humans being tested for GH responses (6, 10). In the human studies, similar FFA levels were reached compared to those in the present study, but the infusions were performed over a more gradual time period. Thus, it is possible that the rapid elevation in FFA in the present study in awake animals could constitute a nonspecific stress associated with gastrointestinal discomfort. Nevertheless, this would probably not explain the increase in plasma corticosterone observed at doses of Intralipid that elevated FFA to the high physiological range, since these levels are achieved with a similar time course and for a similar duration after exposure to stress (14).

It is clear that more work is required to determine the precise site and mode of action of FFA on the HPA axis. However, in view of the clinical usefulness of Intralipid in the treatment of essential FFA deficiency and several other metabolic and respiratory disturbances, it seems appropriate to determine to what extent, if any, the interaction of FFA and the HPA axis in rats is paralleled in the human. In both species, there are several situations that may result in elevations of FFA similar to levels attained in this study. Among these are fasting hypoglycemia and uncontrolled diabetes. The streptozotocin-induced diabetic rat is a model for the study of chronic stress, as elegantly described by Scribner and co-workers (25). The present results suggest that part of the chronic stress responses observed in these animals may be due to chronically elevated FFA levels in blood.

Acknowledgments

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References

1. **Widmaier EP** 1992 Metabolic feedback in mammalian endocrine systems. Horm Metab Res 24:147–200
2. **Widmaier EP, Plotsky PM, Sutton SW, Vale WW** 1988 Regulation of corticotropin-releasing factor secretion *in vitro* by glucose. Am J Physiol 255:E287–E292
3. **Berelowitz M, Ting N-C, Murray L** 1989 Glucopenia-mediated release of somatostatin from incubated rat hypothalamus: monosaccharide specificity and role of glycolytic intermediates. Endocrinology 124:826–830
4. **Imaki T, Shibasaki T, Masuda A, Hotta M, Yamauchi N, Demura H, Shizume K, Wakabayashi I, Ling N** 1985 The effect of glucose and free fatty acids on growth hormone (GH)-releasing factor-mediated GH secretion in rats. Endocrinology 118:2390–2394
5. **Alvarez CV, Mallo F, Burguera B, Cacicedo L, Dieguez C, Casanueva FF** 1991 Evidence for a direct pituitary inhibition by free fatty acids of *in vivo* growth hormone responses to growth hormone-releasing hormone in the rat. Neuroendocrinology 53:185–189
6. **Imaki T, Shibasaki T, Shizume K, Masuda A, Hotta M, Kiyosawa Y, Jibiki K, Demura H, Tsushima T, Ling N** 1986 The effect of free fatty acids on growth hormone (GH)-releasing hormone-mediated GH secretion in man. J Clin Endocrinol Metab 60:290–293
7. **Blackard WG, Boylen CT, Hinson TC, Nelson NC** 1969 Effect of lipid and ketone infusions on insulin-induced growth hormone elevations in rhesus monkeys. Endocrinology 85:1180–1185
8. **Blackard WG, Hull EW, Lopez SA** 1971 Effect of lipids on growth hormone secretion in humans. J Clin Invest 50:1439–1443
9. **Quabbe H-J, Bratzke H-J, Siegers U, Elban K** 1972 Studies on the relationship between plasma free fatty acids and growth hormone secretion in man. J Clin Invest 51:2388–2397
10. **Casanueva FF, Villanueva L, Dieguez C, Diaz Y, Cabranes JA, Szoke B, Scanlon MF, Schally AV, Fernandez-Cruz A** 1987 Free fatty acids block growth hormone (GH)-releasing hormone-stimulated GH secretion in man directly at the pituitary. J Clin Endocrinol Metab 65:634–642
11. **Widmaier EP** 1989 Development in rats of the brain-pituitary-adrenal response to hypoglycemia *in vivo* and *in vitro*. Am J Physiol 257:E757–E763
12. **Etienne MJ, Schillo KK, Green MA, Boling JA** 1989 Free fatty

acids suppress growth hormone, but not luteinizing hormone, secretion in sheep. Endocrinology 125:85–91

13. **Casanueva F, Villanueva L, Penalva A, Vila T, Cabezas-Cerrato J** 1981 Free fatty acid inhibition of exercise induced growth hormone secretion. Horm Metab Res 13:348–350
14. **Hoo-Paris R, Jourdan ML, Moreau-Hamsany C, Wang LCH** 1991 Plasma glucagon, glucose, and free fatty acid concentrations and secretion during prolonged hypothermia in rats. Am J Physiol 260:R480–R485
15. **Davidson MB** 1988 Diabetes mellitus and hypoglycemia. In: Hershman JM (ed) Endocrine Pathophysiology: A Patient Oriented Approach, ed 3. Lea and Febiger, Philadelphia, pp 203–263
16. **Randle PJ, Garland PB, Hales CN, Newsholme EA** 1963 The glucose fatty acid cycle: its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. Lancet 1:785–789
17. **Ferrannini E, Barrett EJ, Bevilacqua S, DeFronzo RA** 1983 Effect of free fatty acids on glucose production and utilization in man. J Clin Invest 72:1737–1747
18. **Clore JN, Glickman PS, Nestler JE, Blackard WG** 1991 *In vivo* evidence for hepatic autoregulation during FFA-stimulated gluconeogenesis in normal humans. Am J Physiol 261:E425–E429
19. **Goodfriend TL, Ball DL, Elliott ME, Morrisson AR, Evenson MA** 1991 Fatty acids are potential endogenous regulators of aldosterone secretion. Endocrinology 128:2511–2519
20. **Oomura Y** 1976 Significance of glucose, insulin, and free fatty acid on the hypothalamic feeding and satiety neurons. In: Novin D, Wyrwicka W, Bray G (eds) Hunger: Basic Mechanisms and Clinical Implications. Raven Press, New York, pp 145–157
21. **Love JA, Saum WR, McGee Jr R** 1985 The effects of exposure to exogenous fatty acids and membrane fatty acid modification on the electrical properties of NG108–15 cells. Cell Mol Neurobiol 5:333–352
22. **Dhopeshwarkar GA, Mead JF** 1969 Fatty acid uptake by the brain. II. Incorporation of [14C] palmitic acid into the adult rat brain. Biochim Biophys Acta 187:461–467
23. **Andrews SS, Lopez SA, Blackard WG** 1975 Effect of lipids on glucagon secretion in man. Metabolism 24:35–44
24. **Mathru M, Dries DJ, Zecca A, Fareed J, Rooney MW, Rao TLK** 1991 Effect of fast *vs* slow intralipid infusion on gas exchange, pulmonary hemodynamics, and prostaglandin metabolism. Chest 99:426–429
25. **Scribner KA, Walker C-D, Cascio CS, Dallman MF** 1991 Chronic streptozotocin diabetes in rats facilitates the acute stress response without altering pituitary or adrenal responsiveness to secretagogues. Endocrinology 129:99–108