Effects of epinephrine, corticotropin, and thyrotropin on lipolysis and glucose oxidation in rat adipose tissue

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ABSTRACT (Log dose)–response curves have been determined for lipolysis and for the conversion of glucose-^{14}C to $^{14}CO_2$ by adipose tissue from rats in the presence of epinephrine, corticotropin, and thyrotropin. The stimulatory effect of epinephrine on lipolysis was greater than that of corticotropin or thyrotropin. Lipolysis induced by epinephrine was inhibited by propranolol but only slightly by phenoxybenzamine, whereas lipolysis induced by corticotropin was inhibited by phenoxybenzamine to a much greater extent than by propranolol. Neither blocking drug had a pronounced effect on the response to thyrotropin.

Epinephrine stimulated the oxidation of glucose-^{14}C to CO_2 more than did either thyrotropin or corticotropin. Moreover, epinephrine stimulated the conversion of glucose-^{14}C to CO_2 and fatty acids even when lipolysis was not increased.

These studies indicate that epinephrine can affect glucose utilization independently of its effect on lipolysis.

KEY WORDS adipose tissue · lipolytic hormones · adrenergic receptors · glucose metabolism · epinephrine · corticotropin · thyrotropin · rat

INCUBATION OF ADIPOSE TISSUE with catecholamines stimulates lipolysis and enhances the uptake and metabolism of glucose (1), but whether these two processes occur by the same mechanism has not been unequivocally established. According to Ahlquist (2), the receptors that mediate the effects of catecholamines can be divided into two types, α and β, on the basis of quantitative differences in the response to different catecholamines. β-Receptors are those with a greater response to isoproterenol than to norepinephrine, and α-receptors those for which the relative magnitudes of these responses are reversed. The experiments of Wenke, Muhlbachova, and Hynie (3), of Rudman, Garcia, Brown, Malkin, and Perl (4), and of Fain (5) demonstrate that adipose tissue contains β-adrenergic receptors since isoproterenol is a more potent stimulator of lipolysis than norepinephrine. The effects of catecholamines on glucose metabolism by adipose tissue have not, however, been similarly defined. The introduction of adrenergic blocking drugs that specifically inhibit α- or β-receptors has permitted further analysis of the receptors that mediate the lipolytic response.

In the present experiments the effects of epinephrine on lipolysis and the metabolism of glucose were measured simultaneously in the presence and absence of adrenergic blocking drugs, and the results of these experiments were compared with those from similar studies in which corticotropin and thyrotropin were the lipolytic agents. A preliminary report of part of these studies has appeared previously (6).

Abbreviation: FFA, free fatty acid.

METHODS AND MATERIALS

Animals

Male Holtzman rats weighing between 200 and 400 g were housed in a constant-temperature room with Purina Labena Chow and tap water available ad lib.

Experimental Procedures

The dose–response relationships for the conversion of glucose-^{14}C to $^{14}CO_2$ and for the release of FFA have been determined simultaneously in four experiments each with epinephrine, corticotropin, and thyrotropin. The inhibitory effect of propranolol was tested in two experiments with each hormone and the inhibitory effect of phenoxy-

benzamine in the other two. For each experiment the epididymal fat pads from six to nine rats were distributed into three Petri dishes that contained physiological saline. Small pieces weighing 5–15 mg were cut from the pooled epididymal fat, and several pieces, with a combined weight of 50–80 mg, were placed in 1 ml of Krebs-Ringer bicarbonate buffer, pH 7.4, containing 40 mg of bovine albumin (Fraction V, Armour Pharmaceutical Co., Kankakee, Ill.) and 1 mg of glucose labeled with 0.1 μc of glucose-^{14}C. Epinephrine was diluted in saline from commercial epinephrine (Adrenalin Chloride 1:1000, Parke, Davis & Co., Detroit, Mich.); thyrotropin (Thytropar, Armour Pharmaceutical Co.) and corticotropin[1] were freshly prepared before each experiment in 150 mM sodium chloride. Phenoxybenzamine[2] was present in a concentration of 40 or 400 μg/ml and propranolol[3] at 10 or 100 μg/ml. After each vial had been incubated for 1 hr at 37°C in an atmosphere of 95% O_2–5% CO_2, 0.5 ml of 0.5 N sulfuric acid was added to it. 0.5 ml of *p*-(diisobutylcresoxy-ethoxyethyl)dimethyl benzyl ammonium hydroxide (Hydroxide of Hyamine 10-X, Packard Instrument Company, Inc., Downers Grove, Ill.) was added to a filter paper wick in a polyethylene collection vessel suspended from the cap of the incubation vial and the vials were shaken for an additional hour. $^{14}CO_2$ was counted in a Packard liquid scintillation spectrometer after the Hyamine and polyethylene cap had been transferred to counting vials containing the liquid scintillator (7). Free fatty acids in the medium were determined by the method of Dole (8).

In the final experiment where glycerol release was measured, the medium was neutralized with 0.5 ml of 0.5 N sodium hydroxide after the CO_2 had been collected, and the fat pad was removed. Glycerol was determined in a 0.5 ml aliquot of the neutralized medium by the method of Wieland with minor modifications (9), and the remainder of the medium was used for determination of FFA. The fat pad was rinsed and crushed in extraction fluid (8) and the triglycerides and FFA were extracted into 4 ml of heptane. A 1 ml aliquot of the heptane phase was added directly to 10 ml of the liquid scintillator (7) for determination of the radioactivity in triglycerides. A 2 ml aliquot of the heptane phase was titrated for FFA and then saponified with 30% alcoholic KOH in a steam bath. The saponified triglycerides were acidified with 6 N sulfuric acid and the fatty acids extracted three times with 2-ml aliquots of ether. The ether extracts were dried, liquid scintillator solution was added, and the radioactivity was counted. Quenching was corrected for by addition of an internal standard.

[1] Oxycel-purified corticotropin—130 USP sc U/mg; control 2 27563, Organics, Inc., Chicago, Ill., obtained through the courtesy of Dr. E. B. Astwood.

[2] Dibenzyline (Smith, Kline, & French, Laboratories, Philadelphia, Pa.) diluted from the ampules containing 50 mg/ml.

[3] Crystalline Inderal (Ayrest Laboratories, New York, N.Y.), kindly supplied by Dr. Sahigian-Edwards.

RESULTS

The release of FFA in response to logarithmically increased doses of epinephrine (Figs. 1*a* and 2*a*) increased in a linear fashion over almost the entire range of epinephrine concentrations tested. Propranolol, 100 μg/ml, inhibited release of FFA altogether (Fig. 1*a*) and 10 μg/ml inhibited it at all concentrations of epinephrine below 30 μg/ml. In contrast, phenoxybenzamine, 40 or 400 μg/ml (Fig. 2*a*) had no influence on the release of FFA at the lower concentrations of epinephrine (less than 3 μg/ml), but decreased it at higher ones.

The release of FFA in response to corticotropin (Figs. 1*b* and 2*b*) was less than in response to epinephrine. Phenoxybenzamine, 400 μg/ml, almost completely blocked the release of FFA induced by corticotropin (Fig. 2*b*), whereas propranolol, 100 μg/ml, (Fig. 1*b*) completely inhibited this release only at doses of corticotropin below 0.3 μg/ml, although some depression of the release was evident for all doses of the stimulator. The lower concentrations of propranolol and phenoxybenzamine did not effect the release of FFA in response to corticotropin.

The release of FFA induced by thyrotropin (Figs. 1*c* and 2*c*) was similar in magnitude to that with corticotropin, but less than that with epinephrine. The release of FFA by thyrotropin was not influenced by the lower concentration of propranolol (Fig. 1*c*) or phenoxybenzamine (Fig. 2*c*). Propranolol and phenoxybenzamine (Figs. 1*c* and 2*c*, respectively) at the higher concentration both reduced the rise in FFA, though these inhibitory effects were much smaller than that of propranolol on the release of FFA by epinephrine or that of phenoxybenzamine on the release of FFA by corticotropin.

The dose–response curves for the conversion of glucose-^{14}C to $^{14}CO_2$ are shown in Figs. 3 and 4. Epinephrine had a much greater effect (3- to 4-fold increase) on the production of $^{14}CO_2$ than either corticotropin or thyrotropin (2-fold stimulation).

The stimulatory effect of epinephrine on the conversion of glucose-^{14}C to $^{14}CO_2$ was diminished by both concentrations of propranolol (Fig. 3*a*); the dose–response curves were shifted to the right in the presence of propranolol but remained parallel to that of epinephrine alone. Epinephrine, both 10 and 30 μg/ml, significantly ($P < 0.05$) increased $^{14}CO_2$ formation in the presence of propranolol, 100 μg/ml, in contrast to the inhibitory effect of the latter on the release of FFA induced by these doses of epinephrine (Fig. 1*a*).

Phenoxybenzamine, 400 μg/ml, blunted the effect of high concentrations of epinephrine on the conversion of glucose to $^{14}CO_2$ (Fig. 4*a*), but at 40 μg/ml it had no effect on $^{14}CO_2$ production.

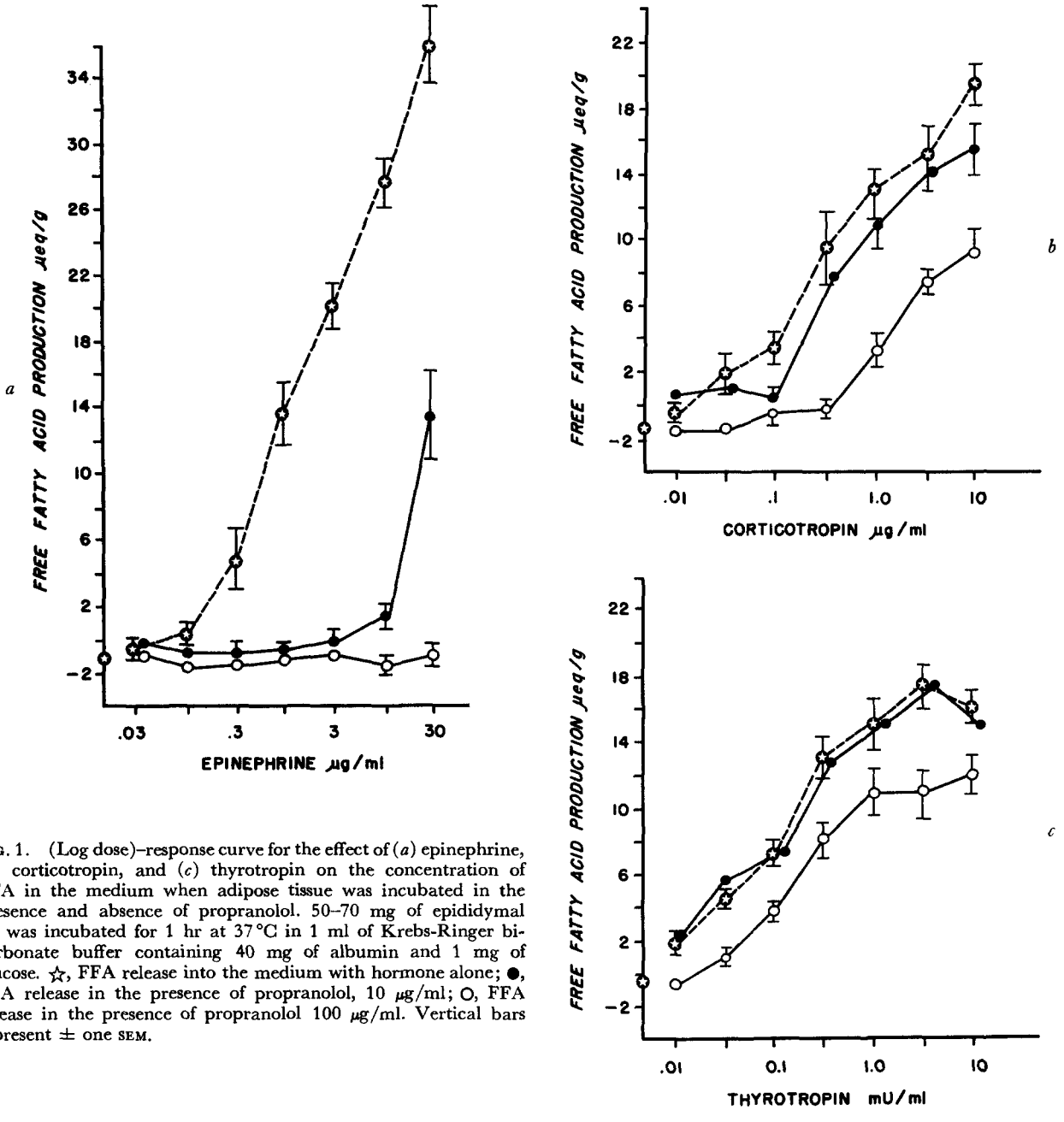

FIG. 1. (Log dose)–response curve for the effect of (*a*) epinephrine, (*b*) corticotropin, and (*c*) thyrotropin on the concentration of FFA in the medium when adipose tissue was incubated in the presence and absence of propranolol. 50–70 mg of epididymal fat was incubated for 1 hr at 37°C in 1 ml of Krebs-Ringer bicarbonate buffer containing 40 mg of albumin and 1 mg of glucose. ☆, FFA release into the medium with hormone alone; ●, FFA release in the presence of propranolol, 10 μg/ml; ○, FFA release in the presence of propranolol 100 μg/ml. Vertical bars represent ± one SEM.

The conversion of glucose to $^{14}CO_2$ in the presence of corticotropin or thyrotropin was less than with epinephrine (Figs. 3 and 4). Propranolol, 100 μg/ml, did not affect the response to thyrotropin but it did reduce the response to some concentrations of corticotropin (Fig. 3). Phenoxybenzamine, 400 μg/ml, slightly inhibited production of $^{14}CO_2$ from glucose at all concentrations of corticotropin and thyrotropin (Fig. 4). The lower concentrations of these drugs had no inhibitory effect on conversion of glucose to CO_2.

The apparent dissociation between lipolysis and the conversion of glucose to CO_2 (Fig. 1*a* vs. Fig. 3*a*) might be explained if propranolol enhanced reesterification. This explanation was tested as follows. The production of FFA, the release of glycerol (10), and the conversion of glucose-^{14}C to glyceride-glycerol were compared in the presence and absence of propranolol, with or without epinephrine (Table 1). Epinephrine, 30 μg/ml, increased lipolysis and enhanced the conversion of glucose-^{14}C to $^{14}CO_2$ and to glyceride-glycerol, but decreased the incorporation of ^{14}C into fatty acids. In the tissue incubated with both propranolol (100 μg/ml) and epinephrine as compared to the unstimulated tissue, the conversion of radioactive glucose to CO_2 and to fatty acids was in-

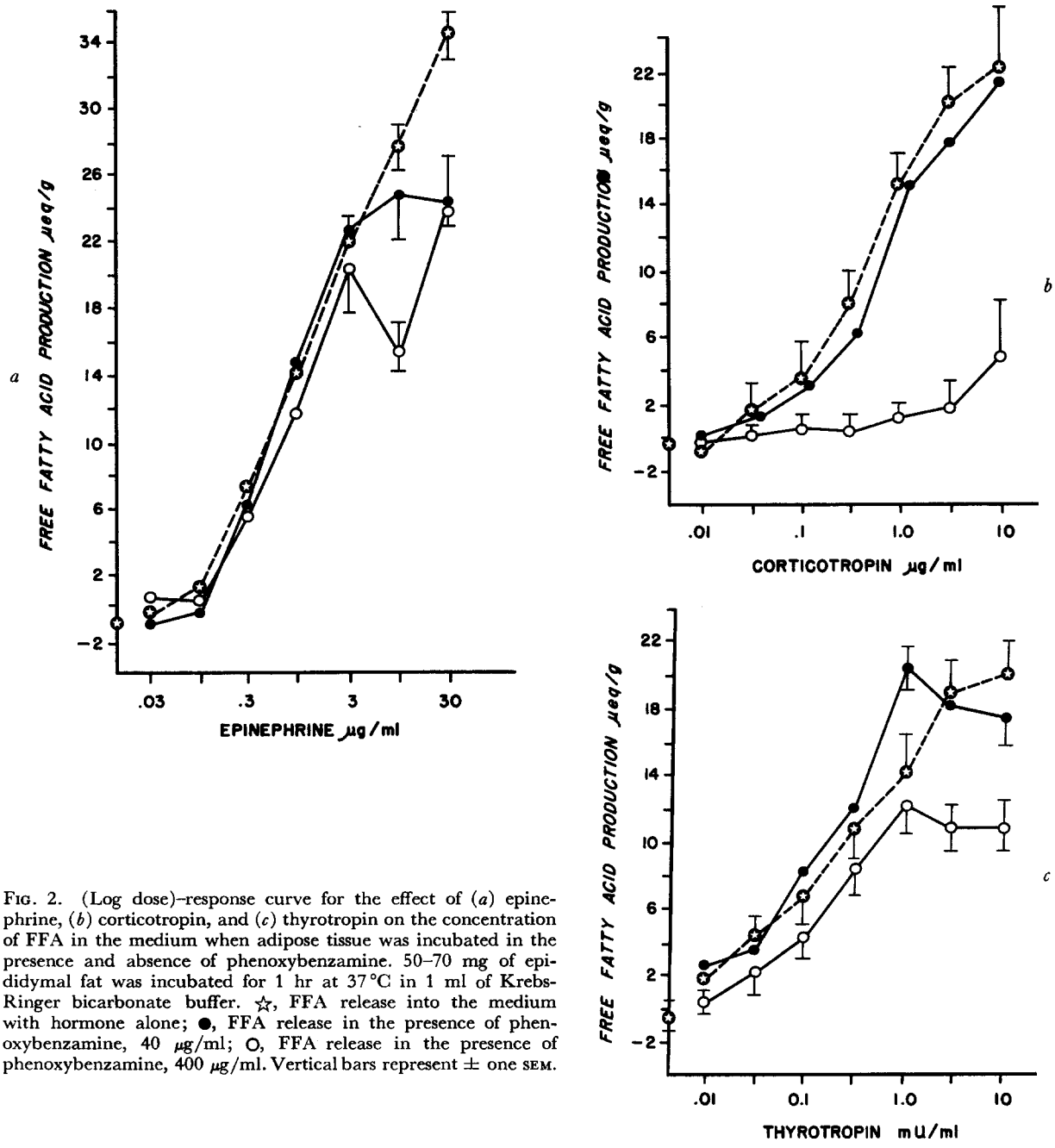

FIG. 2. (Log dose)–response curve for the effect of (*a*) epinephrine, (*b*) corticotropin, and (*c*) thyrotropin on the concentration of FFA in the medium when adipose tissue was incubated in the presence and absence of phenoxybenzamine. 50–70 mg of epididymal fat was incubated for 1 hr at 37°C in 1 ml of Krebs-Ringer bicarbonate buffer. ☆, FFA release into the medium with hormone alone; ●, FFA release in the presence of phenoxybenzamine, 40 μg/ml; ○, FFA release in the presence of phenoxybenzamine, 400 μg/ml. Vertical bars represent ± one SEM.

creased even though glycerol release (lipolysis) was reduced, incorporation of ^{14}C into glyceride-glycerol (esterification) was not changed, and FFA release as well as tissue FFA content remained unchanged.

DISCUSSION

The present experiments have demonstrated that lipolysis and glucose utilization can be dissociated under appropriate experimental conditions. Other laboratories have demonstrated that epinephrine enhances the uptake of glucose and increases the incorporation of radioactivity from glucose into CO_2 and glyceride-glycerol by adipose tissue (11, 12). Cahill, Leboeuf, and Flinn (11) showed that the addition of palmitate to the incubation medium produced a pattern of glucose utilization similar to that seen when epinephrine was added, and they proposed that the effects of epinephrine upon glucose metabolism might be secondary to the high levels of FFA resulting from lipolysis. The effects of thyrotropin and corticotropin on glucose oxidation observed in the present experiments may be explained in this manner since inhibition of FFA release was accompanied by a reduction in the conversion of radioactive glucose to CO_2. The effects of epinephrine,

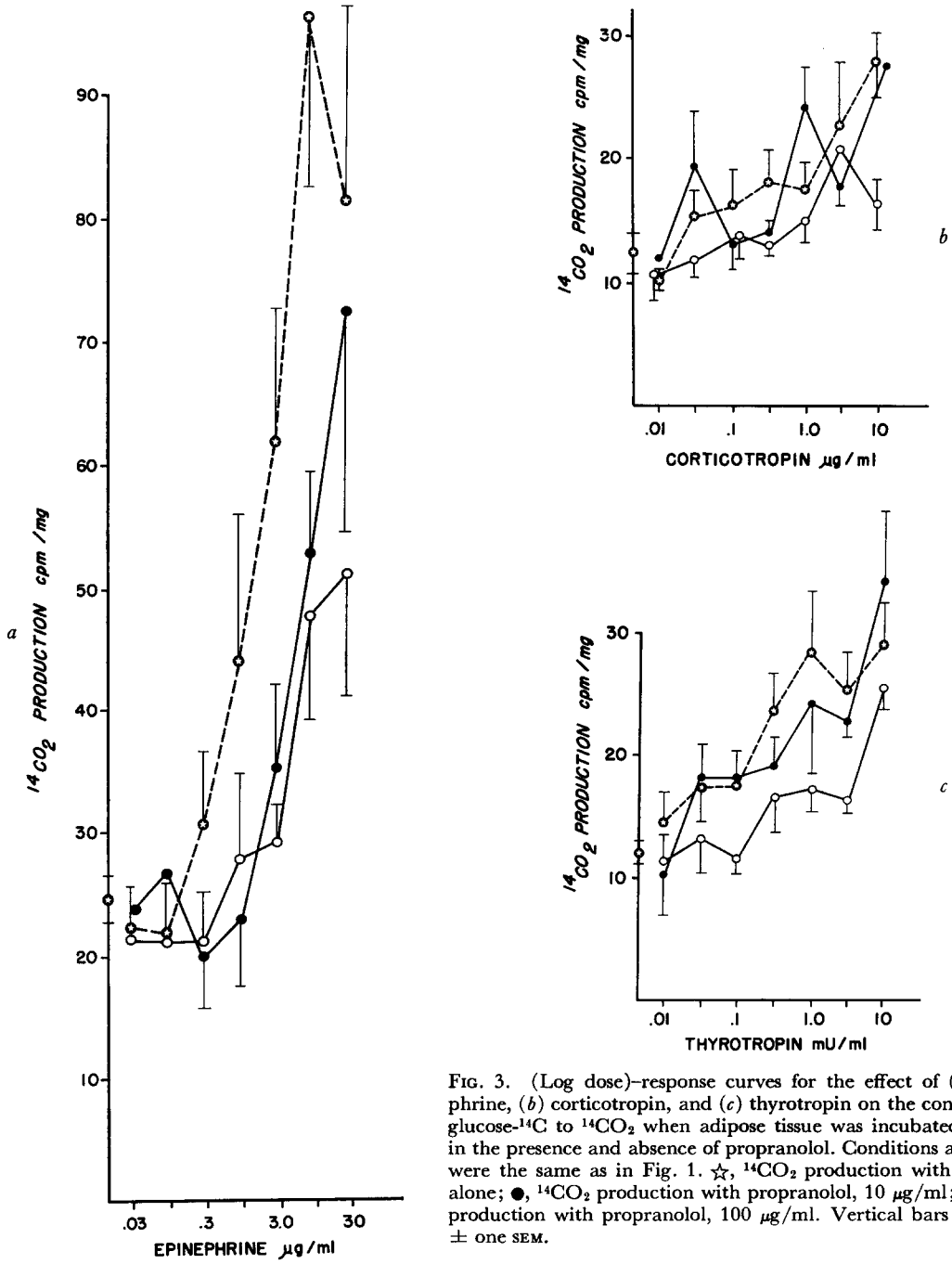

FIG. 3. (Log dose)–response curves for the effect of (*a*) epinephrine, (*b*) corticotropin, and (*c*) thyrotropin on the conversion of glucose-^{14}C to $^{14}CO_2$ when adipose tissue was incubated in vitro in the presence and absence of propranolol. Conditions and tissues were the same as in Fig. 1. ☆, $^{14}CO_2$ production with hormone alone; ●, $^{14}CO_2$ production with propranolol, 10 μg/ml; ○, $^{14}CO_2$ production with propranolol, 100 μg/ml. Vertical bars represent ± one SEM.

however, seem to be more complex. In the presence of propranolol, the (log dose)–response curve for the production of CO_2 was shifted to the right and in the presence of low concentrations of epinephrine, CO_2 production was abolished. Thus, at low concentrations the effects of epinephrine on glucose utilization may be secondary to lipolysis. At higher concentrations, however, as the data in Table 1 show, the incorporation of radioactivity from glucose into CO_2 and fatty acids was significantly increased by epinephrine in the presence of propanolol, whereas the incorporation of ^{14}C into glyceride-glycerol was not increased nor was there an increase in glycerol release or in the concentration of FFA in the medium or tissue.

Further support for the idea that epinephrine has a direct effect on glucose oxidation has come from two sources (13, 14). The work of Love, Carr, and Ashmore (13) has shown that 1-(2′,4′-dichlorophenyl)-2-*t*-butylaminoethanol stimulated conversion of glucose to CO_2, fatty acids, and glyceride-glycerol without enhancing

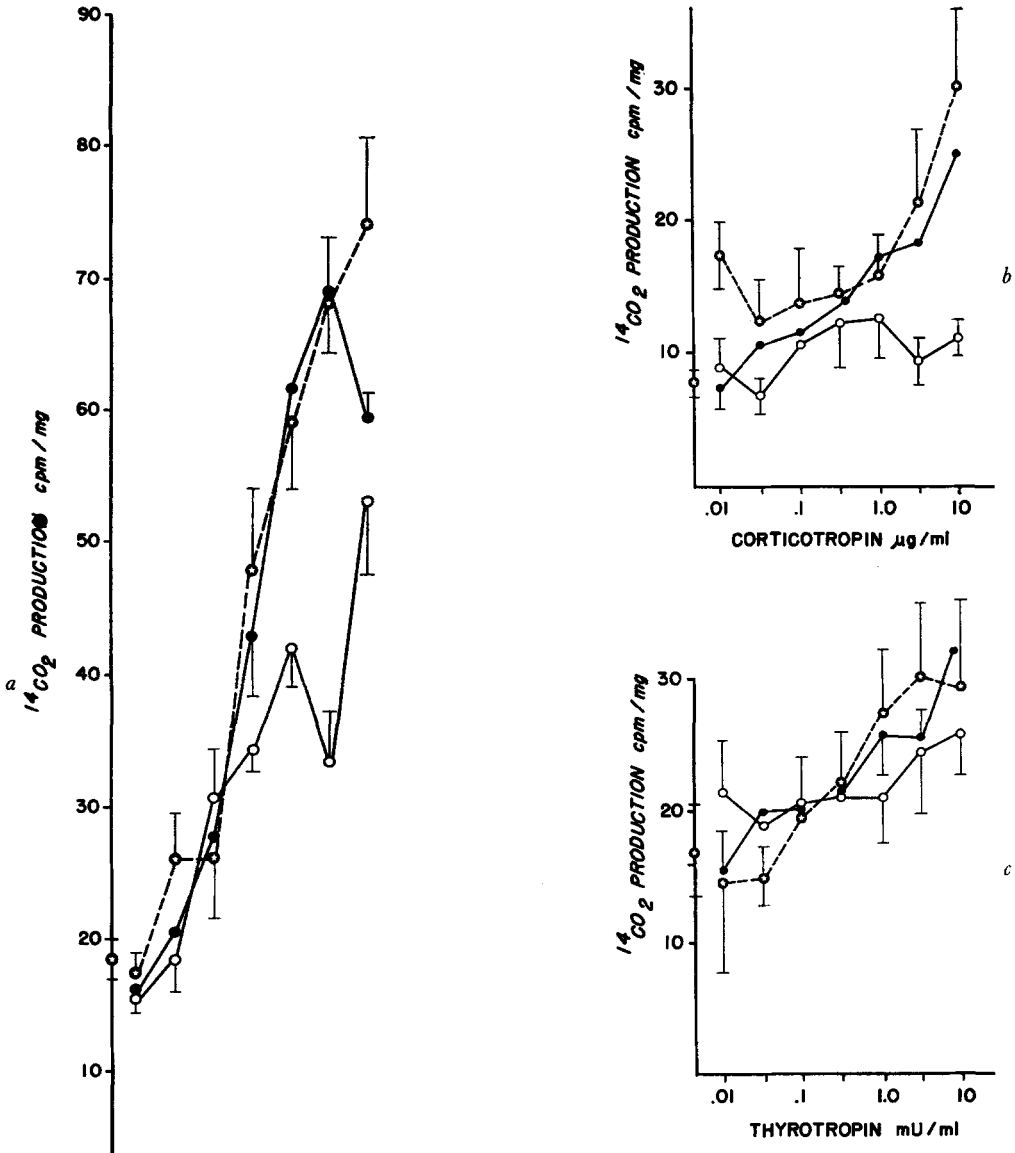

FIG. 4. (Log dose)–response curves for the effect of (*a*) epinephrine, (*b*) corticotropin, and (*c*) thyrotropin on the conversion of glucose-^{14}C to $^{14}CO_2$ when adipose tissue was incubated in vitro in the presence of phenoxybenzamine. Conditions and tissues were the same as for Fig. 3. ☆, $^{14}CO_2$ production with hormones alone; ●, $^{14}CO_2$ production with phenoxybenzamine, 40 µg/ml; ○, $^{14}CO_2$ production with phenoxybenzamine, 400 µg/ml. Vertical bars represent ± one SEM.

lipolysis. Recent studies in our laboratory (14) on adipose tissue from normal and hypothyroid rats have shown that epinephrine-induced lipolysis is diminished in tissue from hypothyroid rats whereas glucose utilization is unimpaired.

It has recently been proposed that lipolysis induced by epinephrine and ACTH is mediated by cyclic AMP (15, 16). Epinephrine and ACTH increase the concentration of cyclic AMP in adipose cells (16), and cyclic AMP can induce lipolysis in broken cell preparations (17). Moreover, dibutyryl cyclic AMP, a derivative of cyclic AMP that is not destroyed by phosphodiesterase, can increase the release of FFA in isolated adipose cells (16). According to the definition of a β-receptor as one in which the response to isoproterenol is quantitatively greater than the response to norepinephrine (2), adipose tissue cells have β-receptors since work from several laboratories (2–4) has shown a greater lipolytic response to isoproterenol than to norepinephrine. Moreover, phenylepinephrine, which is primarily an α-mimetic drug, has much less effect on lipolysis in vitro than does isoproterenol or epinephrine (18). A similar conclusion has been

TABLE 1. EFFECT OF PROPRANOLOL AND EPINEPHRINE ON LIPOLYSIS AND GLUCOSE METABOLISM IN RAT ADIPOSE TISSUE*

	Change in FFA Content		Change in Glycerol Content	Radioactivity Converted into		
	Medium	Tissue		CO_2	Glyceride-Glycerol	Fatty Acids
	μeq/g		*μmoles/g per hr*		*μmoles/g per hr*	
Control	−1.36 ± 0.68†	6.3 ± 0.48	2.14 ± 0.22	6.4 ± 0.84	1.05 ± 0.11	0.21 ± 0.026
Epinephrine	11.8 ± 1.26	17.6 ± 0.80	11.6 ± 0.53	16.0 ± 1.24	2.95 ± 0.20	0.10 ± 0.034
Propranolol	−0.92 ± 0.42	5.4 ± 0.40	1.14 ± 0.25	4.8 ± 0.58	0.55 ± 0.02	0.15 ± 0.021
Propranolol + epinephrine	−1.46 ± 0.33	5.0 ± 0.33	1.74 ± 0.17	9.0 ± 0.62	1.0 ± 0.12	0.38 ± 0.032

* Epididymal fat pads were incubated for 1 hr at 37°C in 1 ml of Krebs-Ringer bicarbonate buffer containing 40 mg of albumin and 1 mg of glucose-^{14}C. The amounts of glucose converted to CO_2, glyceride-glycerol, and fatty acids were calculated from the specific activity of the medium.

† Mean ± SEM (n = 8).

reached from studies on the isoproterenol-induced mobilization of FFA in man: lipolysis is inhibited by the β-adrenergic blocking drug, propranolol, but not by α-adrenergic blockade with phentolamine (19). However, the increase in cyclic AMP and induction of lipolysis may occur by mechanisms other than interaction with β-adrenergic receptors since both α- and β-adrenergic blocking drugs can inhibit lipolysis induced by catecholamines (3, 15, 20–22).

An analysis of the mechanism by which adrenergic blocking drugs inhibit lipolysis has led to the conclusion (15) that the β-adrenergic blocking drugs are competitive antagonists for norepinephrine whereas the α-adrenergic blocking drugs are noncompetitive antagonists. The present data support this concept since propranolol produced a parallel shift of the dose–response curve for epinephrine whereas phenoxybenzamine depressed the maximal response to epinephrine without shifting the dose–response curve. The present data also suggest that the stimulation of glucose oxidation occurred through β-receptors, since propranolol produced a parallel shift in the dose–response curve (i.e., acted as a competitive inhibitor). This also implies that the mechanism for increasing glucose oxidation is more sensitive to epinephrine than the mechanism for stimulating lipolysis since the two processes could be dissociated in the presence of propranolol at certain concentrations of epinephrine.

The nature of the initial hormonal cellular interaction for corticotropin and thyrotropin is less clear than for the catecholamines. For example, corticotropin does not stimulate lipolysis in vitro without calcium in the incubation medium whereas epinephrine does (23). It has been proposed that the response of adipose tissue to corticotropin is mediated by catecholamines since adipose tissue from reserpine-treated rats is alleged not to release FFA when incubated with corticotropin (24). These experiments have been called in question by the work of Edmonson and Goodman (25) and Love, Carr, and Ashmore (20). The present experiments also fail to support the concept that corticotropin-induced lipolysis is mediated by catecholamines. If corticotropin were to act through the release of catecholamines, the pattern of response to the two agents should be similar—but it is not. Moreover, adrenergic blocking drugs produce different types of inhibition with each of these lipolytic agents. Studies on the lipolytic response to corticotropin by Stock and Westerman (15) showed that all of the inhibitors they tested were noncompetitive antagonists of ACTH. The present data are consistent with this formulation. The depression in the dose–response curve for thyrotropin produced by both propranolol and phenoxybenzamine suggests that these drugs may also be noncompetitive antagonists for the lipolytic effects of thyrotropin.

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