Regulation of Human Lipolysis

IN VIVO OBSERVATIONS ON THE ROLE OF ADRENERGIC RECEPTORS

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ABSTRACT Changes in the plasma free fatty acids of a pancreatectomized subject and in free fatty acids and insulin in 10 normal subjects in response to the in vivo infusion of epinephrine alone, epinephrine plus phentolamine, and epinephrine plus propranolol indicate that both alpha and beta adrenergic receptors are present in human adipose tissue. Under the experimental conditions used, adipose tissue appeared to be more responsive to epinephrine than did the cardiovascular system.

INTRODUCTION

It has been postulated that adrenergic receptors are linked to the adenylate cyclase-cyclic AMP system (1). According to this hypothesis an effective interaction of a catecholamine with beta adrenergic receptors causes activation of adenylate cyclase, an increase in cyclic AMP, and an increase in hormone response, while interaction with alpha sites reduces the effective concentration of cyclic AMP leading to an opposite effect on cell function. We have recently tested this hypothesis with a homogenous cell system consisting of human adipocytes, and our findings agreed closely with those predicted by the hypothesis (2, 3). Obviously, the significance of these observations would be enhanced if similar findings were made in the intact human. The in vivo demonstration of alpha site activity in adipose tissue is complicated by the fact that adrenergic agonists and blockers have widespread effects. Particularly troublesome is the influence of catecholamines on the secretion of insulin, a potent antilipolytic hormone. The effects of catecholamines on the cardiovascular system might also obscure or distort metabolic responses. In an attempt to circumvent or minimize these problems, two approaches were used as described below.

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METHODS

Experimental design I. The first approach (see Fig. 1) depended on the absence of regulated insulin secretion. A 20-yr-old man who had undergone a total pancreatectomy a year earlier for an islet cell adenoma located immediatley adjacent to the duodenum was studied over an 8-day period. The subject's acquired diabetes was reasonably well controlled and other than an occasional loose stool, he had no complaints and appeared to be in good health. On days 2 and 6, propranolol was administered; on day 4 and 8, phentolamine was given; no experiments were done on days 1, 3, 5, and 7. On each experimental day, the patient fasted, and his insulin was withheld until after the study was completed. Each study consisted of four 60-min periods. Initially, saline was infused (period I). This was followed by either propranolol, 0.08 mg/min or by phentolamine, 0.5 mg/min (periods II and III); finally epinephrine was infused at a rate of 6 μg/min (periods III and IV). The blockers and epinephrine were obtained from commercial sources; the doses used had been administered without difficulty by Porte (4). Blood was obtained every 15 min for estimation of plasma free fatty acids (5) and glucose. Glucose determinations were made with a prepared reagent, Glucostat obtained from the Worthington Biochemical Corp., Freehold, N. J. Vital signs were closely monitored.

Experimental design II. The rationale of the second experimental design (see Fig. 2) was based in part on the possibility that adipose tissue may be more sensitive to epinephrine than other responsive tissues, such as the islet cells of the pancreas or the cardiovascular system. Accordingly, graded doses of epinephrine were infused into 10 normal fasting young adults. Each subject was studied on 3 different days. Each study was comprised of five 45-min periods. Saline was infused during period I; either propranolol (0.08 mg/min) or phentolamine (0.5 mg/min)

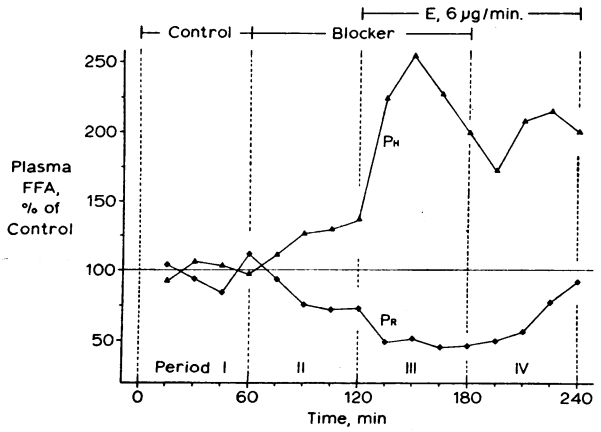

FIGURE 1 The effect of intravenous infusions of propranolol (P_R, diamonds, 0.08 mg/min) and phentolamine (P_H, triangles, 0.15 mg/min) alone and in combination with epinephrine (E, 6 μg/min) on the plasma concentration of free fatty acids (FFA) in a pancreatectomized subject. Each point represents the mean of values obtained on two different days.

was then commenced and infused at a constant rate through periods II, III, IV, and V. In a third series of experiments, saline was continued through period II and no blocker was administered. Epinephrine was started at the outset of period III at a rate of 0.25 μg/min. This was increased to 0.5 μg/min at the beginning of period IV, and then to 1 μg/min (period V). Blood pressure and pulse were determined every 15 min, and blood was obtained for the estimation of free fatty acids, glycerol (6), glucase, and insulin (7).

All subjects studied were hospitalized on the ward of the Clinical Research Center. The protocols followed were approved by the institution's Committee for Projects Involving Human Subjects. The study was explained to each subject and written consent obtained.

Statistical analysis. Standard errors of means were calculated to express the scatter of the data but were not used for comparison purposes. The control values (period I) of the various parameters studied were compared with the values obtained during periods III, IV, and V with the sign test (8).

RESULTS

Experimental design I. During the infusion of saline, propranolol, phentolamine, and the combination of propranolol and epinephrine (period III) the subject was free of symptoms. However, during the administration of epinephrine and phentolamine, the patient was anxious and developed palpitation and mild muscular twitching. Midway during the second phentolamine experiment, the patient complained of chest discomfort and the study was terminated. Except for change in rate, continuous monitoring of the electrocardiograph revealed no changes during any of the four studies.

FFA results expressed as a percent change from the mean of control values are contained in Fig. 1. Each point represents the mean of FFA values obtained on two different days. Propranolol alone depressed the level of FFA in the plasma, and the addition of epinephrine exaggerated this effect, the nadir being less than 50% of baseline values. Phentolamine alone caused a slight increase in FFA and the addition of epinephrine markedly enhanced this effect to a maximum of 250% of the baseline. During the administration of either blocker, plasma glucose concentration gradually rose (data not shown).

Experimental design II. The infusion of epinephrine alone, of either blocker alone, or of the combination of epinephrine and propranolol to the group of normal subjects failed to elicit symptoms. During the administration of the higher doses of epinephrine (0.5 and 1.0 μg/min) in combination with phentolamine, several subjects complained of palpitation, anxiety, nasal stuffiness, and muscle tremor. These symptoms were very mild and in no instance was it necessary to terminate a study. As

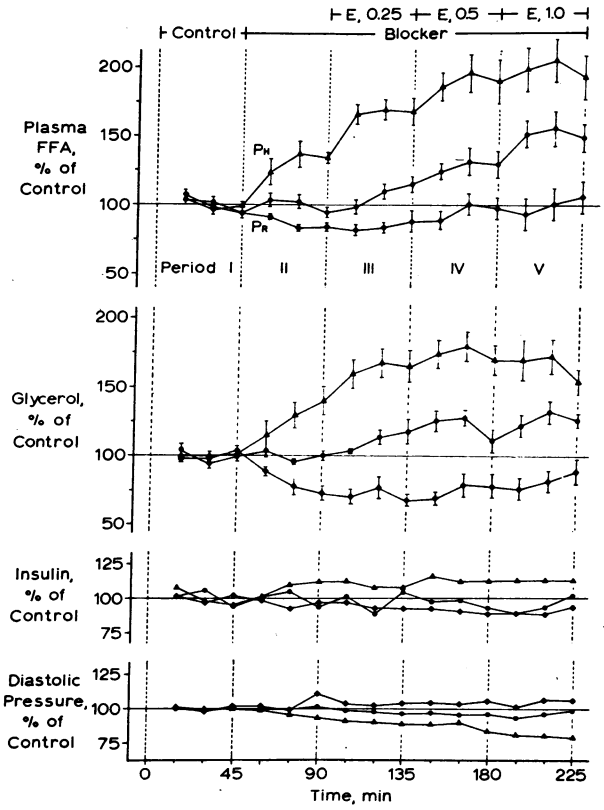

FIGURE 2 The effect of graded quantities of epinephrine (E) alone (closed circles), epinephrine plus propranolol (Pr, diamonds, 0.08 mg/min) and epinephrine plus phentolamine (Ph, triangles, 0.05 mg/min) on the plasma-free fatty acids (FFA), glycerol, insulin and diastolic blood pressure of 10 normal subjects. Each point is the mean of 10 values; the horizontal lines above and below the points are the standard errors of the means.

with the pancreatectomized subject, there were no electrocardiographic changes other than alterations in rate.

Changes in plasma FFA, glycerol, insulin, and diastolic blood pressure are plotted in Fig. 2. The results were calculated as a percent of mean control values in each study; that is, for each subject the average of three samples obtained during saline infusion was considered to be 100%. In Fig. 2, each point is the mean of values taken from each of the 10 subjects. Infusion of phentolamine alone during period II caused an elevation in plasma FFA to about 130% of baseline. When epinephrine was added during periods III, IV, and V, there was a progressive further elevation in FFA to 200%. Epinephrine alone caused a parallel, but much less marked increase in FFA. Propranolol alone depressed FFA, and the addition of epinephrine did not enhance this effect. Alterations in the concentrations of plasma glycerol closely paralleled that of FFA.

These results should be considered in light of the changes in insulin concentration. The infusion of epinephrine plus phentolamine was associated with a modest increase in insulin concentration; this increase was statistically significant (period I vs. IV and V, P values less than 0.05 and 0.01, respectively). The administration of epinephrine plus propranolol caused a slight fall in the level of insulin. Thus, the marked increase in FFA seen with epinephrine plus phentolamine occurred in spite of an augmented level of insulin, and the depression of FFA seen with epinephrine plus propranolol occurred in the face of insulin concentrations below the control base line.

In the presence of propranolol, the lowest infusion rate of epinephrine caused a modest increase in diastolic blood pressure; during the highest infusion rate this increase was statistically significant (period I vs. V, $P < 0.01$). In the presence of phentolamine, epinephrine prompted a small but significant decline in blood pressure (period I vs. III, $P < 0.01$). The latter effect became more marked with higher doses of epinephrine. Changes comparable in degree but opposite in direction were noted in the pulse. Thus, while the lowest infusion rate of epinephrine used in these experiments, 0.25 μg/min, was sufficient, in the face of adrenergic blockade, to alter diastolic blood pressure and pulse, these changes were relatively small compared to those noted in FFA concentration.

The plasma glucose concentration remained unchanged throughout the infusion of the epinephrine-plus-phentolamine combination; during the infusion of epinephrine alone and of epinephrine plus propranolol, it gradually rose. The glucose concentrations in period V were statistically significantly higher than those of period I. The P values for epinephrine alone and for epinephrine plus propranolol were each < 0.01 (data not shown).

DISCUSSION

The results obtained with both experimental design I and II suggest that both alpha and beta adrenergic receptor sites are present in human adipose tissue. When alpha sites are blocked, as during phentolamine administration, the stimulation of beta sites by endogenous catecholamines is unopposed and the level of plasma FFA increases. This effect is substantially enhanced by the administration of epinephrine. When beta sites are blocked, as during propranolol infusion, lipolysis declines and the plasma concentration of FFA tends to fall below the control level. In the pancreatectomized subject this fall was accentuated by the infusion of epinephrine, suggesting that negative alpha sites were being stimulated (experimental design I, Fig. 1). The fact that this accentuation was not observed in normal subjects (experimental design II, Fig. 2) is unexplained.

Other interpretations are, of course, possible. The increase in plasma FFA concentration seen during phentolamine administration conceivably could have been secondary to its effects on muscle. Thus, if the calorigenic action of epinephrine was blocked by phentolamine, presumably the rate of oxidation of FFA in muscle would decline, resulting in a rise in plasma FFA concentration, even though the rate of lipolysis had remained unchanged. Hemodynamic changes might have modified blood flow in adipose tissue resulting in an alteration in the rate of delivery of FFA to the general circulation; conceivably hemodynamic changes might have influenced the disposal rate of released FFA. However, in view of the close parallelism between the in vitro results previously reported and the findings reported here, it would seem unlikely that circulatory changes alone could account for the present data.

Dual adrenergic receptors appear to be operative in other metabolically important tissue. Turtle and Kipnis (9) demonstrated the presence of both alpha and beta receptors in rat pancreatic tissue, and Batzri, Selinger, and Schramm (10) have presented evidence indicating the existence of both alpha and beta sites in rat parotid gland tissue; the former prompted release of potassium ion while the latter stimulated amylase secretion. Propranolol had no effect on the alpha adrenergic response, suggesting that the alpha receptor response in this tissue is not related to cyclic AMP.

If we assume that both beta and alpha adrenergic receptors are present in human adipose tissue, basic questions remain unanswered. Does alpha site stimulation reduce cyclic AMP by inhibiting adenylate cyclase by stimulating phosphodiesterase, or by some other mechanism? More fundamental questions concern the survival value that dual adrenergic sites had primitive man, and the physiological advantage, if any, of such a system to modern man. Of interest are the recent studies of Rosen-

quist (11) demonstrating that adipose tissue from hypothyroid patients fails to respond to the lipolytic action of epinephrine; this effect was not due to impaired beta site activity, but rather to enhanced alpha adrenergic responsiveness.

Of several other species (rat, swine, dogs, rhesus monkey, guinea pig, and hamster) whose adipose tissue we have tested for alpha site activity, only two, the hamster and monkey, were found to have both beta and alpha receptors.

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