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Research Article

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The Effect of Adrenergic Blockade on the Glucagon Responses to Starvation and Hypoglycemia in Man

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ABSTRACT In an attempt to ascertain whether the sympathetic nervous system modulates glucagon release in man during starvation and hypoglycemia, the influence of alpha and beta adrenergic blockade on glucagon responses was studied in young, healthy men subjected to fasting and insulin-induced hypoglycemia. Six volunteers fasted for 84 h on three separate occasions. Plasma immunoreactive glucagon (IRG), measured initially at 12 h, climbed gradually from mean levels of 54 pg/ml to a zenith of 124 pg/ml at 48 h, with maintenance of these levels for the duration of the fast. The infusion of propranolol or phentolamine throughout the terminal 24 h of the second and third fasts failed to alter the pattern of IRG release. After an overnight fast, five volunteers received insulin intravenously, which evoked a mean rise in plasma IRG levels from 63 pg/ml to a maximum of 256 pg/ml at 30 min. The concurrent administration of propranolol or phentolamine did not modify the glucagon responses to insulin-induced hypoglycemia. These data suggest that the augmented glucagon release in man during starvation or after hypoglycemia is not significantly regulated by signals from the adrenergic nervous system.

INTRODUCTION

It is generally accepted that the sympathetic nervous system modulates insulin secretion through interaction with alpha and beta adrenergic receptors in the pancreatic beta cell (1). The extensive ramification of adrenergic nerve endings in pancreatic islets of sub-

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primate species (2, 3) also raises the possibility that the alpha cell may be influenced by sympathetic activity. Indeed, in states of stress, such as infection, exercise, and diabetic ketoacidosis, where elevated urinary and plasma catecholamines have been recorded (4-6), plasma glucagon levels are increased (7-10). Furthermore, infusion of catecholamines and their agonists have been reported to stimulate glucagon secretion in a variety of animals, both in vivo and in vitro (11-15). However, it is unclear whether this effect is mediated through alpha or beta adrenergic receptors, since the results in various species are inconsistent and contradictory. A regulatory role of the adrenergic nervous system in human glucagon secretion has yet to be clarified. In man, both enhanced glucagon release and activation of the sympathetic nervous system are sequels of starvation and insulin-induced hypoglycemia (16-23); therefore, the present investigations were designed to determine whether the increases in plasma glucagon levels under these circumstances were influenced by alpha or beta adrenergic blockade. In addition, these studies also examined the possibility of a circadian rhythm in glucagon release during fasting.

METHODS

Subjects and procedures. After a 12-h overnight fast, healthy nonobese men between the ages of 18 and 25, without a family history of diabetes mellitus, were admitted to beds in the Clinical Research Center of the University Hospital. An 18-gauge butterfly needle was inserted into an antecubital vein of each arm and kept patent with 0.9% saline. Food was withheld from six subjects for an additional 72 h on three separate occasions. Throughout the final 24 h of the second and third fasts, propranolol (Inderal, Ayerst Laboratories, New York) and phentolamine (Regitine, Ciba Pharmaceutical Co., Summit, N. J.) were infused at 0.08 and 0.5 mg/min, respectively. 2 liters of 0.9% saline was given during each 24 h period, and, ad lib. oral intake of water was permitted, as was ambulation on the ward. After 12 h of fast, blood was obtained at intervals of 2 h throughout the study. Five different subjects received

regular insulin (0.15 U/kg) by rapid intravenous push on three separate mornings. On the second morning, 30 min before insulin administration, propranolol was given as a 5-mg bolus, and then instilled at 0.08 mg/min for 150 min by Holter pump. Similarly, on the third morning, phentolamine was given as a 5-mg bolus, followed by an infusion at 0.5 mg/min for 150 min.

Analytic techniques. All blood samples were withdrawn into heparinized tubes containing benzamidine at a final concentration of 0.05 M to prevent degradation of glucagon (24). The tubes were kept in ice, the plasma separated by centrifugation within 30 min, and replaced in ice for up to 3 h before freezing at -20°C . Immunoreactive glucagon (IRG)¹ was measured by modification (24) of the cellulose adsorption method of Nonaka and Foa (25), using antiserum 30-K² and benzamidine at a final concentration of 0.01 M. [¹²⁵I]Glucagon (sp act = 500–600 mCi/mg) was purchased from New England Nuclear, Boston, Mass., and before use was purified by gel filtration on Sephadex G-25. Employing charcoal to adsorb glucagon, Weir, Turner, and Martin (26) have shown that 30-K antiserum measures substances in plasma of higher molecular weight than glucagon, which result in spuriously high basal levels under conditions of their assay. Although this has not been universally observed, we have also found that basal IRG levels measured in native plasma varied widely among individuals, and, using charcoal adsorption, we have confirmed the findings of Weir. To separate the interfering plasma factor(s) from IRG in our immunoassay, we have used the following procedure modified from Manns (27), in which glucagon is extracted into acetone. 2.3 ml of analytic grade acetone was added to 1 ml cold plasma which was mixed immediately by Vortex, centrifuged at 2,000 rpm for 4 min at 4°C , and the supernate decanted. The pellet was re-extracted twice with 1 ml of deionized water-acetone solution (3:7; vol/vol). The three combined supernates were evaporated to dryness *in vacuo* and stored at -20°C . At the time of assay, the extract was dissolved in 0.4 ml of water containing 20 mg of bovine serum albumin (Pentex Biochemical, Kankakee, Ill.). The recovery of crystalline pork glucagon added to plasma was 70%, with a reproducibility of $\pm 5\%$, and all samples have been corrected to 100%. Interassay coefficient of variation was ± 20 pg and intrassay variance was ± 13 pg. Insulin (IRI) was measured by radioimmunoassay, using human standards and separation of bound from free by cellulose adsorption (28). Glucose was determined by an autoanalyzer adaptation of the Hoffman ferrocyanide technique (29). Conventional statistical analysis including standard errors (SEM) and comparisons of paired difference by Student's *t* test were calculated on a Wang model 600 computer (Wang Laboratories, Tewksbury, Mass.) (30).

RESULTS

IRG responses during fasting without and with adrenergic blockade. Fig. 1 shows the mean plasma levels of IRG, IRI, and glucose in the six volunteers throughout 84 h of fast. The first samples obtained at 12 h after initiation of the fast serve as reference points for comparison with subsequent changes and

¹Abbreviations used in this paper: IRI, immunoreactive insulin; IRG, immunoreactive glucagon.

²Purchased from Diabetes Research Foundation, University of Texas Southwestern Medical School, Dallas, Tex.

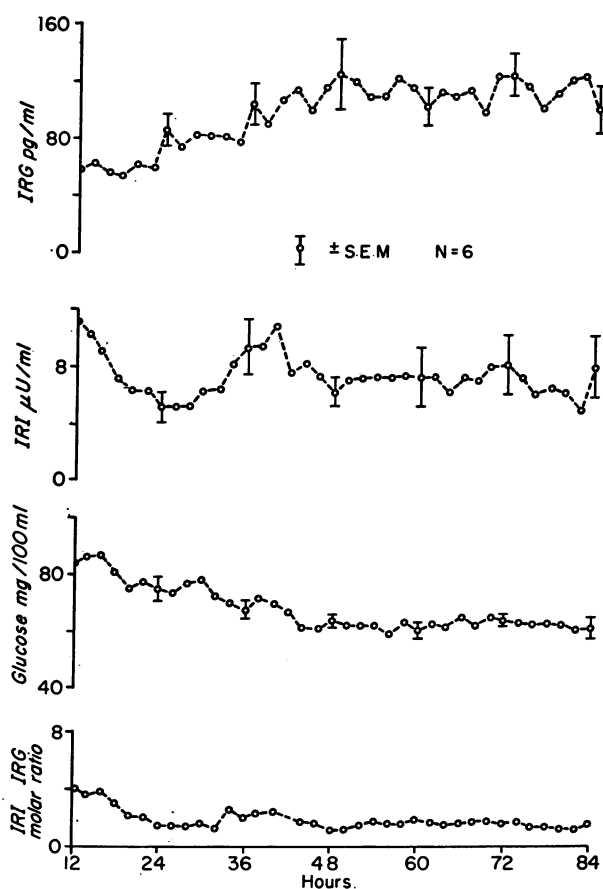


FIGURE 1 Mean levels of plasma IRG, IRI, glucose, and the IRI:IRG during fasting in six normal young men throughout 84 h of fast.

are termed basal. Basal plasma IRG levels varied from 37 to 81 pg/ml (mean, 59 pg/ml) among the six subjects and with progression of the fast, in all, there was a gradual irregular rise in IRG with an eventual plateau which was achieved at different times for each individual. Nevertheless, in all instances, this had been reached by 48 h at which time the mean zenith was 124 pg/ml. Greater than a twofold increase in IRG concentrations from basal occurred in five of six men and subsequently, each person tended to maintain these elevated levels. Though there was a tendency to oscillate throughout the period of observation, no regular periodicity could be discerned for the individual or for the group, particularly since no reliance could be placed on differences of IRG of 20 pg/ml which fell within assay variance. Basal plasma glucose levels ranged from 78 to 92 mg/100 ml (mean 84 mg/100 ml) and progressively fell and reached a mean level of 63 mg/100 ml by 54 h after beginning of the fast which was subsequently maintained. Similarly basal IRI levels,

ranging from 6–15 $\mu\text{U/ml}$ (mean 11 $\mu\text{U/ml}$), declined during the fast achieving a mean nadir of 5 $\mu\text{U/ml}$ within 28 h. In general, the rise in IRG tended to coincide with the fall in both plasma glucose and IRI, however, because of the temporal variation in decrement of glucose and insulin and rise in glucagon in any given subject, there was no statistically significant correlation between onset of glucagon response and rate or magnitude of fall of plasma glucose or insulin. Similarly, individual variation in glucose and IRI levels precluded meaningful direct correlations. As predicted from the previous studies of Aguilar-Parada, Eisentraut, and Unger (16), the mean insulin to glucagon molar ratio slowly declined to a plateau by 48 h.

In Figs. 2 and 3 are compared the effects on IRG levels of propranolol and phentolamine, which were infused separately throughout the last 24 h of the sec-

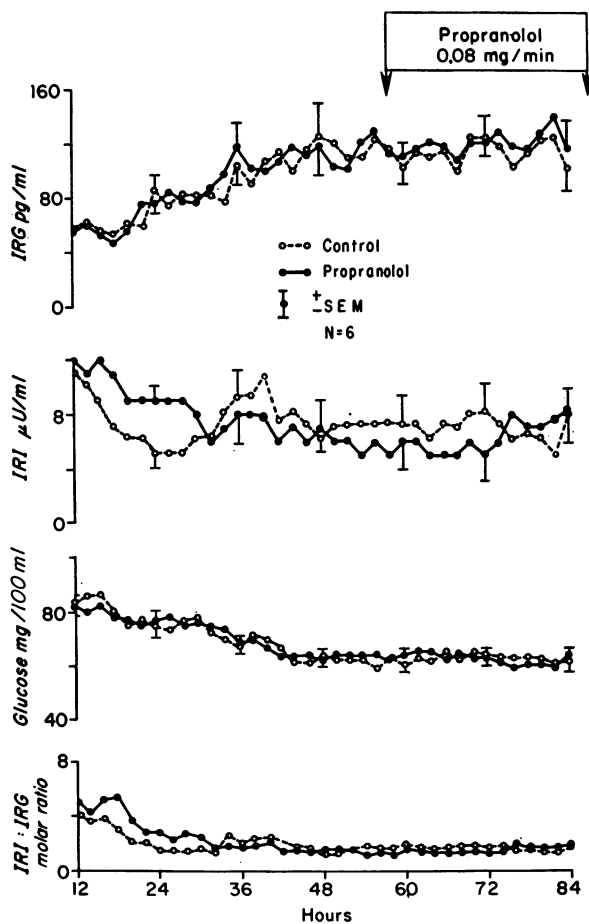


FIGURE 2 The effect of propranolol on plasma IRG, IRI, glucose, and IRI:IRG during fasting. The mean values of six normal young men are shown throughout two separate 84-h of fasts. Propranolol was infused during the terminal 24 h of the fast indicated by the solid line.

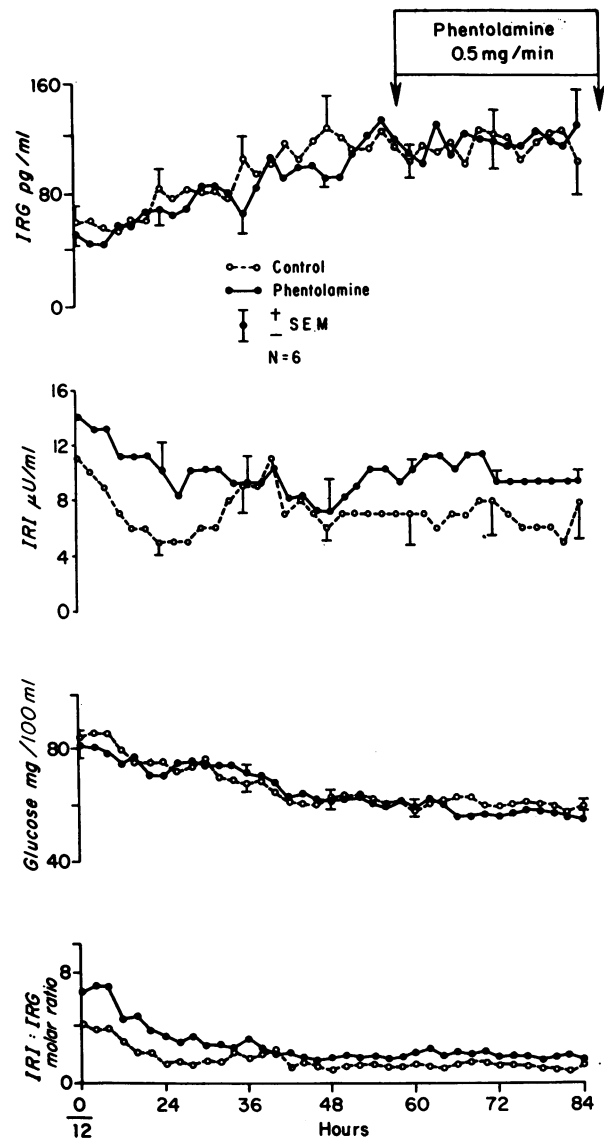


FIGURE 3 The effect of phentolamine on plasma IRG, IRI, glucose, and IRI:IRG levels during fasting. The mean values of six normal young men are shown throughout two separate 84-h fasts. Phentolamine was infused during the terminal 24 h of the fasts indicated by the solid line.

ond and third fasts, respectively. Paired comparisons of the mean IRG values for each subject during the first 60 h in the three repetitive starvation periods, demonstrated that both the onset and magnitude of rise were highly reproducible. With the continuous 24 h infusion of propranolol or phentolamine, there were no significant changes in the IRG patterns when analyzed by paired comparisons with those in the terminal 24 h period of the control fast. Plasma IRI and glucose concentrations were also not different from those ob-

served at the same time interval in the control fast, nor were they significantly altered by the infusion of either propranolol or phentolamine. Similarly, the decline in the insulin to glucagon molar ratio was not significantly affected by adrenergic blockade. That blockade of conventional alpha and beta adrenergic receptors had been accomplished by the phentolamine and propranolol was judged by the characteristic alterations in cardiovascular dynamics.

IRG responses to insulin-induced hypoglycemia without and with adrenergic blockade. The effects of administration of insulin on plasma glucose and IRG levels in five healthy young men are shown in Fig. 4. Mean plasma glucose concentrations ranged between 81 and 85 mg/100 ml during a 45-min control period and dropped rapidly to a nadir of 31 mg/100 ml at 15 min after insulin injection. These levels persisted for an additional 15 min and then gradually rose. Mean plasma IRG levels, ranging between 57 and 66 pg/ml throughout the control periods, abruptly rose to a mean zenith of 256 pg/ml 30 min after insulin injection and slowly declined thereafter. All subjects demonstrated at least a threefold increase in IRG concentration between 30 and 35 min after insulin.

As also shown in Fig. 4, the simultaneous infusion of propranolol in the same five subjects did not significantly alter the IRG responses to insulin-induced hypoglycemia. Similarly, when phentolamine was concurrently instilled, IRG values were not appreciably changed after insulin (Fig. 5). Neither the rate of fall

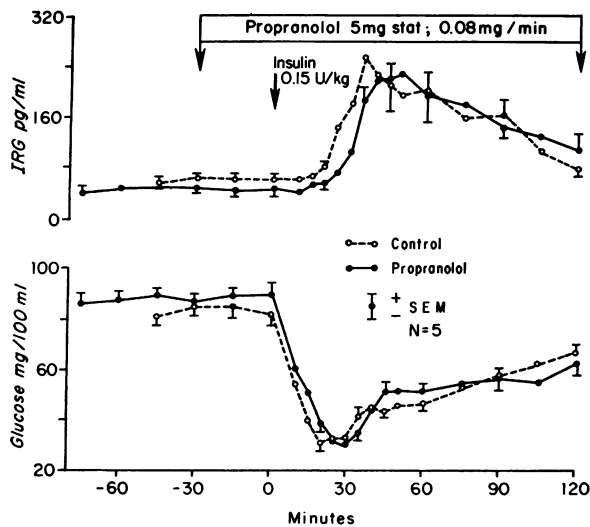


FIGURE 4 The effect of propranolol on the response of plasma IRG and glucose to insulin-induced hypoglycemia. The mean values during two studies of five normal young men are shown. Propranolol, beginning 30 min before insulin, was infused during the studies indicated by the solid line.

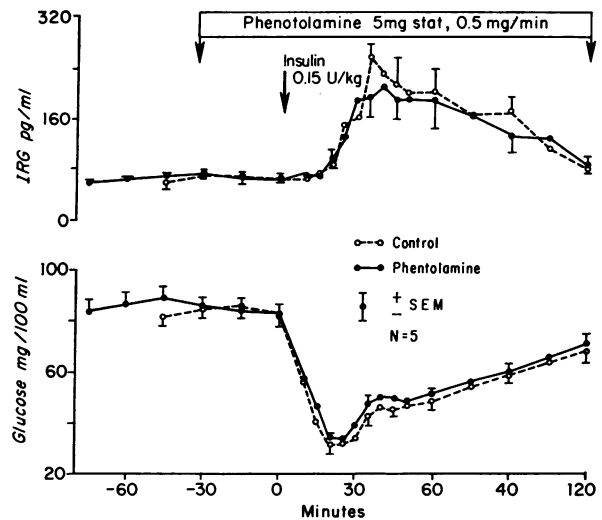


FIGURE 5 The effect of phentolamine on the response of IRG and glucose to insulin-induced hypoglycemia. The mean values during two studies of five normal young male volunteers are plotted. Phentolamine, beginning 30 min before insulin, was infused during the studies indicated by the solid line.

nor absolute level of plasma glucose achieved was modified by the concomitant administration of either of the two sympathetic blocking agents.

DISCUSSION

Though involvement of the adrenergic nervous system in the control of insulin and growth hormone secretion has been confirmed frequently (1, 31, 32), its role in glucagon release is not established. In particular, discordant results after administration of catecholamines and specific adrenergic antagonists have been obtained depending upon the species examined. In ducks, epinephrine infusion has been reported to elicit a glucagon release which is attenuated by phentolamine and augmented by propranolol (11-13). Furthermore, glucagon secretion is allegedly enhanced by norepinephrine and suppressed by isoproterenol suggesting mediation by alpha adrenergic receptors in this bird (12, 13). In contrast, one group of investigators has shown in the rat an increase in basal glucagon levels after phentolamine and an inhibition of exercise-induced glucagon release after propranolol, thus implicating beta receptor mechanism in effecting release (33); however, others have reported, in rats swimming to exhaustion, that phentolamine inhibited glucagon secretion (34). In dogs, it has been claimed that the glucagon response to alanine is potentiated by concurrently infused phentolamine (35). In the isolated perfused canine pancreas, glucagon release is evoked by epinephrine, norepinephrine, and isoproterenol, which in turn is blocked by propranolol, thereby implying that these changes in glu-

cagon secretion are mediated through a beta adrenergic receptor (15). That glucagon secretion may be augmented through neural pathways is indicated by the hyperglucagonemia after electrical stimulation of the ventromedial nucleus of the hypothalamus (36), and after stimulation of the splanchnic nerves in calves (37) and mixed sympathetic pancreatic nerve trunk in the anesthetized dog (38). Nevertheless, the rise in glucagon levels after hypothalamic stimulation in the rat was not abolished by adrenalectomy (36), or by pharmacologic doses of phentolamine or propranolol (36).^{*} Studies of the interrelationship between the adrenergic nervous system and the alpha cell secretion in man have also been conflicting. The infusion of epinephrine at 6 $\mu\text{g}/\text{min}$ has been reported to cause a rise in plasma glucagon levels, which is attenuated by phentolamine, implicating an alpha adrenergic-mediated effect (39). In contrast, we have been unable to demonstrate any meaningful alteration in plasma glucagon levels in six men after a 60-min infusion of norepinephrine or epinephrine at 6 $\mu\text{g}/\text{min}$, isoproterenol at 2–6 $\mu\text{g}/\text{min}$, or methoxamine at 200 $\mu\text{g}/\text{min}$. During arginine administration, concomitant alpha receptor blockade and beta receptor stimulation has been reported to cause enhanced glucagon release beyond that achieved by arginine alone (40, 41). In the present studies, conventional doses of propranolol or phentolamine failed to modify the glucagon responses during fasting or after insulin-induced hypoglycemia, thereby suggesting that the release of glucagon under these circumstances is not primarily mediated through alpha or beta adrenergic receptors. Furthermore, we have noted a glucagon rise after hypoglycemia in a 20-yr-old man after bilateral adrenalectomy, while on maintenance glucocorticoid, which was indistinguishable from that found in healthy young men. This is in keeping with the observations in the adrenalectomized rat after hypothalamic stimulation (36), and in calves after splanchnic nerve section (42), and indicates that epinephrine is not responsible for glucagon secretion during glucopenia. It is difficult to reconcile the diverse findings with regard to relationships of the adrenergic nervous system to glucagon release in man and other species. Subject and protocol differences, as well as the possibility that the various glucagon radioimmunoassays measure substances other than pancreatic glucagon, may contribute to the divergent results.

The factors responsible for glucagon release with hypoglycemia or during starvation remain unclear. The abrupt increase in plasma glucagon levels after insulin-induced hypoglycemia occurs immediately after a precipitous drop in glucose. Though the sensing by the alpha cell of low extracellular glucose concentra-

^{*}Frohman, L. A. Personal communication.

tions seems a likely mechanism for glucagon release in this setting, the possibility that the cholinergic nervous system is responsible for glucagon discharge cannot be discounted (42). In general, the rise of glucagon with fasting coincides temporally with the gradual decline of glucose (43); yet, it is uncertain whether intracellular alpha cell glucopenia is the major signal (43).

Studies of prolonged starvation have consistently shown that glucagon levels reach a zenith on the 3rd day of the fast and subsequently decline to values slightly above those before the fast. Glucose, however, reaches its nadir by the 2nd or 3rd day of fast and remains at this level; thus, the drop in glucagon cannot be attributed to alterations in extracellular glucose. It is possible that free fatty acids or ketones, which are elevated at this time, may inhibit glucagon release, as has been demonstrated in isolated rats islets and in the anesthetized dog (44–45). Several amino acids have been shown to stimulate glucagon secretion (46), and in man, it has been postulated that one or more amino acids may be responsible for augmented glucagon release in fasting (17). Leucine, isoleucine, and valine rise during the first 5 days of starvation, but subsequently fall (47). In dogs a response to leucine, but not to isoleucine, has been found in one study (48), but not confirmed in another (49). As suggested by the exaggeration of the glucagon response to arginine after 3 days of starvation, it is possible that endogenous amino acid levels enhance glucagon release from an alpha cell previously sensitized by low intracellular glucose levels. Despite these speculations, our results suggest that in man, the adrenergic nervous system is unlikely to exert a significant modulating influence on the hyperglucagonemia occurring during fasting or after insulin-induced hypoglycemia.

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