Stimulation of Glucagon Secretion by Ethanol-induced Hypoglycemia in Man

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SUMMARY

In fasted man, ethanol lowers plasma glucose by inhibiting gluconeogenesis with concomitant suppression of insulin release. Since glucose regulation of glucagon (IRG) secretion may be insulin dependent, we have evaluated IRG secretion in this setting of hypoglycemia and insulin deficiency. Mean IRG levels in six men fasted for fifty-six hours rose gradually from a basal level of 57 pg/ml. to 101 pg/ml. During the subsequent four-hour ethanol infusion, mean glucose concentration fell only 18 mg. per 100 ml. (26 per cent of pre-infusion values) yet IRG tripled to 265 pg/ml. Insulin (IRI) fell to unmeasurably low values. Alcohol given after only eight hours of fasting has no effect on plasma levels of glucose, IRG and IRI. These results suggest that the small decrease in extracellular glucose combined with relative insulin deficiency may cause inordinate intra-alpha cell glucopenia and result in exaggerated glucagon release. DIABETES *24*:295-300, March, 1975.

Glucagon release in normal man is suppressed during hyperglycemia and stimulated during low glucose concentration.[1-3] In diabetic patients neither their elevated fasting blood sugar nor further elevation by glucose administration diminishes their glucagon levels.[4,5] Dogs made diabetic with alloxan also have hyperglucagonemia in the face of hyperglycemia and in this experimental model insulin treatment restores the capability of glucose to inhibit glucagon secretion.[6] Similarly, in insulin deficient isolated islets and in pancreatic slices from streptozotocin treated rats, the normal suppressive effect of glucose on glucagon release is lost and is regained by the addition of insulin.[7] Thus, it has been postulated that glucose-mediated inhibition of glucagon release is insulin dependent[6,7] so that the alpha cell, in the absence of insulin, is "*glucose blind*." In addition, insulin, independent of glucose, may inhibit the alpha cell.[8] Stimulation of glucagon secretion by hypoglycemia is usually induced by the administration of insulin such that the alpha cell is exposed simultaneously to low glucose concentration and high levels of insulin. Under these conditions, normal man responds to the abrupt fall in sugar by a two- to threefold increase in glucagon levels.[2,3] It has been reported that insulin in high concentrations prevents the stimulation of glucagon release by glucopenia.[9] Thus, simultaneous lowering of glucose level and concomitant hypoinsulinemia, thereby removing the suppressive effect of insulin on the alpha cell, might be expected to result in an exaggerated glucagon response compared to that seen during reduction of glucose concentration induced by insulin administration. The infusion of ethanol into fasted subjects, by inhibiting gluconeogenesis, lowers blood glucose and consequently further diminishes insulin levels.[10,11] This has enabled us to evaluate glucagon secretion during glucopenia when insulin levels were low in an attempt to examine further the role of insulin in the glucagon response to hypoglycemia.

METHODS

Six twenty- to thirty-year-old male volunteers, within 20 per cent ideal body weight,[12] with no family history of diabetes, normal fasting plasma glucose concentrations and no concurrent medical problems were studied on the Clinical Research Center after an overnight fast. They continued to fast for the following forty-eight hours and had blood drawn every six hours.

On the third morning 15 per cent ethanol in normal saline was infused through an indwelling needle in an antecubital vein by Holter pump over four hours at a rate of 125 ml./hr. Four baseline specimens were collected over one hour prior to the infusion and then

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during the infusion every half-hour blood was withdrawn from a vein in the opposite arm for insulin (IRI), glucagon (IRG) and glucose determinations. To assess if there was a glucagon response to alcohol in the absence of hypoglycemia the subjects were given the same ethanol infusion after only an overnight fast. Each subject was informed of the nature, purpose, expected reactions and potential risks prior to their voluntary participation.

All blood samples were collected in heparinized tubes and immediately placed on ice; those for IRG also contained benzamidine at a final concentration of 0.05 M to prevent degradation of the glucagon.[13] Plasma was separated by centrifugation within thirty minutes of collection and frozen at -20° C.

Employing charcoal to adsorb glucagon, Weir et al.[14] have shown that 30-K antiserum* measures substances in plasma of higher molecular weight than pancreatic glucagon, which result in spuriously high basal values under conditions of their assay. Although this has not been universally observed, we have confirmed these findings and therefore in our assay have employed the following acetone extraction procedure modified from Manns[15] to remove the interfering plasma factor(s).

To 1 ml. cold plasma 2.3 ml. of analytic grade acetone was added, mixed immediately by Vortex, centrifuged at 2,000 RPM for four minutes at 4° C, and the supernatant decanted. The pellet was re-extracted twice with 1 ml. of deionized water-acetone solution (3:7; V/V). The three combined supernatants were evaporated to dryness in vacuo and stored at -20° C. At the time of assay these extracts were dissolved in 0.4 ml. of water containing 20 mg. of bovine serum albumin.

We use benzamidine at a final concentration of 0.01 M and antiserum 30-K in our glucagon assay and separate bound from free antibody by cellulose adsorption.[16]

The recovery of crystalline pork glucagon added to plasma was 70 per cent, with a reproducibility of ± 5 per cent, and all samples have been corrected to 100 per cent. Interassay coefficient of variation was ± 20 pg and intra-assay variance was ± 13 pg.

Insulin was measured by double antibody radioimmunoassay[17] and glucose by a modification of the cupric-neocuproine reduction method.[18] Conventional statistical analysis including standard errors (SEM) and Student's t-test were calculated on a Wang model 600 computer.[19]

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

RESULTS

Figure 1 depicts the IRG, IRI and glucose responses to the fast and the subsequent ethanol infusion. At the beginning of the study basal glucagon concentrations in the six subjects averaged 57 pg./ml. and in each volunteer glucagon levels progressively rose during the continued forty-eight hours of fasting to a mean value of 101 pg./ml. Insulin concentrations measured in five of the six volunteers declined during the two days of fasting coincident with the fall in plasma sugar. The ethanol infusion resulted in a further reduction in plasma glucose with five out of six falling to 50 mg. per 100 ml. or less and insulin levels, measured in four out of six, declined from a mean of 6.6 μU/ml. to 2.2 μU/ml., a value indistinguishable from 0 in our assay. Four of the six volunteers experienced clinical neuroglucopenia and all felt dramatically better with the administration of 25 gm. glucose intravenously at the end of the alcohol infusion. Even though the fall in plasma sugar concentration was slight (mean of 18 mg. per 100 ml.) there was a dramatic two- to threefold increase in glucagon levels from a mean of 101 pg/ml. to a maximum of

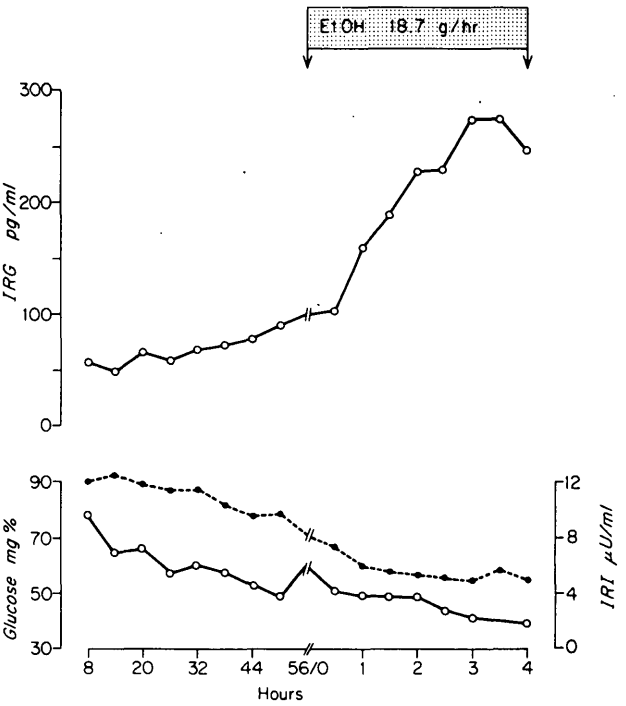

FIG. 1. Glucagon, insulin (o———o), glucose values during the fifty-six-hour fast and subsequent four-hour ethanol (ETOH) infusion in six men. Glucose (●----●) was measured in four of six subjects during the fast.

274 pg/ml. at three and a half hours. Quantitatively this rise in glucagon was similar to that observed during insulin-induced hypoglycemia[2,3] where the fall in glucose was larger (60 mg. per 100 ml. and 52 mg. per 100 ml.) and more abrupt: nadir at twenty to thirty minutes after insulin injection versus two to three hours during alcohol infusion. The glucagon elevation was sustained coincident with the sustained lowered sugar induced by ethanol. One subject (D.H.), whose plasma glucose concentration was relatively high after the forty-eight hour fast (87 mg. per 100 ml.) and fell to only 72 mg. per 100 ml. with the ethanol infusion, had the smallest rise in glucagon values (table 1). He was slightly heavier than the other subjects and this is in accord with the observation that obesity confers a relative resistance to the hypoglycemic effects of alcohol.[20] As shown in figure 2, when the increase in glucagon during the four hour infusion was expressed as the area above baseline a direct correlation was found with the decrement in glucose expressed as the area below basal ($p<0.001$). In addition, when the subjects were considered individually, the rise in glucagon was temporally related to the fall in sugar (table 1). During the ethanol infusion after an overnight fast, there was no change in glucose, insulin or glucagon values, thereby eliminating a direct stimulatory effect of the ethanol or one of its metabolic products on glucagon secretion (figure 3).

Two minor observations deserve mentioning. The endogenous hyperglucagonemia did not reverse the ethanol-induced block of hepatic glucose production similar to the resistance of ethanol-induced hypoglycemia to exogenous glucagon.[21] The suppressive effect of alcohol on the insulin response to intravenous

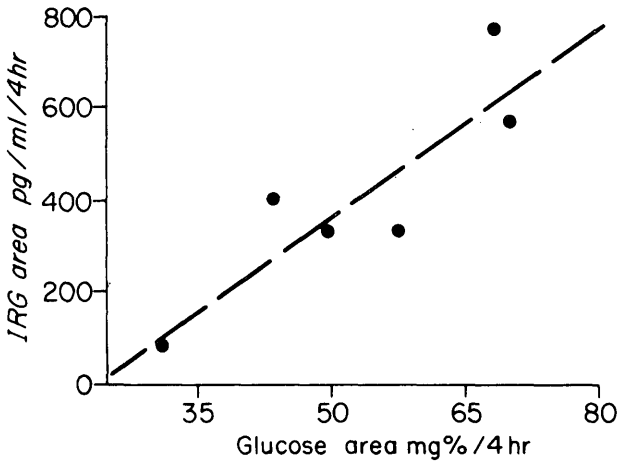

FIG. 2. Glucagon area above basal vs glucose area below basal over the four-hour ethanol infusion. (r=0.88)

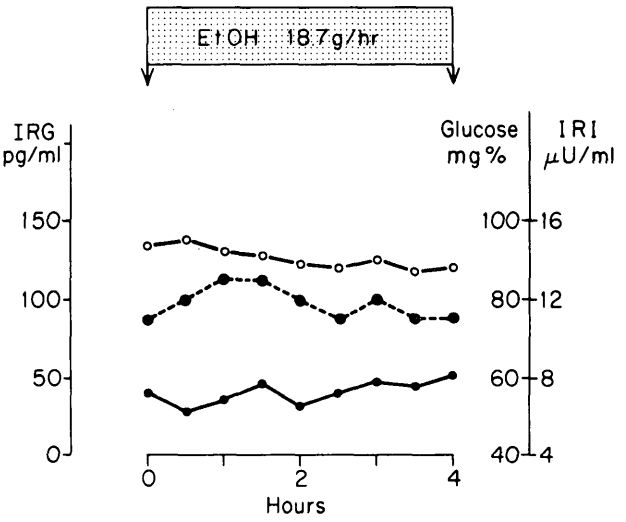

FIG. 3. IRG (●——●), IRI (●----●) and glucose (○——○) during ethanol infusion, after overnight fast in six men.

TABLE 1

IRG (pg/ml.) and glucose (glu-mg. per 100 ml.) for each subject during ethanol infusion after fifty-six-hour fast

Subject		Control	½ hr.	1 hr.	1½ hr.	2 hr.	2½ hr.	3 hr.	3½ hr.	4 hr.
B.S.	IRG	201	217	211	274	416	490	576	576	581
	GLU	75	70	68	61	56	—	49	—	45
D.W.	IRG	104	100	325	251	259	268	268	322	274
	GLU	70	—	50	—	—	—	—	—	50
D.G.	IRG	73	103	120	219	188	174	191	265	180
	GLU	65	70	55	50	50	50	50	—	55
D.H.	IRG	78	83	86	94	100	103	123	114	88
	GLU	87	84	84	79	79	77	77	74	72
B.M.	IRG	69	63	66	114	225	182	217	194	222
	GLU	65	58	53	50	50	50	50	50	55
M.K.	IRG	79	57	160	182	182	160	268	171	137
	GLU	65	51	49	49	49	49	49	51	51
$\bar{X}$ SEM	IRG	101±20	104±24	161±39	189±30	228±43	230±56	274±64	274±67	247±72
	GLU	71±4	67±6	60±6	58±6	57±6	57±7	55±6	58±8	55±4

doses of glucagon,[22] plus the inhibition of beta cell secretion by hypoglycemia[23] may explain the complete failure of the elevated glucagon levels to stimulate insulin secretion during the ethanol infusion.

DISCUSSION

The inability of glucose to suppress glucagon release in dogs with alloxan diabetes[6] and in isolated islets from streptozotocin-treated rats[7] plus the return of suppression with insulin administration[6,7] strongly supports the hypothesis that insulin is required for the alpha cell's response to hypoglycemia. Although insulin, independent of glucose, has been postulated to inhibit glucagon secretion,[8] the inhibition of glucagon release by glucose is probably not merely secondary to induced-insulin secretion since other insulin secretogogues such as isoproterenol[24] and arginine[25] unrelated to their effects on blood sugar stimulate rather than inhibit glucagon secretion.

In contrast to the numerous studies evaluating the role of insulin in glucose suppression of alpha cell function, the possible role of insulin in the cell's recognition of hypoglycemia as a stimulus to glucagon secretion has been little investigated. In this study the administration of ethanol to fasted subjects lowered both glucose and insulin concentrations and elicited a similar glucagon response to that observed during insulin-induced hypoglycemia.[2,3] Preliminary results on glucagon secretion during ethanol-induced hypoglycemia in fasting pigs[26] are in agreement with our findings in man.

In our studies, ethanol increased glucagon secretion only after the two-day fast. The metabolic alterations induced by ethanol are minimally affected by fasting since alcohol elicits similar changes in alanine, lactate, and acetate in either the post-absorptive or fasted state;[27-29] therefore ethanol itself or the secondary increased acetate or lactate is probably not the cause of the glucagon rise since there was no change in glucagon when ethanol was administered after an overnight fast. The resultant lowering of insulin during ethanol-induced hypoglycemia might be expected to raise the branched chain amino acids, valine, leucine and isoleucine,[30] but these amino acids, at least in the dog, are devoid of glucagon stimulating ability. Free fatty acids were not measured and so their possible contribution to the glucagon rise cannot be evaluated. Fasting is known to enhance the glucagon response to arginine[32] and possibly fasting for fifty-six hours primed the alpha cell and partially accounted for the exaggerated glucagon response.

In addition, since insulin may directly inhibit the alpha cell,[8] suppression of IRI to levels indistinguishable from zero may be partially responsible for the increased glucagon, just as insulin deficiency even in the face of marked hyperglycemia is presumably responsible for the hyperglucagonemia of experimental diabetes. Hypoglycemia is well recognized to result in increased parasympathetic[33] and sympathetic[34] nervous system activity and increased adrenal medullary discharge.[34,35] Both in vitro and in vivo studies have suggested a possible role for adrenergic[24,36] and/or vagal[37,38] modulation of glucagon secretion. Epinephrine and isoproterenol infusions in man have been reported to stimulate glucagon secretion with blockade of the isoproterenol stimulation by propranolol (β blocker)[36,41] but the dose of epinephrine used probably exceeded adrenergic stimulation achieved by endogenous epinephrine release.[42] Employing insulin-induced hypoglycemia as the stimulus for glucagon release we have observed normal IRG responses in normal man during pharmacologic alpha and beta adrenergic blockade,[3] in sympathectomized man secondary to cervical cord transection[39] and in bilateral adrenalectomized patients.[40] Additionally, others have demonstrated that propranolol did not block the alpha cell response to hypoglycemia in rats.[43] Therefore, although under certain other conditions the adrenergic system may modulate glucagon secretion, it is unlikely that the sympathetic nervous system response to hypoglycemia, in this case ethanol induced, mediated the glucagon increase that we observed. Bloom has reported that truncal vagotomy or the administration of atropine markedly attenuated the glucagon response to insulin-induced hypoglycemia.[44] Using a different antibody for pancreatic glucagon and different assay technics we have been unable to reproduce these results.

Even though the fall in sugar over the four-hour ethanol infusion was slight, averaging 18 mg. per 100 ml. or 26 per cent of basal, and in no one to levels less than 45 mg. per 100 ml. if insulin translocates glucose or influences its metabolism in the alpha-2 cell thereby suppressing glucagon release, then the associated hypoinsulinism may have resulted in exaggerated intra-alpha cell glucopenia and thereby markedly enhanced the glucagon secretion. The direct correlation between net lowering of glucose and degree of glucagon stimulation (figure 2) and the temporal correlation between the fall in blood sugar and the rise in glucagon levels seen in most subjects (table 1) strongly suggests that the alpha cell response was directly due to the glucopenia.

In summary, the administration of ethanol to normal volunteers after a fifty-six-hour fast resulted in a slight fall in glucose levels, suppression of circulating insulin to values indistinguishable from zero in our assay, and a dramatic stimulation of glucagon secretion comparable to that seen with the much larger and more abrupt decrement in glucose after insulin administration.[2,3] The hypoinsulinemia probably potentiated the hypoglycemic effect on the alpha cell of the simultaneous decline in sugar and accentuated the glucagon response.

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