Effects of Long-term Infusion of Glucagon on Carbohydrate Metabolism, Insulin and Growth Hormone Secretion in Patients with Congestive Heart Failure

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SUMMARY

Glucagon was given by continuous intravenous infusion for ninety-six hours to eight patients with severe congestive heart failure. The dose was 4 or 2 mg. per hour. Results were similar in both groups and are presented together.

Mean fasting blood sugar increased significantly during infusion (from 87 to 103 mg./100 ml.). Mean fasting serum immunoreactive insulin (IRI) increased considerably (from 9.1 to 32.0 μU./ml.) and mean fasting serum immunoreactive growth hormone (HGH) increased from 2.8 to 11.9 ng./ml.

Intravenous glucose tolerance tests were performed before and during glucagon infusion. The glucose disappearance rate (K-value) declined significantly during the infusions (from a mean of 0.85 to 0.46), although serum IRI responses to the intravenous glucose were much higher than before. Serum HGH levels were not suppressed by the glucose and sometimes even showed a rise.

A carbohydrate-rich meal induced the same rises in blood sugar during glucagon infusion as before, but serum IRI responses were much higher during glucagon, and elevated serum HGH values were not normalized.

It is concluded that long-term infusion of pharmacological doses of glucagon to patients with cardiac insufficiency leads to a state of mild carbohydrate intolerance, characterized by some increase in fasting blood sugar and decrease in glucose disappearance rate, in spite of very considerable rises in serum IRI. This diabetes-like state may be accounted for by elevated levels of serum HGH, although other insulin-antagonistic factors may be involved. DIABETES *21*:939-45, September, 1972.

We have recently treated eight patients with chronic congestive heart failure by continuous intravenous infusion of glucagon for ninety-six hours, and have reported the (negative) results elsewhere.[1] In these patients we have measured blood glucose, serum immunoreactive insulin (IRI) and serum immunoreactive growth hormone (HGH) in the fasting state and during glucose tolerance tests before and during glucagon infusion.

Effects of long-term infusion of glucagon on IRI and HGH in man are unknown. Some information may be gathered from observations of patients with glucagon-producing tumors, but only two such patients have been well described. In the first case[2] the patient had mild diabetes mellitus in spite of high fasting IRI and increased IRI response to glucose. In the second case[3] the diabetes was more severe. IRI was not measured, but the patient was resistant to exogenous insulin. In neither case could it be determined whether the diabetes was a result of the secretory products from the tumor, or represented a concomitant disease. Nor was HGH measured.

In animals, several attempts have been made to induce a state of "meta-glucagon diabetes" by long-term infusions. Reptiles have proved the most sensitive to such influence. In turtles, for example, Marques,[4] after administration of 0.1 mg. glucagon/kg. body weight for thirty days noted increasing fasting blood sugar and serum insulin-like activity, and these changes were reversible. Neither in man nor animals have observations of insulin/glucose ratios been reported.

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PATIENTS AND METHODS

The patients have been described in detail elsewhere.[1] Three were females and five males. Their mean age was sixty-five years, their median age sixty-three. They were seriously ill, with edema and poor appetite. One patient (F.S.) had diabetes mellitus, well controlled on 32 units of protamine insulin/day, which he received during the whole treatment period with glucagon. This patient had metabolic responses to glucagon which were qualitatively and quantitatively similar to those of the others, and which have been included in the calculation of results.

The patients were on a constant-dosage cardiac insufficiency regime before and during the whole treatment period with glucagon. In addition, perphenazine in doses of 4 mg. b.i.d. was added two days before infusions started in order to reduce nausea. The dose of furosemide was either 40 or 80 mg./day.

On the morning of *day 1* an indwelling intravenous cannula was inserted in an arm vein and infusion started with 2.5 per cent fructose, which was later used as the vehicle for glucagon. By a constant-rate infusion pump, 30 ml./hr. (equivalent to 0.75 gm. fructose/hr.) was delivered during the entire treatment period.

At 8 a.m. the same morning an intravenous glucose tolerance test was carried out according to Kienholz.[5] 0.33 gm. glucose/kg. body weight was injected in the course of four minutes, and blood drawn for determination of glucose, IRI and HGH after 0, 15, 30, 45 and 60 minutes.

At 11 a.m. the patient received his first feeding, which was a standardized carbohydrate-rich meal containing 49.7 gm. of carbohydrate, 11.5 gm. of fat and 4.5 gm. of protein, a total of 320 calories. Blood was drawn before eating and 30, 60, 90 and 120 minutes later, and analyzed as above.

At 2 p.m. the glucagon infusion was started, and continued for ninety-six hours. We used glucagon which had been generously supplied free of charge by "Novo Industri." This preparation contains less than 0.1 per cent of insulin. The dose was 2 mg./hr. in the first four patients, and 4 mg./hr. in the next four.

On *day 2* and *day 5* intravenous glucose tolerance test and carbohydrate-rich meal was given as above. On days 3 and 4 only fasting blood samples were drawn. In one patient (A.R.) free fatty acids (FFA) in plasma were examined at all the above-indicated hours.

The infusion was usually a considerable stress to the patients, with nausea as the main problem.

Blood sugar was determined according to Hyvärinen and Nikkiliä.[6] IRI and HGH were determined as described by Norman and Turter.[7] Plasma FFA were determined with the method of Dole.[8] We are indebted to S. Skrede, M.D., for performing the FFA analyses. Serum potassium was measured by a Beckman automatic flame-photometer with internal lithium standard. Serum inorganic phosphate was measured with SMA 12/60 (AutoAnalyzer). Blood pH was measured with Astrup-equipment, Radiometer.

RESULTS

1. *Fasting state*

Values for blood glucose, serum IRI and HGH are shown in table 1. Because the results were qualitatively and quantitatively similar regardless of dose level, they have been calculated and presented together.

Fasting blood sugar. The mean value increased slightly and on the fourth day differed significantly from the pretreatment mean: $0.02 < p < 0.05$. The rise, however small, was present in all patients.

IRI. The mean value rose significantly ($p = 0.01$) on day 2, with a decline again on day 5 when the value was not significantly higher than on day 1 ($0.1 < p < 0.2$). The pre-infusion mean is somewhat high compared to our laboratory normal, this being due to the finding in two patients of values 13 and 18 μU./ml. The diabetic patient, F.S., had a pre-infusion value of 13 μU./ml., rising on day 2 to 29.

HGH. The levels rose in all patients. The lowest mean, on day 4, was still significantly higher than before infusion ($0.01 < p < 0.02$).

TABLE 1

Blood glucose, serum (immunoreactive) insulin, serum growth hormone, serum potassium (K), serum inorganic phosphorus (P) and blood pH in the fasting state on each morning of the treatment period. Day 1 is before glucagon infusion was started. n = 8. S.E. = standard error of the mean.

		Treatment day				
		1	2	3	4	5
Blood glucose (mg./100 ml.)	Mean	87	96	100	103	90
	S.E.	5.8	10.1	4.9	3.0	9.1
Serum insulin (μU./ml.)	Mean	9.1	25.3	32.0	26.4	16.9
	S.E.	1.6	5.4	13.2	11.2	4.5
Serum growth hormone (ng./ml.)	Mean	2.8	11.9	7.0	6.1	6.5
	S.E.	0.6	2.3	1.0	1.1	1.4
Serum K (mEq./L.)	Mean	4.2	3.8	4.3	4.1	4.5
	S.E.	0.2	0.1	0.3	0.2	0.2
Serum P (mg./100 ml.)	Mean	3.2	3.8	—	—	3.4
	S.E.	0.2	0.3	—	—	0.2
Blood pH	Mean	7.45	7.46	7.47	7.46	7.45
	S.E.	0.02	0.01	0.02	0.02	0.02

TABLE 2

Serum immunoreactive insulin and growth hormone during intravenous glucose tolerance test before (day 1) and during (day 2 and day 5) glucagon infusion. n = 8. S.E. = standard error of the mean

		Day 1					Day 2					Day 5				
Minutes after I.V. glucose		0	15	30	45	60	0	15	30	45	60	0	15	30	45	60
Serum insulin (μU./ml.)	Mean	9.1	37.3	27.6	23.4	18.6	25.3	86.8	94.9	95.6	131.1	16.1	55.7	79.4	64.1	74.4
	S.E.	1.6	9.6	6.7	4.8	3.4	5.4	21.9	28.9	28.0	40.0	4.9	17.8	28.1	23.2	28.7
Serum growth hormone (mg./ml.)	Mean	2.8	2.6	6.6	13.7	16.1	11.9	10.0	27.9	42.9	44.9	7.1	5.7	9.4	12.1	14.3
	S.E.	0.6	0.7	2.1	5.2	6.4	2.3	1.4	5.5	6.9	13.1	1.5	1.1	3.5	4.2	4.3

2. Intravenous glucose

The results are presented in table 2 and figure 1.

Blood sugar. The values rose from fasting levels to approximately 160 mg./100 ml. on all days without reaching baseline again during the one hour of blood sampling. Mean K-value on day 1 (before glucagon) was 0.85 (S.E.M. = 0.13) which is in the latent diabetic range for this age group.[5] On day 2 it decreased significantly ($0.001 < p < 0.005$) to 0.46 (S.E.M. = 0.08) and on day 5 was still very low: 0.49 (S.E.M. = 0.08). These last values are in the manifest diabetic range.

IRI. The mean insulin response to intravenous glucose before glucagon was in the normal range for our laboratory. In patient F.S. it rose from a fasting value of 13 μU./ml. to a maximum of 28 after thirty minutes. During infusion of glucagon, insulin responses to glucose were greatly increased. In two patients the increase was slight, in four marked and in two excessive, with maxima of 280 and 320 μU./ml. on day 2.

HGH. An unexpected rise in HGH following intravenous glucose took place before glucagon was started. The high mean values were due to very considerable increase in three patients, of which the highest was 47 ng./ml. after sixty minutes. During glucagon, all patients had much higher rise in HGH after intravenous glucose than they had before, and the rise was greatest in the three patients mentioned above. On day 5 the values were lower again, but still showed a rise in response to glucose.

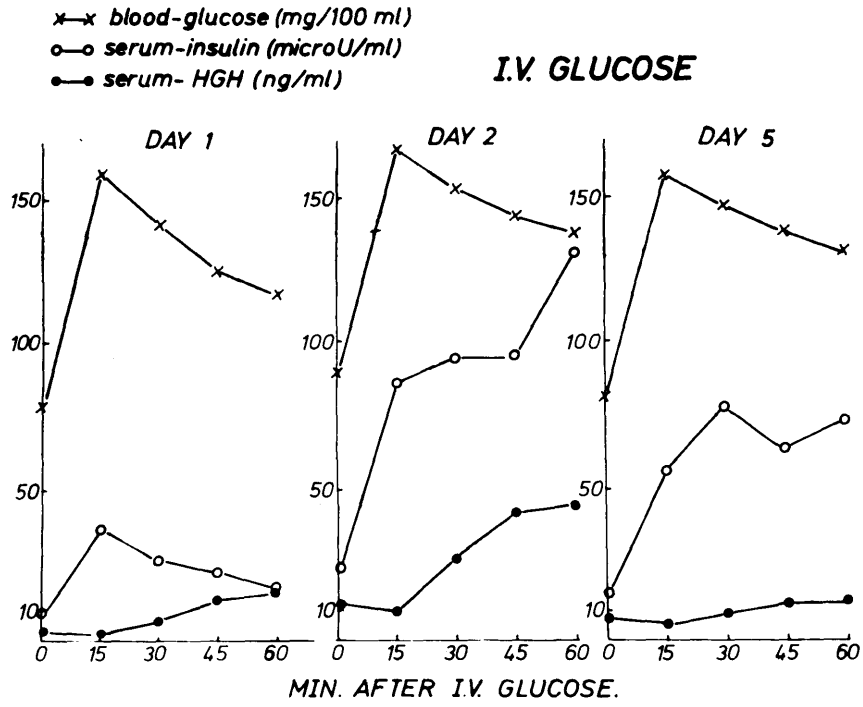

FIGURE 1

Mean values for blood glucose, serum insulin and serum growth hormone during intravenous glucose tolerance tests before (day 1) and during (day 2 and day 5) glucagon infusion. n=8.

3. Carbohydrate-rich meal

Only five patients are included in the calculation, because in the other three negligible rise took place in blood sugar in the first two hours following the meal, probably because of nausea with reduced gastrointestinal motility and absorption. The results are presented in table 3 and figure 2.

The increase in *blood sugar* in these five patients was of the same magnitude before and during glucagon infusions, but the maximum was reached somewhat later on days 2 and 5.

IRI. Mean IRI response to the meal before glucagon was in accord with our normal values. On days 2 and 5 the rise in IRI after the meal was very much greater in four patients and unchanged in one.

HGH. Before glucagon, serum HGH was at a low level (normal) during the two hours following the meal. On day 2 the prefeeding value was definitely elevated, and did not fall after the meal. On the contrary there was a tendency to a further rise. On day 5 these changes were less pronounced. There was, however, still a high prefeeding value, and no suppression by the meal could be observed.

In one patient blood was drawn for analysis of FFA at all the indicated hours. In this patient there was no definite change in the fasting level (940 μM/L.) after glucagon was started. Intravenous glucose and meals resulted in a two-thirds reduction of FFA before glucagon infusion, and a similar fall was observed on days 2 and 5.

Fasting values for serum potassium, serum inorganic phosphorus and blood pH are included in table 1. No significant change took place in any of these parameters during glucagon infusion.

DISCUSSION

The results indicate that in our patients long-term infusion of glucagon led to mild glucose intolerance in spite of raised levels of IRI, and that HGH might contribute to this metabolic derangement, possibly through insulin antagonism. We wish to point out, however, that these effects are the result of very large doses of glucagon, probably representing a maximal stimulation, because 4 mg./hr. did not elicit greater responses than 2 mg./hr. Pharmacologic doses of glucagon are known to induce large increases in plasma cyclic AMP,[9] which probably is the common mediator for the various hormonal effects that we have observed in our patients. It is not unlikely that other hormones also respond to intense stimulation by glucagon, but of necessity our study is limited to insulin and growth hormone.

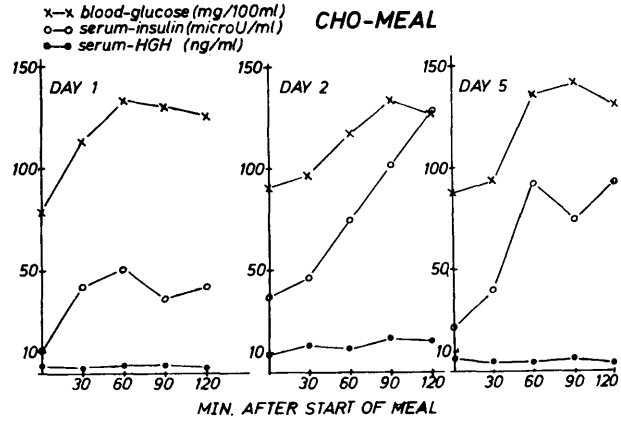

FIG. 2. Mean values for blood glucose, serum insulin and serum growth hormone after a carbohydrate-rich meal before (day 1) and during (day 2 and day 5) glucagon infusion. n=5.

Blood glucose.

Mean glucose disappearance rate (K-value) in our patients was in the latent diabetic range before glucagon infusion started, although only one of our patients was a known diabetic. It should, however, be recognized that they were all quite ill, had a low caloric intake, used diuretics and belonged to an older age group.

TABLE 3

Blood glucose, serum immunoreactive insulin and growth hormone after a carbohydrate-rich meal before (day 1) and during (days 2 and 5) glucagon infusion. n = 5. S.E. = standard error of the mean.

		Day 1					Day 2					Day 5				
Minutes after start of meal		0	30	60	90	120	0	30	60	90	120	0	30	60	90	120
Blood glucose (mg./100 ml.)	Mean	79	114	134	131	127	90	97	118	133	127	88	94	136	142	131
	S.E.	4.5	8.2	4.6	5.2	6.9	8.0	8.1	8.1	8.1	6.5	7.7	7.2	6.1	10.4	12.4
Serum insulin (μU./ml.)	Mean	11	42	51	36	43	37	47	75	102	129	22	40	93	75	93
	S.E.	2.1	6.7	15.8	6.0	16.8	8.8	16.1	21.4	34.8	63.4	5.9	8.5	31.0	18.5	31.0
Serum growth hormone (ng./ml.)	Mean	3.0	2.2	3.7	4.2	2.9	9.2	13.9	12.2	16.7	15.4	6.2	4.1	4.5	5.8	4.3
	S.E.	0.9	0.5	1.2	1.6	0.9	1.6	5.0	3.9	4.4	4.1	2.3	1.3	1.6	1.8	1.2

These are all factors which tend to reduce carbohydrate tolerance. Even bedrest alone may have this effect.[10] During glucagon treatment a marked reduction of glucose tolerance took place, as indicated by the low K-value, which persisted throughout the treatment period. To what extent these metabolic changes are determined by our particular selection of patients is hard to determine, and caution should be exercised in drawing conclusions that would also include normal persons and their reaction to glucagon infusions.

Several metabolic events are set in motion during glucagon infusion that cause a subsequent interference with glucose utilization. The well-known acute hyperglycemic effect wears off within two to three hours in spite of continued infusion.[11,12] In the first two hours following a bolus injection of glucagon in man, an increase in peripheral glucose uptake has been demonstrated as judged by an increment in arteriovenous glucose difference.[13-15] These early effects of glucagon administration, therefore, cannot explain our findings of reduced carbohydrate tolerance.

Two studies are available on prolonged, intermittent glucagon administration in man, furnishing information on glucose tolerance beyond the acute stage. Salter et al.,[16] during intravenous infusion of pharmacologic doses for ten hours daily during two to four days, observed decreased glucose tolerance and, in addition, increased urinary nitrogen excretion, probably reflecting accelerated hepatic deamination of amino acids and augmented gluconeogenesis. Such a mechanism might well be at work also under the conditions of our experiment, thus contributing to a lower K-value.

On the other hand, Van Itallie et al.,[17] by repeated intramuscular injections for two to five days, found evidence for reduced peripheral glucose uptake, and maintained that the glucagon-induced diabetes-like state might be due to the presence of insulin antagonistic substances (other than glucagon) in the circulation. Since we have demonstrated the presence of one such antagonist, namely HGH, we agree that this explanation is a probable one.

The blood glucose curves following meals do not allow conclusions regarding glucose tolerance, because the gastrointestinal side-effects of glucagon probably involve delayed absorption.

Insulin. In our patients an undisputed rise in fasting serum IRI took place during glucagon infusion. The admixture of insulin to the glucagon preparation is too small to account for this finding.

The effects of glucagon upon insulin release were first demonstrated by Samols et al.,[18] and have later been repeatedly confirmed by Deckert[19] and others. It has further been shown[11] that the hyperinsulinemia continues during glucagon infusion for eight hours *and* after the hyperglycemia has normalized completely. Even in juvenile diabetics glucagon was able to stimulate insulin release[20] while tolbutamide failed to do so. It was therefore not unexpected that serum IRI rose even in our diabetic patient.

Even more impressive was the potentiating effect of glucagon on the insulinemia following intravenous glucose or oral carbohydrate. This effect has also been demonstrated in short-term experiments.[12,21,22] Our results show that it lasts undiminished for at least ninety-six hours.

Previous authors have neglected to comment upon the rising insulin/glucose ratio. We want to point out that hyperinsulinemia in the presence of elevated fasting blood sugar and diminished glucose disappearance rate implies a state of relative insulin resistance.

Growth hormone. In our patients a uniform and sustained increase in fasting serum HGH levels took place. The small amounts of fructose infused cannot explain this finding.[23] The first reports on the HGH-stimulating effect of glucagon were those of Drosk et al.[24] in diabetic children and of Mitchell et al.[25] in normals. It appears that the route of administration is essential for a reliable response, intravenous bolus injection having often failed to induce HGH release, whereas intravenous infusion and intramuscular injection seem to represent reliable stimuli.[26,27]

Considering the great variation in stimuli that may produce growth hormone release, it is difficult to have a definite opinion on the cause or causes for the observed high levels of growth hormone in our patients. We tend, however, not to think of it as a nonspecific stress reaction. For one thing, we have regarded "stress" as a poor HGH stimulator since we failed to demonstrate elevated HGH levels in patients with acute myocardial infarction.[7] Second, the HGH elevation in the patients reported here seemed to decline toward the end of the infusion period, when stress probably was greatest. Whatever the mechanism, the growth hormone-releasing stimulus must be very potent as it was not suppressible by glucose infusion. The complexity of the metabolic situation and our defective understanding are illustrated by the rise in serum HGH concentration during intravenous glucose in three of our patients even before the glucagon was administered. This was

not caused by a rapidly falling blood sugar.*

Although our study has been carried out with pharmacologic doses of glucagon, it is tempting to consider our findings of HGH elevation in the light of the results reported by Molnar et al.[30] and of Johansen.[31] These workers observed elevated HGH levels in patients with brittle diabetes[30] and juvenile diabetes.[31] Recently, Unger et al.[32] have reported pancreatic alpha-cell hyperactivity in diabetes mellitus. It is thus possible that the diabetes-like state induced in our patients may represent a closer analog to genuine diabetes mellitus than is realized today.

Administration of HGH has a diabetogenic effect both in the intact animal[33] and human[34] organism, probably due to insulin antagonism.[35] It is therefore reasonable to consider the glucose intolerance and relative insulin resistance encountered in our patients at least partly as an effect of the increased serum HGH level. Other insulin antagonistic factors may, however, also be of importance.

High levels of plasma FFA may induce a state of insulin resistance.[36,37] Glucagon has a lipolytic effect in vitro,[38] but the results of short-term experiments in the intact human organism have been contradictory. Dreiling et al.[39] and Crockford et al.[12] observed a reduction in plasma FFA lasting up to three to four hours following injection of glucagon, while Lipsett et al.[40] and Warembourg et al.,[41] after an initial fall, noted a rise above pre-injection level. In one of our patients, plasma FFA during glucagon infusion was not changed in the fasting state, and followed the normal pattern of suppression after intravenous glucose and meals. The importance of plasma FFA for the development of glucagon-induced diabetes has not been settled by our study.

In short-term experiments in dogs[42,43] and man,[44] glucagon stimulates catecholamine release from the adrenal, but results from long-term experiments are lacking. High plasma catecholamine level could account for a state of insulin resistance.[45] We have not measured these hormones in our patients, but have failed to observe clinical evidence of increased adrenal medullary influence, such as rapid heart rate, hypertension[1] and reduced insulin secretion, though admittedly the heart failure itself and various drugs given would modify the response to catecholamines considerably.

A fall in serum potassium and phosphorus levels has been reported following administration of glucagon,[46] but did not take place in our patients, nor was there any fall in blood pH. Thus neither acidosis nor derangement of the ionic equilibrium could account for the insulin resistance.[45]

Finally, the comment should be made that the immunoreactive insulin measured in these experiments might represent biologically hypo-active insulin, in other words that there exists no insulin resistance at all. It has been shown[47] that insulinomas secrete disproportionately high quantities of proinsulin, and the possibility exists that the beta-cell hyperactivity induced by exogenous glucagon could have similar consequences. We have not undertaken any separation between insulin and proinsulin, and are thus not able to answer this question.

* In order to determine whether this paradoxical HGH response was a fortuitous occurrence in these three patients, or if it was a more common finding in this disease state, we repeated the intravenous glucose tolerance test in eight normal individuals and in eight other patients with congestive heart failure, though less severe than in the patients treated with glucagon. In fifteen the serum HGH concentration stayed at a minimum level throughout the procedure. In one patient with congestive failure the values were: before glucose injection and fifteen minutes after, less than 2 ng./ml.; thirty minutes after injection it rose to 4.6 ng./ml., forty-five minutes after 10.6 ng./ml. and sixty minutes after 11.8 ng./ml. These values correspond quite well to the mean HGH concentration on day 1 of table 2, indicating that a paradoxical HGH response to glucose may not be uncommon in this condition. No explanation of this finding is readily available, but similar observations have been reported in other severely ill patients, such as in uremia.[28,29]

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