Portal and Peripheral Vein Immunoreactive Glucagon Concentrations After Arginine or Glucose Infusions

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SUMMARY

Catheterization of the portal vein via the umbilical vein was performed under local anesthesia in eight nondiabetic subjects before exploratory laparotomy for a variety of conditions. Levels of immunoreactive glucagon (IRG) and immunoreactive insulin (IRI) were determined in simultaneously obtained portal and peripheral vein plasma before, during and after a fifteen minute arginine infusion (30 gm.) in five subjects. The mean portal vein to peripheral vein glucagon ratio in the postabsorptive state was 1.7 ± 0.5. A biphasic portal vein IRG response to arginine was observed, with the initial glucagon peak occurring within one minute of the beginning of the infusion. Peripheral IRG concentrations did not reflect the biphasic response. The portal vein IRI response to arginine was also mildly biphasic, and the first phase occurred before a detectable increase in blood glucose. The portal vein IRG peak either preceded or coincided temporally with the portal vein IRI peak. In three nondiabetic subjects, portal vein IRG decreased rapidly to its nadir within two minutes after a two minute glucose infusion (25 gm.) was started. DIABETES *23*:199-202, March, 1974.

Portal vein insulin concentrations have provided useful information regarding insulin secretion in man. Previous investigations from this institution employing umbilical vein catheterization to obtain portal blood samples have depicted the portal vein insulin response to glucose and tolbutamide in healthy and diabetic subjects.[1-3]

Recent availability of sensitive and specific assays for plasma glucagon make portal vein glucagon studies in man feasible also. The portal and peripheral plasma glucagon profiles after either glucose or arginine administration were examined in this investigation. In addition, the portal vein insulin response to arginine in man is shown for the first time.

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METHODS

Eight nondiabetic subjects requiring abdominal exploration were selected for study. The patients had a variety of conditions requiring surgery. Five had peptic ulcer disease; two had carcinoma (stomach and colon) and one had chronic cholecystitis with cholelithiasis. All subjects were males and their ages ranged from thirty-eight to sixty-three. None was obese. Three of the subjects had lost over twenty pounds during the preceding year. Fasting plasma glucose concentrations were normal in all subjects, and two hour postprandial plasma glucose values were normal in all subjects tested (five of the eight). A family history of diabetes was related by only one subject, and this patient had a normal two hour postprandial plasma glucose concentration. Results of liver function tests were normal in all subjects.

After an overnight fast, the patients were taken to the operating room and prepared in the usual fashion for an abdominal exploration. No general anesthetic was given although some of the patients received 5 mg. Valium or 50 mg. Demerol prior to the procedure. A catheter for withdrawal of blood samples was placed in an antecubital vein before the operation, and the catheter was kept patent by a saline infusion delivered slowly. Under local anesthesia with 1 per cent Xylocaine, the umbilical vein was exposed through an extraperitoneal upper abdominal midline incision and cannulated with a size 9 ureteral catheter. The catheter was then passed into the left branch of the portal vein just past its entrance into the liver. After the catheter had been secured in place, blood samples were easily obtained from the portal vein. The catheter was rinsed with saline before each blood sample was obtained.

After two baseline blood samples one minute apart had been obtained simultaneously from the portal and antecubital veins, the patients received either a glucose or an arginine infusion. In three subjects, 25 gm.

glucose was infused over two minutes and blood samples were obtained simultaneously from the portal and antecubital veins 60, 90 and 120 seconds after the start of the glucose infusion and 1/2, 1, 3, 5, 8, 10 and 15 minutes after completion of the infusion.* In five subjects 30 gm. arginine (10 per cent solution as L-arginine monohydrochloride) was administered at a constant rate (2 gm. per minute) for fifteen minutes and blood samples were obtained 1, 3, 5, 8, 10, 12, 15, 17 and 20 minutes after the start of the infusion. In two subjects, blood samples were taken more frequently, at 30 second intervals, for the first two minutes.

The blood samples were discharged into heparinized tubes and special tubes for glucagon preservation containing 500 U. Trasylol and 1.2 mg. Na_2 EDTA per milliliter of blood collected. Blood glucose, plasma IRI and plasma IRG were measured in all blood samples. Blood glucose was determined by the glucose oxidase method.[4] Plasma IRI was assessed by a double antibody radioimmunoassay method.[5] Plasma glucagon (IRG) was assayed by the radioimmunoassay method described by Unger and coworkers, using their pancreatic glucagon specific antibody 30-K.[6]

RESULTS

Figure 1 demonstrates the portal and peripheral vein IRG responses to a 10 per cent arginine infusion delivered at a rate of 2 gm. per minute. Baseline portal vein IRG values were approximately 60 per cent higher than peripheral vein IRG concentrations. Portal vein IRG concentrations rose early, with peak glucagon concentrations occurring within one minute of the start of the arginine infusion. The portal vein IRG response was biphasic, with the nadir between the two phases occurring at three minutes. Peripheral vein IRG concentrations, which were approximately half the portal vein IRG values, except during the first three minutes, rose slowly throughout the twenty minute period of observation.

The effect of arginine on portal and peripheral plasma IRI concentrations is shown in figure 2. The portal vein IRI concentrations peaked one minute after the start of the arginine infusion, while the peak peripheral IRI level occurred at five minutes. The

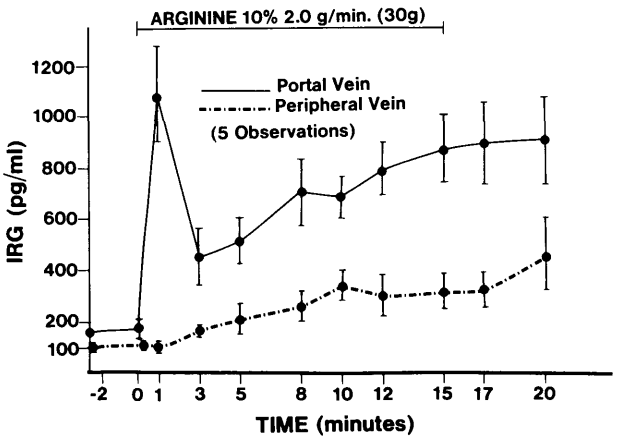

FIG. 1. Effect of arginine on portal and peripheral vein immunoreactive glucagon concentrations in nondiabetic subjects. Means ± S.E.M. are shown.

magnitude of the portal vein response to arginine and the timing of the two phases are almost identical to that previously reported with glucose as a stimulus.[1] As can be seen in table 1, the portal vein IRI response occurred before any elevation in blood glucose.

In two subjects blood samples were obtained at more frequent intervals to determine more precisely the timing of the initial glucagon and insulin peak in response to arginine. One patient exhibited a peak portal vein glucagon response, which occurred ninety seconds earlier than the peak portal vein insulin response (figure 3). In the other subject in whom frequent early blood samples were obtained, the peak portal vein glucagon and insulin concentrations occur-

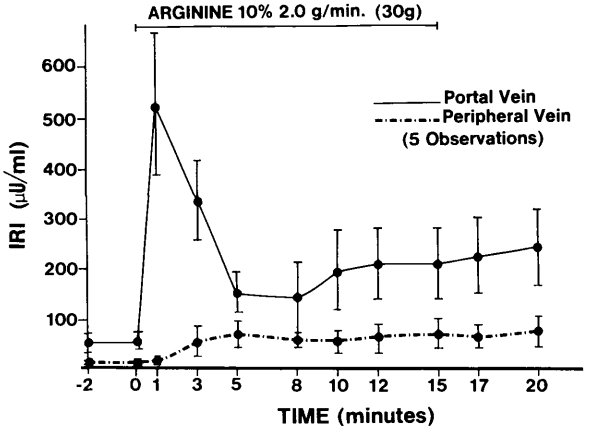

FIG. 2. Effect of arginine on portal and peripheral vein immunoreactive insulin concentrations in nondiabetic subjects. Means ± S.E.M. are shown.

*Plasma samples from our previous portal vein studies using glucose could not be used for this investigation as the samples had not been collected in Trasylol, which is necessary for glucagon preservation.

TABLE 1

Portal and peripheral vein blood glucose

Time	(30 gm. of arginine) Blood glucose (mg./100 ml.)	
	Peripheral	Portal
Baseline	83 ± 3	87 ± 1
Baseline	81 ± 2	85 ± 1
1 min.	82 ± 4	88 ± 5
3 min.	83 ± 6	89 ± 3
5 min.	88 ± 5	92 ± 4
8 min.	86 ± 5	94 ± 5
10 min.	86 ± 4	98 ± 5
12 min.	94 ± 7	97 ± 5
15 min.	89 ± 3	99 ± 5
17 min.	91 ± 4	102 ± 6
20 min.	94 ± 5	105 ± 6

Means ± S.E.M. (five observations)

red simultaneously at sixty seconds after start of the arginine infusion (figure 4).

Figure 5 shows the portal and peripheral vein IRG response to a two minute glucose infusion. As in the arginine studies portal vein IRG values are approximately twice those in the peripheral plasma. A rapid decrease in portal vein glucagon concentrations occurs during the two minute period of glucose infusion.

Figure 6 illustrates the portal vein IRI and IRG response to glucose. The nadir in IRG concentrations coincided temporally with the maximum rise in IRI concentrations.

DISCUSSION

Studies in dog and man have indicated higher glucagon concentrations in portal plasma than in peripheral plasma.[7-9] However, considerable variation in the portal to peripheral vein glucagon ratios have

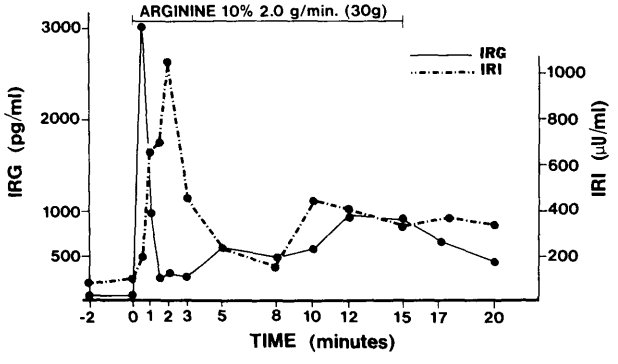

FIG. 3. Arginine-induced portal and peripheral vein IRI and IRG elevations in a single subject.

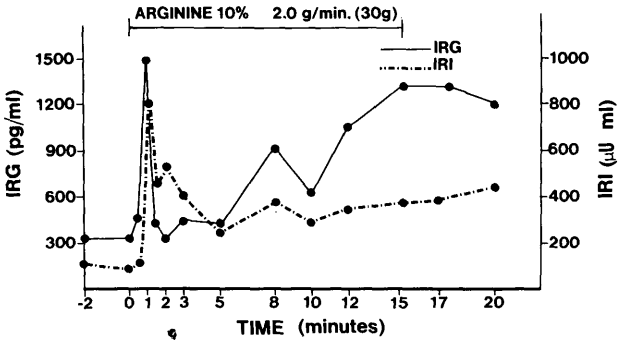

FIG. 4. Arginine-induced portal and peripheral vein IRI and IRG elevations in a single subject.

been observed in these studies. The mean portal vein to peripheral vein glucagon ratio in the basal postabsorptive state in the eight subjects studied in the report was 1.7 ± 0.5.

In this investigation performed in man, blood samples were obtained sufficiently early and frequently enough to assess multiphasic aspects of glucagon secretion, as manifested by portal vein hormone concentrations. Arginine, which was chosen as the stimulatory agent to assess the portal vein glucagon response, fortuitously stimulates both glucagon and insulin release, thus permitting simultaneous observations on the release of the hormones.

Previous portal vein studies by us, using the umbilical vein catheterization procedure, have demonstrated a biphasic insulin secretory response to glucose in man,[1] almost identical to that reported in rats.[10] The separation of two phases of insulin secretion during

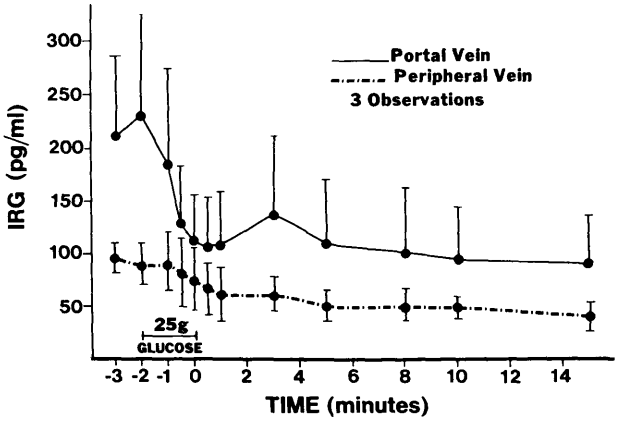

FIG. 5. Portal and peripheral vein immunoreactive glucagon concentrations during and following a two minute glucose infusion (25 gm.). Means ± S.E.M. are shown.

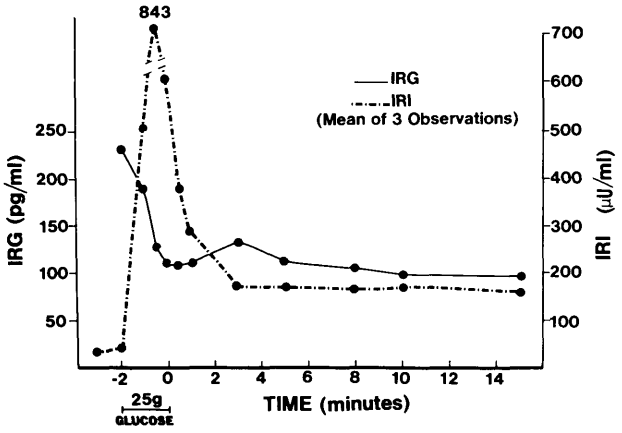

FIG. 6. Portal vein IRG and IRI values during and following a two minute glucose infusion. Means ± S.E.M. are shown.

arginine stimulation was somewhat less impressive than that occurring after glucose administration.[1] However, in a closed system, such as the present experimental design necessitates, insulin recirculation tends to obscure the nadir between the two secretory phases. The early phase of insulin release during arginine infusion cannot be attributed, even in part, to the hyperglycemic response which occurs after arginine administration, since significant blood glucose elevations did not occur until after the first phase of insulin secretion had been completed.

The portal vein glucagon response to arginine in this study was more impressively biphasic than the insulin response to this amino acid. Peripheral glucagon concentrations alone gave no indication of the biphasic response. In a recent publication Grodsky and co-workers have also observed a biphasic glucagon secretory response in the perfused rat pancreas.[11] The timing of the two phases of glucagon secretion in our studies in man and in the latter studies in rats are remarkably similar. Our studies in human beings, in which the stimulus is given in a peripheral vein, should dispel any concern that the early response might be an artifact.

The possibility that glucagon may play a major contributory role in the insulin response to amino acids cannot be excluded by our observations. The stimulatory effect of glucagon on insulin secretion is well known.[12] In addition, the present study has shown that glucagon release occurs as early as insulin release and in some cases may occur even earlier. If communication between the alpha and beta cells exists, as seems likely from morphologic observations by Zimny and co-workers,[13] arginine-induced glucagon release could conceivably be partly responsible for the insulin release after this stimulus. However, dissociation between glucagon and insulin release after various amino acids[14] would suggest that the insulin secretory response to amino acids cannot be attributed entirely to glucagon. The very early glucagon response to arginine, however, probably causes the slight hyperglycemic response to arginine and protects man from hypoglycemia, which might occur as a result of hyperinsulinemia, if glucagon were not secreted simultaneously.

ACKNOWLEDGMENT

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