The Effect of Intraportal and Peripheral Infusions of Glucagon on Insulin and Glucose Concentrations and Glucose Tolerance in Normal Man

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Summary. The effect of peripheral and intraportal infusions of $0.86 \text{ pmol/kg} \cdot \text{min}^{-1}$ of glucagon on plasma glucose, plasma insulin, and glucose tolerance was examined in four normal subjects. Peripheral glucagon concentrations increased by 60–90 pmol/l during intraportal and 70–180 pmol/l during peripheral infusions. The infusions caused increases in plasma glucose levels of approximately 1 mmol/l, and in plasma insulin levels of 75–100%, regardless of route of administration. Intravenous glucose tolerance tests carried out during the glucagon infusions showed that glucose tolerance remained within the normal range and was uninfluenced by the route of administration.

Key words: Glucagon, insulin, glucose tolerance, diabetes mellitus.

Recently Sherwin et al. [1] reported that elevation of plasma concentrations of glucagon of three to six times basal levels caused only transient and inconspicuous rises in blood glucose levels and was without effect on plasma insulin and glucose tolerance. These important findings led the authors to conclude that glucagon is not itself diabetogenic, a statement which called forth comments from several groups [2–8]. The elevation of glucagon concentrations, however, was brought about by infusion into peripheral veins, which may have different effects than elevations caused by glucagon production in the splanchnic system. This is so, because 1) the liver is probably the principal site of glucagon action and is also responsible for some glucagon removal, and 2) when infused peripherally the glucagon will

affect the pancreatic islets before it reaches the liver by the portal route and thereby initiate an insulin production which might obscure any effect of hyperglucagonaemia. This insulin production, furthermore, could very well escape notice in peripheral blood, the liver being of great importance for the removal of insulin from blood [9].

We therefore made the following experiments in which elevated glucagon concentrations were induced by peripheral as well as by intraportal infusions, thus allowing a study of the importance of the route of administration on the effect of glucagon on glucose tolerance.

Material and Methods

Four patients (all female, aged 43–57 years) scheduled for a cholecystectomy for uncomplicated cholelithiasis volunteered for the study after the nature, purpose, and possible risks had been carefully explained to them. None were obese (within 90–110% of ideal body weight) or suffered from any known disease except for the cholelithiasis, and none had first degree diabetic relatives. None were receiving any drugs. At laparotomy, a catheter (Argyle® feeding tube CH 8, Sherwood Medical Industry, St. Louis, Miss., USA) was inserted into the obliterated umbilical vein and advanced so that its tip was positioned close to the entrance of the vessel into the portal vein. The catheter was exteriorized through a small separate incision and anchored with a skin suture. The catheter was kept patent with a slow infusion of heparinized saline. Recovery from operation was uneventful in all cases. Experiments were performed on the fifth postoperative day by which time the patients had resumed their normal diet and were feeling completely well. After an

Table 1. The effect of intraportal (Po) or peripheral (Pe) infusions of glucagon ($0.86 \text{ pmol/kg} \cdot \text{min}^{-1}$) on the plasma glucose, glucagon, and insulin responses to a 25 g intravenous glucose tolerance test (IVGTT) in four normal subjects. The glucagon infusion was started at "0" min, and the IVGTT at 60 min. Individual values are shown

Time (min)			-20	-10	15	30	45	60	70	80	90	100	110	120
Patient no. 1	glucose	Po	4.4	4.5	5.2	5.8	5.4	5.4	11.1	9.3	7.4	6.0	5.8	5.0
		Pe	4.5	4.8	5.5	5.9	4.2	4.9	13.5	8.9	8.7	7.5	7.0	6.3
	glucagon	Po	31	22	86	71	100	94	91	80	94	91	93	87
		Pe	34	25	86	91	64	96	88	63	97	93	77	88
	insulin	Po	63	58	88	85	90	88	270	320	214	146	123	94
		Pe	52	48	66	82	75	67	260	300	246	222	168	130
Patient no. 2	glucose	Po	4.8	4.8	5.0	5.2	5.0	5.1	12.7	11.2	9.6	8.0	6.9	6.3
		Pe	4.2	4.4	4.8	4.8	4.8	-	12.7	10.3	9.0	8.1	7.2	6.4
	glucagon	Po	25	22	83	80	87	88	68	90	84	91	88	71
		Pe	24	25	77	97	97	-	74	88	83	80	97	94
	insulin	Po	108	94	198	266	210	180	1700	1370	1150	980	780	440
		Pe	107	116	184	176	204	-	1490	1200	890	850	760	460
Patient no. 3	glucose	Po	4.3	4.4	4.9	5.3	5.4	5.3	13.5	12.1	11.2	11.0	9.8	8.3
		Pe	4.2	4.1	5.0	5.7	5.9	5.7	14.7	12.9	11.6	9.8	9.1	8.0
	glucagon	Po	22	38	87	120	117	103	129	129	91	86	143	117
		Pe	8	12	131	180	149	189	172	177	154	154	129	127
	insulin	Po	39	37	62	72	70	76	188	154	160	180	214	203
		Pe	33	32	44	55	65	64	154	150	143	194	300	230
Patient no. 4	glucose	Po	4.7	4.0	5.5	5.8	5.7	5.8	13.4	11.1	8.7	7.9	7.1	6.1
		Pe	4.7	4.7	5.2	5.7	5.6	5.6	12.2	9.5	8.3	7.2	6.2	5.5
	glucagon	Po	24	22	91	80	86	94	111	86	109	112	109	86
		Pe	22	15	87	114	91	137	121	106	127	103	114	98
	insulin	Po	118	100	92	180	132	106	480	478	500	1170	800	450
		Pe	118	116	180	254	208	154	790	700	960	1070	1070	830

overnight fast glucagon (NOVO) (1 mg dissolved in 1 ml sterilized water and diluted with saline containing 10 g/l human serum albumin) was infused at 0.86 pmol/kg body wt/min with a finger pump (Ole Dich, Copenhagen) in a volume of 2.0 ml/min. Peripheral blood samples were obtained from a catheter inserted into an antecubital vein. After two basal samples were obtained (Table I) the infusion of glucagon was started into the portal vein catheter and blood samples were taken at intervals as indicated in Table 1. After 60 minutes of infusion, 25 g of glucose (500 g/l) were given intravenously over 2 minutes. At 120 minutes the glucagon infusion was terminated. On the next day the experiment was repeated, but this time the glucagon was infused via a catheter inserted into an antecubital vein. Otherwise the conditions were exactly the same, including the overnight fast. The patients received full, carbohydrate-rich meals the day before both experiments.

No complications were encountered secondary to the portal catheterizations or to the infusions.

Blood was collected in ice-chilled tubes containing heparin (50 U) and aprotinin (Trasylo[®], Bayer, GRF) 500 KIU/ml blood. Plasma glucose was determined on an autoanalyzer using a glucose-oxidase-system [10]. Plasma insulin was determined according to Albano et al. [11] and glucagon concentrations in plasma by radioimmunoassay, as pre-

viously described [12]. The antiserum used is directed against the C-terminal sequence of glucagon and crossreacts less than 0.1 per cent with high concentrations of enteroglucagon. Detection limit of the assay is 5 pmol/l, the within-assay coefficient of variation is 6.7 per cent, and the inter-assay coefficient is 16 per cent, determined from 16 consecutive assays over a period of 4 months.

Results

All individual results of glucose, glucagon, and insulin determinations are shown in Table 1. The mean values are shown in Figure 1. Individual glucose disappearance rates [13] are shown in Table 2.

All patients had normal basal glucose, insulin, and glucagon concentrations in plasma. The infusion of glucagon, $0.86 \text{ pmol/kg} \cdot \text{min}^{-1}$ raised peripheral glucagon concentrations 70–180 pmol/l. In two of the patients (Nos. 3 and 4) peripheral administration brought about larger increments in glucagon concentrations than intraportal administration. It also appears that a stable plateau was reached after 15–30 minutes' infusion. The glucagon infusion led to an average increase in plasma glucose concentrations of 1 mmol/l, coincident with the attainment of a stable glucagon plateau. Intraportal and peripheral

Fig. 1. Effect of intraportal (○—○) and peripheral (●—●) infusions of $0.86 \text{ pmol/kg} \cdot \text{min}^{-1}$ glucagon on the plasma glucagon, insulin, and glucose responses to a 25 g intravenous glucose tolerance test (IVGTT) in 4 normal subjects. The IVGTT was performed after 60 minutes' glucagon infusion. Mean values are shown

Table 2. Glucose disappearance rates during intraportal or peripheral infusion of glucagon

Disappearance rate (per cent $\cdot \text{min}^{-1}$)	Patient no. 1	Patient no. 2	Patient no. 3	Patient no. 4	Mean
Portal infusion	1.08	1.47	1.35	1.23	1.28
Peripheral infusion	1.22	1.35	1.11	1.21	1.21

infusions caused identical changes in glucose concentrations. The glucose tolerance test, carried out after 60 minutes infusion, showed that none of the patients had diabetic glucose tolerance [13] during the glucagon infusion and that there was no difference between the results obtained during intraportal as opposed to peripheral infusion (Table 2). All patients showed an increase in peripheral insulin concentrations during the glucagon infusion with a maximum after 30 minutes. The glucose infusion led to further increases of 5–17 times the basal values.

Marked hyperinsulinaemia persisted throughout the experiment. Again the route of administration of the glucagon infusion did not influence the results.

Discussion

The experiments of Sherwin et al. [1] showed that "physiological" increases in glucagon concentrations brought about by peripheral infusions did not influence glucose tolerance in normal man. It was argued later that the route of administration might be of great importance, the intraportal route being preferable to the peripheral. Our results, however, clearly demonstrate that the hyperglycaemia and the glucose tolerance after glucagon infusions were not influenced by the route of administration. The peripheral infusions, on average, led to slightly higher concentrations of glucagon in peripheral plasma than the intraportal infusions, consistent with the concept that the liver only moderately degrades the glucagon presented to it [14–15]. However, the concentrations of glucagon in the portal venous blood during intraportal infusion must have been some four times the portal glucagon concentration in the control experiment, assuming a portal flow of one fourth of the cardiac output. The plasma insulin concentrations resemble very much those reported by Sherwin et al. [1], who also found increases of 50–100% with a maximum after 30 minutes infusion; again our results from the two experiments are very similar.

Unfortunately it was not possible to extend the experiments to include a glucose tolerance test without glucagon infusion. A comparison with IVGTT-values from other newly operated patients was considered to be insufficient, because of the large variation in a normal population [13]. Such a comparison, however, was beyond the scope of the present investigation.

We chose the intravenous route for the test of glucose tolerance, because, thus determined, the results are uninfluenced by differences in absorption, secretion of gastrointestinal hormones and nervous activity elicited by oral loading. The intravenous test may, however, be less sensitive than the oral, when used to assess the effects of a hormone of which the principal site of action is the liver.

Our results support the conclusions of Sherwin et al. [1] that the hyperglycaemic activity of glucagon will not be displayed unless the insulin levels in blood are depressed, as in insulinopenic diabetes [6], during exercise [17], ethanol-induced hypoglycaemia [18], and hypotensive stress [19].

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