The Effect of Glucagon Infusion on Kidney Function in Short-term Insulin-dependent Juvenile Diabetics

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Summary. Kidney function was studied in nine, metabolically well controlled, short-term insulin-dependent male diabetics before and during glucagon infusion of 4 to 5 and 8 to 10 ng/kg/min. Glomerular filtration rate, effective renal plasma flow (steady-state infusion technique, with urinary collections, using ^{125}I -iothalamate and ^{131}I -iodohippurate), and urinary albumin and β_2 -microglobulin excretion rates were measured. The mean plasma glucagon concentration increased during infusion from 254 ± 19 pg/ml to 440 ± 31 pg/ml (low dose) and 730 ± 52 pg/ml (high dose). Glomerular filtration rate increased in all subjects from 133 ± 5 before the glucagon infusion to 141 ± 4 with the low dose, and 148 ± 7 ml/min/1.73 m² with the high dose ($p < 0.01$). The increase in glomerular filtration rate correlated with the rise in plasma glucagon concentration ($r = 0.67$; $p < 0.01$). Renal plasma flow increased from 530 ± 21 before the glucagon infusion to 555 ± 20 with the low dose and 572 ± 29 ml/min/1.73 m² with the high dose ($p < 0.01$). Urinary β_2 -microglobulin excretion rate rose from 5.8 ± 1.0 before infusion to 8.7 ± 1.7 with the low dose, and 17.9 ± 5.7 $\mu\text{g} \times 10^{-2}$ /min with the high dose ($p < 0.01$). Urinary albumin excretion remained unchanged during the glucagon infusion. These results suggest that glucagon may contribute to the reversible elevation of glomerular filtration rate typically found in poorly regulated insulin-dependent diabetics, but not to the moderate elevation found in well controlled diabetics.

Key words: Albumin, β_2 -microglobulin, glomerular filtration rate, glucagon, insulin-dependent diabetes, kidney function, renal plasma flow.

Pronounced alterations in kidney function are present in poorly controlled short-term insulin-dependent diabetics. These include increased glomerular

filtration rate (GFR), increased filtration fraction (FF), increased urinary albumin and β_2 -microglobulin excretion rates, and normal or slightly elevated renal plasma flow (RPF) [4, 13, 15]. The elevations in GFR and FF are less, and the urinary albumin and β_2 -microglobulin excretion rates are normal in clinically well controlled short-term insulin-dependent diabetics [13, 15]. Hyperglycaemia [2], hypoxia [5], and growth hormone [13] have all been suggested as causes of the GFR elevation, but we could find no evidence for the involvement of these proposed mediators [15].

Glucagon infusion in pharmacological doses causes enhanced GFR in animals and man [3, 6, 11], and recently we have shown that moderate glucagon infusions increase GFR in normal man [16]. Since the infusion induced moderately elevated plasma glucagon levels, as typically found in diabetics in ketoacidosis, we suggested that hyperglucagonaemia might contribute to the reversible alterations of kidney function in poorly controlled insulin-dependent diabetics.

To test this hypothesis, we have determined GFR, RPF, urinary albumin and β_2 -microglobulin excretion rates before and during glucagon infusion in metabolically well controlled, short-term juvenile diabetics.

Material and Methods

Nine male juvenile diabetics, aged 18 to 33 years (mean 27 years), all of whom had been fully informed of the nature of the study before giving their consent, were investigated. All patients were "ketosis-prone". Duration of diabetes ranged from 1 year to 10 years (mean 3 years). Clinical signs of diabetic retinopathy, nephropathy and neuropathy were absent in all patients. None of the patients were taking any drugs other than insulin. Mean percent ideal body weight was 97 (range 87 to 103%). Ketonuria was not present. Average daily urinary glucose excretion during the last three days before the investigation was 23g (range 0–77g). Mean daily insulin dose was 41 Units (range 12 to 80). None of the subjects received insulin on the day of investigation.

Table 1. Kidney function during glucagon infusion in 9 insulin-dependent diabetics

Subject no.	Plasma glucagon (pg/ml)			GFR (ml/min/1.73 m ²)			RPF (ml/min/1.73 m ²)			Plasma glucose (mmol/l)		
	I	II	III	I	II	III	I	II	III	I	II	III
1	260	410	870	116	122	128	513	533	533	6.4	6.3	7.1
2	210	290	510	140	149	152	628	624	623	4.1	4.6	5.4
3	180	360	670	146	153	159	575	586	597	6.7	7.9	8.1
4	230	390		134	141		470	497		8.7	9.1	
5	260	520	790	123	136	160	482	504	622	6.4	8.2	7.6
6	300	520	810	140	147	161	553	582	614	11.5	13.4	14.4
7	370	570		132	139		570	617		13.0	17.5	
8	210	400		154	159		551	597		13.4	15.9	
9	270	500	730	113	120	126	424	458	445	8.8	10.3	11.3
p	<0.01		<0.05	<0.01		<0.05	<0.01		NS	<0.01		NS

I = Mean control values (4 periods)

II = Mean values during low dose glucagon infusion (4–5 ng/kg/min) (last 3 periods)

III = Mean values during high dose glucagon infusion (8–10 ng/kg/min) (last 3 periods)

p values show comparisons between I and II, and II and III respectively. Wilcoxon's test

Investigations were started at 0900h in the fasting state. Subjects were supine during the investigation, standing up only during urinary voiding. To promote diuresis 250 ml of water was given every 20 min, starting one h before the experiment and continuing throughout the clearance procedure. All the variables mentioned below were measured in each 20-min clearance period.

GFR and RPF were measured using a steady-state infusion technique with ¹²⁵I-iothalamate and ¹³¹I-iodohippurate, respectively [13]. Urinary albumin and β_2 -microglobulin were measured by radioimmunoassays [7, 12]. Plasma glucagon concentration was measured, after extraction, by radioimmunoassay, using antiserum K964 [9]. Plasma glucose was measured by a glucose oxidase method on an Autoanalyser. Total plasma protein concentration was measured by refractometry. Blood pressure and pulse rate were determined during each clearance period.

The mean intra-individual coefficient of variation in the four consecutive control clearance periods was as follows: 3.3% (GFR), 3.7% (RPF), 30.2% (albumin excretion), 22.4% (β_2 -microglobulin excretion).

Protocol

After 40 min of constant infusion, 4 control clearance periods of 20 min each were performed. Glucagon (Novo, Copenhagen) infusion (4–5 ng/kg/min) was then started and continued for 4 further 20 min clearance periods, followed immediately by another 3 twenty min clearance periods with glucagon given at 8 to 10 ng/kg/min.

The effect of high dose glucagon infusion could not be evaluated in 3 patients: one was unable to pass urine and two patients developed nausea and vomiting during the high dose infusion.

The results were compared with those obtained during glucagon infusion (10 ng/kg/min) in six normal male subjects (mean age 25 years) by exactly the same technique [16]. Wilcoxon's non-parametric test for paired comparison was used for statistical analysis, excluding the values for the transitional clearance periods (5 and 9), the first periods after hormone infusion was initiated and when it was later increased. Wilcoxon's test for unpaired comparison was used to test differences between diabetic and control subjects.

Results

Table 1 shows the individual changes in plasma glucagon and kidney function induced by glucagon infusion in 9 short-term insulin-dependent diabetics. Plasma glucagon concentration increased rapidly, reaching a steady level of about 440 ± 21 pg/ml within 30 min after the start of the low dose infusion. A new plateau level of about 730 ± 52 pg/ml was obtained 30 min after infusion with the high glucagon dose. The average control glucagon concentration was 254 ± 19 pg/ml. GFR increased in all subjects after the start of the low dose infusion and remained elevated during the infusion. The mean value in the last 3 clearance periods was 141 ± 4 ml/min/1.73 m² compared to a control value of 133 ± 5 ml/min/1.73 m² ($p < 0.01$). A further increase took place during the high dose infusion, with a mean GFR in the last 3 periods of 148 ± 7 ml/min/1.73 m² ($p < 0.01$). RPF increased in all patients, save one: average values, 530 ± 21 ml/min/1.73 m² (before), 555 ± 20 ml/min/1.73 m² (low dose), and 572 ± 29 ml/min/1.73 m² (high dose), ($p < 0.01$). Filtration fraction remained unchanged during the infusion with mean values of 0.25 ± 0.01 , 0.26 ± 0.01 , and 0.26 ± 0.01 respectively.

RPF and GFR before and during the glucagon infusion showed a close correlation; ($r = 0.58$; $p < 0.01$) (Fig. 1).

Figure 2 shows the individual changes in GFR in relation to the changes in plasma glucagon concentration. A significant positive correlation was found between the two variables ($r = 0.67$; $p < 0.01$). No

significant correlation was found between plasma glucagon concentration and RPF.

Urinary albumin excretion rate remained unchanged during the glucagon infusion; 5.5 ± 1.0 (before), 5.6 ± 1.2 (low dose), and 5.9 ± 1.7 $\mu\text{g}/\text{min}$ (high dose). Urinary β_2 -microglobulin excretion rose during the infusion to mean of 8.7 ± 1.7 $\mu\text{g} \times 10^{-2}/\text{min}$ during high dose infusion, low dose glucagon, and 17.9 ± 5.7 $\mu\text{g} \times 10^{-2}/\text{min}$ during high dose infusion as from a mean control value of 5.8 ± 1.0 $\mu\text{g} \times 10^{-2}/\text{min}$ ($p < 0.01$). An increase in urine volume also occurred during the infusion: 11.5 ± 0.9 ml/min (before), 15.3 ± 0.8 ml/min (low dose), and 17.4 ± 1.1 ml/min (high dose), ($p < 0.01$).

Blood pressure and pulse rate were the same before and during the low and high dose glucagon infusion. There was a slight decrease in plasma protein levels during the investigations: 65 g/l (before), 64 g/l (low dose) and 63 g/l (high dose), ($p < 0.05$).

The basal GFR was significantly elevated in the diabetics (133 ± 5 ml/min/ 1.73 m^2) compared to our normal value of 115 ± 3 ml/min/ 1.73 m^2 [16], ($p < 0.01$). Despite this, the increase in GFR for a given increase in plasma glucagon concentration was nearly 2 times greater in the diabetics compared to normals (Fig. 2), ($p < 0.05$). RPF was about the same in the diabetics, mean 530 ± 21 ml/min/ 1.73 m^2 , and in the normals 552 ± 28 ml/min/ 1.73 m^2 . RPF remained unchanged during the glucagon infusion in normals [16], while a significant increase in RPF occurred in the diabetics (Table 1). The basal plasma glucagon concentration was slightly elevated in the diabetics, mean 254 ± 19 pg/ml, compared to the normal value of 200 ± 16 pg/ml.

Discussion

The major novel observation in the present study is that a low rate of infusion of glucagon, resulting in slightly elevated plasma glucagon levels, induced a rise in GFR in well controlled diabetics (mean rise, 8 ml/min/ 1.73 m^2). The plasma glucagon elevation achieved is comparable with that found in poorly controlled insulin-dependent diabetics without ketoacidosis [8]. It is conceivable therefore that glucagon contributes to the reversible GFR elevation typically found in poorly controlled insulin-dependent diabetics. Glucagon apparently does not contribute to the elevated GFR found in most well controlled insulin-dependent diabetics where, as in the present study, the basal plasma glucagon concentration was only slightly elevated compared to control [16].

The demonstrated increase in glomerular filtration surface area in newly diagnosed insulin-dependent (+ 80 percent) and short-term insulin-dependent

Fig. 1. Renal plasma flow and glomerular filtration rate before and during intravenous glucagon infusion in nine short-duration insulin-dependent diabetics. $r = 0.58$ ($p < 0.01$)

Fig. 2. Correlation between the rise in plasma glucagon concentration and the increase in glomerular filtration rate in nine short-duration insulin-dependent diabetics ($\bullet$). ($r = 0.67$; $p < 0.01$). Note that the increase in glomerular filtration rate for a given increase in plasma glucagon concentration is nearly 2 times greater in the diabetics compared to normals ($\circ$), ($p < 0.05$). There was no significant correlation between Δ GFR and Δ plasma glucagon in normal subjects

diabetics (+ 40 percent) has been suggested as the dominant mechanism for the GFR elevation found before and during insulin treatment [10, 19]. However, the rapid reversibility of GFR induced by changing metabolic control, suggests functional alterations to be quantitatively important for the GFR elevation. Mogensen [13] clearly demonstrated that GFR in newly diagnosed untreated insulin-dependent juvenile diabetics could be reduced considerably during the first 2 weeks of insulin treatment, e.g., 158 (before) and 137 (during insulin therapy) ml/min/1.73 m². Parving et al. [17] measured GFR in 7 short-term insulin-dependent juvenile diabetics during good and poor metabolic regulation (mean glucose 6.4 and 14.3 mmol/l, respectively). The interval between the two investigations was less than 10 days. GFR was elevated in all patients during poor metabolic control, mean 121 ml/min/1.73 m², but only 101 ml/min/1.73 m² during good control. Thus short-term changes in metabolic control induce marked alterations in GFR. The metabolically induced alteration in GFR can hardly be explained by the demonstrated increase in glomerular filtration surface area, since this abnormality is only slowly reversible and essentially unchanged after 5 weeks of insulin treatment in newly diagnosed juvenile diabetics [10]. The enlargement of the glomerular filtration surface area might of course be important for the more moderate and slowly reversible GFR elevation found in clinically well controlled insulin-dependent juvenile diabetics.

Recently Brenner and Humes [1] reviewed the variables regulating GFR. These include RPF, hydraulic and oncotic pressures and the glomerular permeability-surface area-product. The finding of an unchanged urinary albumin excretion rate in spite of a slightly reduced tubular protein reabsorption does not suggest an increased permeability-surface area-product to be present during glucagon infusion. The effect on GFR of the slight decrease in total plasma protein (oncotic pressure) is negligible [1]. The elevated GFR can hardly be explained by increased intraglomerular filtration pressure, since the filtration fraction remained unchanged during the glucagon infusion. The present finding of an elevated RPF and a relationship between RPF and GFR, suggest RPF as the main mechanism of the observed GFR elevation. Increased RPF and GFR has been demonstrated in man and dog, using much larger amounts of glucagon than in the present study [3, 6, 11]. Finally it should be mentioned that RPF is quantitatively the dominant factor in the regulation of GFR [1]. Since arterial blood pressure remained unchanged in the present and previous studies, this suggests that glucagon induces a dilatation of the glomerular arterioles [18].

The finding of an increased β_2 -microglobulin excretion confirms our previous observation in normal subjects that glucagon reduces the tubular reabsorption of protein [16]. Insulin intravenously has exactly the opposite effect on the tubular handling of protein [14].

It is not likely that the small rise in plasma glucose contributed to the above mentioned kidney function alterations, as discussed previously [13].

It should be mentioned in conclusion that glucagon induces a more pronounced increase in GFR and RPF in the diabetic than in the normal subjects [16]. This difference may be due to the glucagon induced enhancement of insulin secretion in normal subjects leading to a reduced glucagon/insulin ratio. This suggestion is supported by the finding that 7 to 8 IU of insulin given intravenously in short-term juvenile diabetics have the opposite effect to those noted during glucagon infusion, i.e., reduced GFR and RPF [14].

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