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Studies of renal autoregulation in pancreatectomized and streptozotocin diabetic rats

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Studies of renal autoregulation in pancreatectomized and streptozotocin diabetic rats. We studied renal autoregulation in pancreatectomized Munich-Wistar diabetic rats and in their sham-operated controls. In a second experiment we studied renal autoregulation in untreated and insulin treated streptozotocin diabetic Munich-Wistar rats and their nondiabetic controls. In the first experiment the diabetic rats had higher baseline renal blood flows (RBF). There was a fall in renal vascular resistance (RVR) and sustained RBF in both diabetic and control rats as renal perfusion pressures (RPP) was reduced from 130 and 110 mm Hg. As RPP was reduced from 110 and 80 mm Hg, there was no significant change in RVR in control rats and RBF began to fall. Below RPP of 80 mm Hg RVR rose and RBF fell sharply in these rats. In contrast, there was a progressive fall in RVR as RPP was lowered to 60 mm Hg in the diabetic rats and, thus, RBF was much better sustained in these animals. In the second experiment the plasma glucose level was 502 ± 52 mg/dl ($\bar{X}$ ± SD) in the untreated diabetic rats and only modestly reduced to 411 ± 49 mg/dl in the insulin treated animals. Untreated streptozotocin diabetic rats had moderately reduced and insulin-treated diabetic rats had mildly reduced baseline RVR and RBF. However, in these animals as in the pancreatectomized rats the increases in RVR noted in control rats at subautoregulatory RPPs were not seen. Thus, regardless of whether baseline RBFs were increased or decreased, diabetic rats sustained RBF at markedly reduced RPPs far more efficiently than did nondiabetic rats. The pathogenesis of these abnormalities in diabetic rats was not learned in these studies. However, it is likely that further study of autoregulation in diabetic rats could uncover factors influencing renal vascular tone which would be helpful in understanding the renal hemodynamic perturbations which may attend this experimental model.

Many juvenile onset diabetic patients have large kidneys and elevated glomerular filtration rates (GFR) and renal blood flow (RBF) very early in the disease [1]. Similar findings may be present in moderately diabetic animals [2–5]. Furthermore, some [2] but not all [4] strains of moderately diabetic rats have elevated glomerular capillary pressures, although severely diabetic rats have normal or reduced glomerular capillary pressures and flows [2, 4, 6]. It has been suggested that perturbations in glomerular hemodynamics might influence the rate of development of renal injury in diabetes [2, 7, 8], although effects of these functional perturbations on rates of glomerular basement membrane widening or on fractional mesangial volume increases have not yet been documented in animals or man.

Since GFR and RBF are independent of renal perfusion pressure (RPP) over the autoregulatory range [9], we wanted to know if, in the early diabetic state, the renal perfusion pressure-blood flow relationship was altered.

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Methods

Two noncontemporaneous studies were performed. In the first (Experiment I) Munich-Wistar rats were made diabetic by subtotal pancreatectomy while in the second (Experiment II) streptozotocin was used to induce diabetes in this same strain of rats.

Experiment I

The subtotal pancreatectomy model of Foglia, Mancini and Cardeza [10] was used. Fifteen male Munich-Wistar rats (Simonsen, California, USA) weighing approximately 250 g were anesthetized with an intraperitoneal injection of 50 mg/kg/body weight of Brevital. Six of these rats underwent subtotal pancreatectomies (95 to 98% pancreatic tissue removal) and splenectomies. The latter was done to shorten the time of surgery. Eight age and weight matched (± 10 g) rats received a sham operation, which included splenectomies. After the surgery, the animals were returned to individual cages and fed a liquid diet for 48 hours. Thereafter, they received Purina lab chow diet and water ad libitum. Animals were tested daily for urinary glucose to confirm the onset of diabetes mellitus, and they were weighed once a week. Food intake was not measured.

Experiments were performed two to six weeks after the appearance of the glycosuria which generally was first seen from 15 to 30 days post-pancreatectomy. Since all animals did not develop glycosuria at the same time, a successful experiment on a diabetic (D) rat was followed by an experiment on an age-matched sham operated control (C) rat. In the group of diabetic rats reported herein plasma glucose levels were not measured except at the end of the renal physiologic studies and, thus, these were not considered representative. These plasma glucose levels were 616 ± 18 mg/dl ($\bar{X}$ ± SD). However, in 16 rats prepared in parallel to those presented here non-fasting plasma glucose levels were 379 ± 99 mg/dl ($\bar{X}$ ± SD) in unmanipulated animals as compared to 137 ± 7 mg/dl in 19 control animals. Rats were allowed free access to food and water prior to experimentation.

Table 1A. Model characteristics in experiment I in subtotal pancreatectomized diabetic and sham control rats

	Body wt	Kidney wt	Kidney wt/ body wt ratio	HCT	AP	RBF	RVR
	g			*%*	*mm Hg*	*ml/min/rat*	*mm Hg/ml/min*
Diabetics $N = 6$	251 ± 7	2.8 ± 0.2	0.0056	49 ± 1.5	129 ± 5	10.4 ± 0.7	12 ± 0.7
Controls $N = 8$	283 ± 8	2.4 ± 0.1	0.0042	49 ± 0.6	144 ± 3	8.6 ± 0.6	17 ± 1.2
$2P$ values	< 0.025	NS	NS	NS	< 0.05	NS	< 0.001

Abbreviations are: Body wt, body weight; Kidney wt, weight of kidney per rat; HCT, hematocrit; AP, arterial pressure; RBF, renal blood flow, RVR, renal vascular resistance; N = number of animals studied.

Experiment II

Male Munich-Wistar rats (Bioproducts Division of Harlan Sprague-Dawley, Madison, Wisconsin, USA) aged 50 days and weighing 100 to 120 g were made diabetic by the intravenous injection of 65 to 70 mg/kg of streptozotocin (supplied by Upjohn Corp., Kalamazoo, Michigan, USA) prepared in citrate buffer, pH 4.0. Diabetes was confirmed by the development of nonfasting plasma glucose levels exceeding 400 mg/dl. These levels were determined by the glucose oxidase method on a Beckman Glucose Analyzer (Beckman Instruments Inc., Fullerton, California, USA).

Two groups of diabetic rats were studied. The first group (D) was untreated with insulin ($N = 7$). The second group (D + I, $N = 11$) received a special heat treated, long-acting ultra lente insulin (supplied by Novo Industry, Copenhagen, Denmark) in a daily dose of 0.3 to 1.4 IU (0.94 ± 0.32) given at 3:00 to 5:00 p.m. Plasma glucose levels to monitor the insulin treatment were obtained at 8:00 to 11:00 a.m. When the insulin dose was stabilized, glucose levels were measured every other day. The aim was to maintain plasma glucose levels at approximately 350 mg/dl, and this was generally achieved. The glucose levels presented in **Results** (Table 1B) are from unmanipulated animals just prior to the autoregulatory studies. Eight littermates served as controls.

All rats were also given free access to water and to standard Purina rat chow. Food intake was measured in rats individually housed for two consecutive days. Daily food intakes were recorded and the average intake for the two days calculated.

Kidney physiology studies

Renal function studies were performed six to eight weeks after induction of diabetes in Experiment II and as noted above in Experiment I. On the day of these studies, animals were anesthetized with intraperitoneal Inactin (80 mg/kg body wt). Animals were placed on a temperature-regulated micropuncture table. A tracheostomy was performed. Urine flow was monitored through a bladder catheter. In Experiment I a solution of 1/3 plasma (obtained from littermates) to 2/3 Krebs-Henseleit solution was infused via a femoral vein catheter at a rate of 5 ml/hr for 30 minutes, thereafter the rate was reduced to 1.2 ml/hr. In Experiment II rats were infused via an external jugular vein with 5% bovine serum albumin, 0.5% of body weight over 20 minutes. This was followed by a bolus of 0.5 ml of a mixture of 6.6 μCi of methoxy ^{3}H-inulin (ICN Radiochemicals Division of ICN Biomedicals, Inc., Irvine, California, USA) diluted 1:1 in Ringers solution over 10 minutes followed by a constant infusion of the same mixture at a rate of 1.2 ml/hr.

In Experiment II two 30-minute urine collections were obtained for the measurement of GFR following a 30-minute equilibration period, and blood samples were withdrawn from the femoral artery catheter at the midpoint of these collection periods. GFR was not measured in Experiment I.

In both experiments femoral arterial pressure (AP) was considered as the renal perfusion pressure (RPP) and was monitored with a Statham P23 Db pressure transducer (Statham Instruments, Los Angeles, California, USA) connected to a Beckman Dynograph (Beckman Instruments). The left kidney and the aorta were exposed through a midline and left lateral abdominal incision and the left renal artery was dissected with special care in avoiding disturbance of the renal nerves.

Blood flow in the left renal artery was measured continuously by a small diameter flow probe (1.5 or 1.8 mm circumference lumen size) connected to a square wave electromagnetic flowmeter (Model 501, Carolina Medical Electronics, King, North Carolina, USA) and a Beckman Dynograph. The flow probe and flowmeter were calibrated using an in vitro technique [9]. The electronic zero of the flowmeter system was calibrated in vitro and in vivo. Absolute zero flow through the renal artery was determined at the end of the experiment. Positioning of the flow probe around the renal artery was judged acceptable when there was a snug fit and the pulsatile flow recorded was free from mechanical "noise". After 15 minute equilibration, baseline values for hematocrit (Hct), renal blood flow (RBF) and arterial pressure (AP) were obtained for 15 minutes. Thereafter, the relationship of RBF to AP was studied over a pressure range from 140 to 50 mm Hg.

Progressive decrements in AP of approximately 10 mm Hg were achieved and maintained at a stable level (± 3.5 mm Hg) by using a constrictor clamp around the aorta above the renal arteries. The degree of aortic constriction was obtained manually through a knob connected to a tube filled with distilled water. The pressure exerted by the hydraulic column descended the plate of the aortic clamp. This device allowed for adjustment of renal perfusion pressure without disturbing the animal. Each level of perfusion pressure was maintained for 3 minutes. When the 50 mm Hg pressure period ended, the aortic constrictor was slowly released and AP was allowed to return to basal levels.

After a 15-minute equilibration period, Hct, RBF, and AP were again obtained for a period of 15 minutes and a new

pressure-flow curve performed. At the end of this curve, AP and RBF were allowed to return to baseline for 10 minutes before occluding the renal artery to obtain the absolute zero flow ± 0.5 ml. Experiments that had greater deviations were not used. Finally, the kidney was removed and weighed and blood samples were obtained for Hct determinations.

Seventy-seven percent of the physiology experiments were successfully completed. Experiments failed because the main renal artery branched too close to the aorta to allow probe placement, instability of systemic blood pressure, low urine flow during GFR measurements, or failure of the hematocrit to remain stable. There was no tendency for experimental failure to cluster in any of the animal groups.

Calculations

Intrarenal vascular resistance (RVR) was calculated from the arterial venous pressure difference and blood flow. Renal venous pressure was assumed to be constant and assigned a value of 5 mm Hg in the calculations [9]. Thus,

$$\text{RVR} = \frac{\text{RPP} - 5 \text{ mm Hg}}{\text{RBF}} \text{ in mm Hg/ml/min}$$

based on total RBF assuming equal RBF in both kidneys

$$\text{RBF} = \frac{\text{RPF}}{(1 - \text{Hct})} \text{ in ml/min}$$

$$\text{FF} = \frac{\text{GFR}}{\text{RPF}}$$

where FF = filtration fraction.

To compare the efficiency of autoregulation among the groups studied, the autoregulation factor was calculated as

$$\frac{\text{RBF}_2 - \text{RBF}_1}{\text{RBF}_1} \Big/ \frac{\text{RPP}_2 - \text{RPP}_1}{\text{RPP}_1}$$

where RPP_1 and RPP_2 are selected perfusion pressures and RBF_1 and RBF_2 are the respective measured flow rates at the given perfusion pressures [11, 12]. A value of 1 indicates absence of autoregulation while a ratio of less than 1 indicates presence of autoregulation. RBF_1 equal to RBF_2 indicates perfect autoregulation.

Curvilinear relationships between variables were fitted with a polynomial equation (order of three). The value of the independent variable when the independent variable reached a maximum or minimum (inflexion or break point) was determined in two different ways: by repeated calculations of the equation and variation of the independent variable and by calculating the value of the independent variable when the first derivative of the polynomial was equal to zero.

Statistical analyses

The autoregulatory factor for a given range of RPP was compared between groups by a two-tailed Student's *t*-test for unpaired data. Comparisons of different ranges of RPP within a group was done by Student's *t*-test for paired data. Linear regression analysis was done by the method of least squares. A value of $2P < 0.05$ was considered statistically significant. However, due to the number of statistical comparisons made, caution should be used in interpreting values for $2P$ between 0.01 and 0.05.

Results

Model characteristics—Experiment I

Body weights in diabetics were about 11% lower than in sham operated controls ($2P < 0.025$). The mean 17% increase in kidney weight and the increased kidney wt/body wt of diabetics did not reach statistical significance (Table 1A). AP was about 15 mm Hg lower in diabetic animals ($P < 0.05$). Hct values were similar. The average basal levels of RBF was not different between diabetic and sham rats but RBF/100 g body wt was significantly higher in diabetic rats. Renal vascular resistance was decreased by 42% in diabetic rats ($P < 0.001$).

Model characteristics—Experiment II

Rats with untreated diabetes had severe hyperglycemia (502 ± 52 mg/dl) which was only modestly ameliorated by the insulin treatment (411 ± 49 mg/dl, $2P < 0.0002$, Table 1B). Food intake after two weeks of diabetes increased by about 54% in untreated diabetic and insulin treated diabetic rats compared to controls ($P < 0.03$ for each comparison). Food intake after eight weeks of diabetes was about 63% greater in both untreated and treated diabetic rats compared to controls ($2P < 0.0001$ for each comparison). There were no differences in food intake at any time comparing untreated and insulin treated diabetic rats.

Body wt at eight weeks was lowest in untreated diabetic rats (66% of control body wt, $2P < 0.001$) but was still reduced in insulin treated rats (81% of controls, $2P < 0.002$). Kidney wt/body wt were increased by about 50% in both groups of diabetic rats compared to controls (Table 1B) but were not significantly different when untreated and insulin treated rats were compared. There were no significant differences in baseline Hct among any of the groups. Severely diabetic but not insulin treated diabetic rats had reduced AP compared to controls at baseline (Table 1B). GFR was reduced by 32% in severely diabetic rats compared to controls ($2P < 0.04$) and by 16% in insulin treated rats ($2P < 0.04$, Table 1B). GFR was 16% higher in insulin treated compared to untreated diabetic rats ($2P < 0.002$). GFR per 100 g body weight was similar in all three groups. RBF was lowest in severely diabetic rats and highest in control rats but, again, based on body weight, was not different between the three groups (Table 1B). Baseline RVR was highest in the severely diabetic group and lowest in the controls (Table 1B) while filtration fraction was not different among these groups of rats.

Autoregulation studies—Experiment I

Renal blood flow (RBF) at 120 mm Hg perfusion pressure (RPP) was higher in the diabetic rats and remained higher through the stepwise reduction in RPP to 50 mm Hg compared to controls (Fig. 1). The break point for autoregulation in the control animals occurred at 100 mm Hg RPP. Although there was no clear break point in the diabetic rats, the best estimate for this was between 50 and 60 mm Hg RPP (Fig. 1). Expressed as a percent of flow at 120 mm Hg the RBFs were greater in diabetic rats at RPPs below 110 mm Hg, and these differences increased in magnitude as RPP was further reduced (Fig. 2). RVR initially fell in both groups but began to increase in controls at RPP below 70 mm Hg while continuing to decrease in the diabetic rats (Fig. 3).

Table 1B. Model characteristics in experiment II in streptozotocin insulin treated and untreated diabetic rats and in controls

	Glucose *mg/dl*	Body wt *g*	Kidney wt *g*	Kidney wt/ body wt ratio	HCT %	AP *mm Hg*	GFR/kidney *ml/min*	GFR/kidney *ml/100 g body wt*
Untreated diabetics (D) $N = 7$	502 ± 52	186 ± 31	1.2 ± 0.2	0.0066 ± 0.0005	0.48 ± 0.02	115 ± 7	0.75 ± 0.05	0.84 ± 0.1
Insulin treated diabetics (D + I) $N = 11$	411 ± 49	229 ± 24	1.3 ± 0.1	0.0058 ± 0.0005	0.47 ± 0.02	125 ± 12	0.96 ± 0.15	0.85 ± 0.1
Controls (C) $N = 8$	123 ± 9	281 ± 37	1.0 ± 0.1	0.0035 ± 0.0005	0.49 ± 0.02	129 ± 7	1.1 ± 0.15	0.79 ± 0.1
$2P$ values								
D vs. C	0.0001	0.0001	0.002	0.02	NS	0.002	0.04	NS
D + I vs. C	0.0001	0.0002	0.0001	0.0001	NS	NS	0.04	NS
D vs. D + I	0.0002	0.004	NS	NS	NS	NS	0.002	NS

Abbreviations are: Body wt, body weight; Kidney wt, weight of a single kidney; HCT, hematocrit; AP, arterial pressure; GFR, glomerular filtration rate; RBF, renal blood flow; RVR, renal vascular resistance; FF = filtration fraction; NS = not significant.

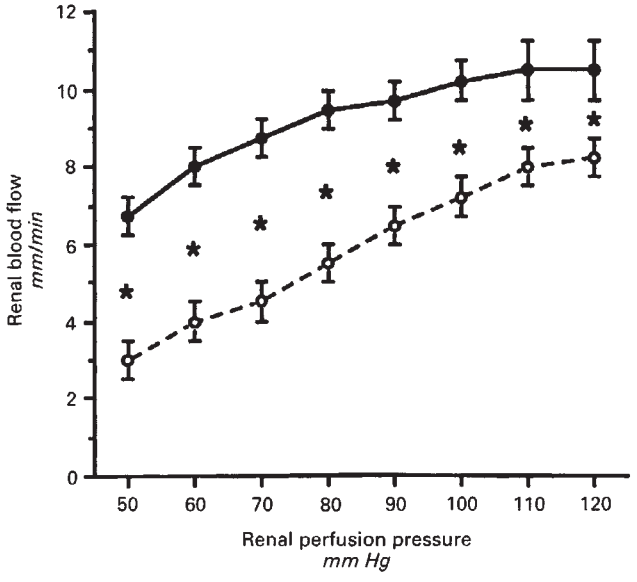

Fig. 1. *Renal blood flows at varying renal perfusion pressures in pancreatectomized diabetic (D, –●–) and sham operated control (C, --○--) animals in Experiment I.* * = significant difference between D and C.

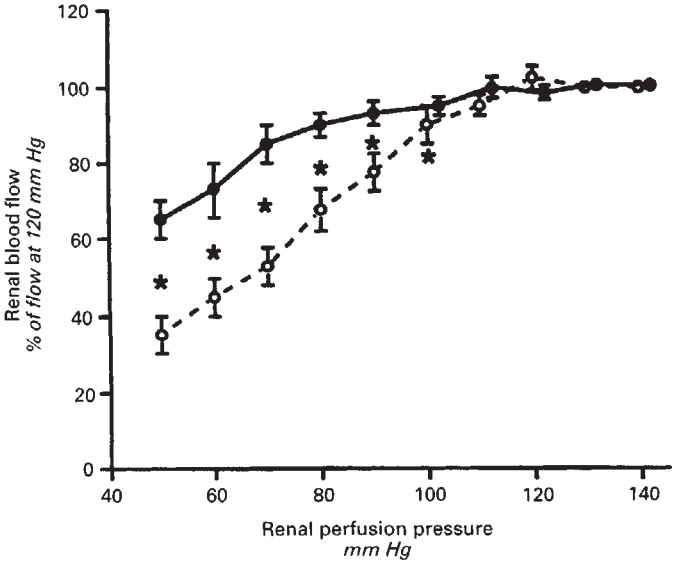

Fig. 2. *Renal blood flows at varying renal perfusion pressures expressed as a percent of the flow at 120 mm Hg in pancreatectomized diabetic (D, –●–) and sham operated control (C, --○--) animals in Experiment I.* * = significant difference between D and C.

Calculations of the autoregulation factor for RPP 90 to 120 mm Hg indicated that autoregulation was more efficient in diabetic rats ($2P < 0.005$). Autoregulation factor was not different from unity or from each other in diabetic and control rats for RPP 60 to 90 mm Hg (Fig. 4).

Autoregulation studies—Experiment II

In this experiment RBF was lower in untreated diabetic rats compared to controls at 110 mm Hg, with intermediate values in the insulin treated rats. In control rats the break point in the autoregulatory curve appeared at 115 mm Hg while both diabetic groups continued to sustain their respective basal RBFs below this RPP. Thus, at RPPs of 60 and 50 mm Hg, RBF was now significantly higher in insulin-treated diabetic rats compared to controls (Fig. 5). Expressed as percent of RBF at 110 mm Hg, the values were higher in the treated and untreated diabetic rats compared to the control rats at all RPP below 110 mm Hg, except at 90 mm Hg where insulin treated diabetics were not significantly different from controls (Fig. 6).

RVRs were initially significantly higher in untreated diabetic rats compared to controls at all RPP between 100 and 80 mm Hg (Fig. 7). However, below RPRs of 95 mm Hg RVR began to rise in controls while still falling in the untreated diabetic rats, so that at RPPs of 60 and 50 mm Hg RVRs tended to be higher than in the controls. Similarly, RVR was higher in insulin treated diabetics compared to controls at 120, 110, and 100 mm Hg RPPs, and significantly lower than controls at RPPs of 60 mm Hg RPR ($P < 0.04$) and 50 mm Hg RPP ($2P = 0.04$, Fig. 7). Autoregulation was significantly more efficient between the RPPs of 90 to 110 mm Hg for severely diabetic rats compared to

Table 1B. *Continued*

RBF/kidney *ml/min*	RBF/kidney *ml/min/100 g* body wt	RVR *mm Hg/ml/min*	FF
4.8 ± 0.6	2.6	23.2 ± 2.1	0.33 ± 0.05
6.2 ± 0.7	2.7	19.9 ± 3.1	0.31 ± 0.02
7.7 ± 1.1	2.8	16.3 ± 2.3	0.29 ± 0.03
0.0001	NS	0.0001	NS
0.001	NS	0.014	NS
0.002	NS	0.028	NS

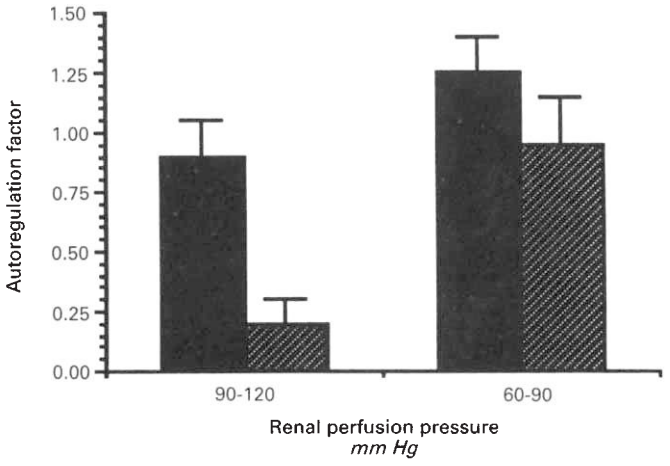

Fig. 4. *Autoregulation factor results in Experiment I in pancreatectomized diabetic (D, ▨) and sham operated control (C, ■) animals.*

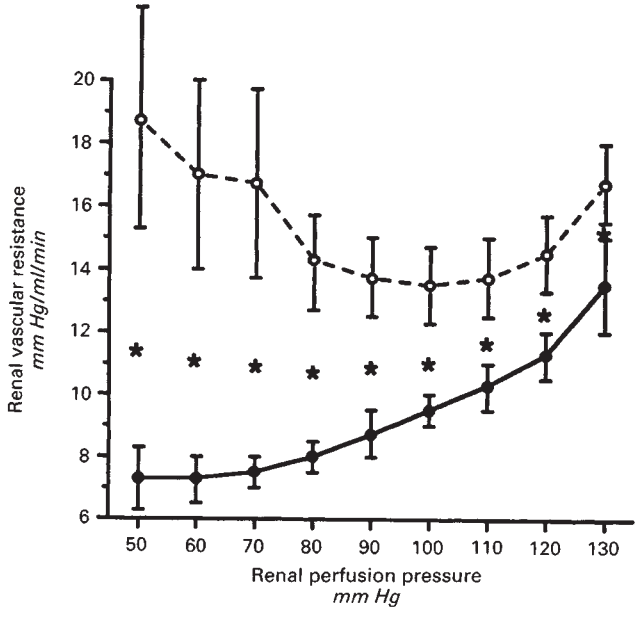

Fig. 3. *Renal vascular resistances at varying renal perfusion pressures in pancreatectomized diabetic (D, –●–) and sham operated control (C, ––○––) animals in Experiment I.* * = significant difference between D and C.

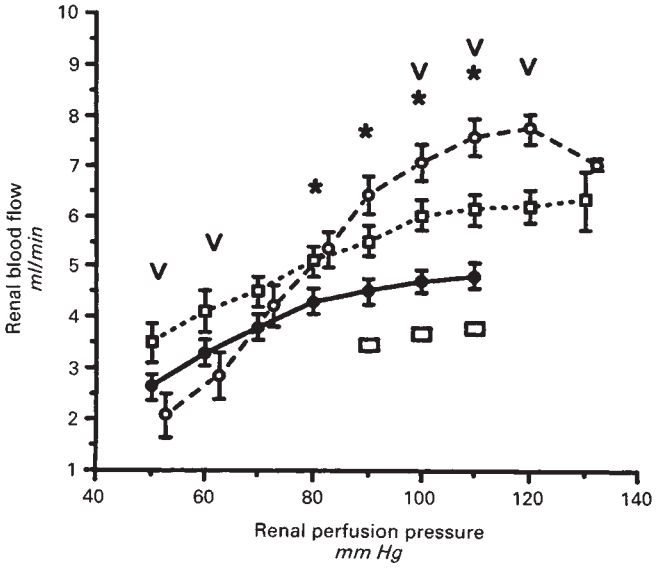

Fig. 5. *Renal blood flows at varying renal perfusion pressures in untreated diabetic (D, –●–), insulin treated diabetic (D + I, ––□––) and control (C, ––○––) animals in Experiment II.* * = significant difference between D and C; v = significant difference between D + I and C; □ = significant difference between D and D + I.

controls ($2P < 0.005$) and showed a similar tendency for insulin-treated diabetic rats ($P < 0.08$). The autoregulation factor for RPP 60 to 90 mm Hg in control rats was greater than unity and greater than that in severely diabetic rats ($P < 0.003$) and insulin-treated diabetic rats ($2P < 0.001$; Fig. 8).

Discussion

These studies show that the renal hemodynamic responses to graded reduction in renal perfusion pressure are markedly different in diabetic as compared to control rats. The results indicate that regardless of whether baseline renal blood flow is increased or decreased in the diabetic animals, renal blood flow was remarkably sustained in association with progressive decrements in renal vascular resistance. In contrast, renal vascular resistance initially declined in nondiabetic rats, then became level and finally, at perfusion pressures below 80 mm Hg, began to rise sharply. This pattern of response to reductions in renal perfusion pressure in normal rats, including the variability in the onset and magnitude of the increase in renal vascular resistance at low perfusion pressures, has been seen in numerous studies [9, 11, 13–17]. However, the diabetic pattern of response in which the progressive increase in RVR in the lower range of subautoregulatory pressures fails to occur has not been previously described except in young rats treated with prostaglandin inhibitors [16] and in salt depleted rats treated with either saralasin or captopril or with kinin infusion [18].

As expected [2, 7], body weights were reduced in the diabetic animals, most severely in untreated streptozotocin diabetic

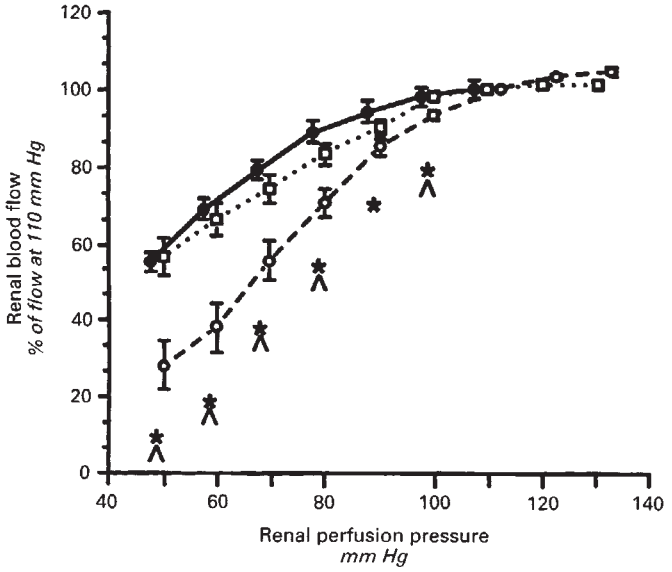

Fig. 6. *Renal blood flows at varying renal perfusion pressures expressed as a percent of the flow at 110 mm Hg in untreated diabetic (D, –●–), insulin treated diabetic (D + I, ··□··) and control (C, ––○––) animals in Experiment II.* * = significant difference between D and C; ∧ = significant difference between D + I and C.

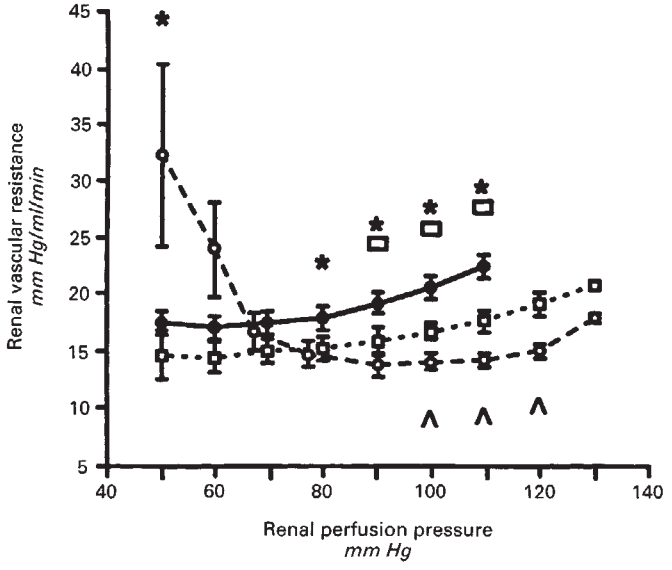

Fig. 7. *Renal vascular resistances at varying renal perfusion pressures in untreated diabetic (D, –●–), insulin treated diabetic (D + I, ––□––) and control (C, ––○––) animals in Experiment II.* * = significant difference between D and C; ∧ = significant difference between D + I and C; □ = significant difference between D and D + I.

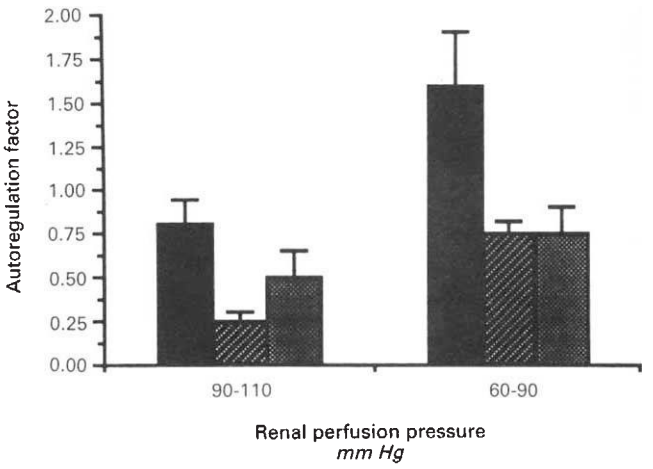

Fig. 8. *Autoregulation factor results in Experiment II in untreated diabetic (D, ▨), insulin treated diabetic (D + I, ▩) and control (C, ■) animals.*

rats. However, all were in positive nitrogen balance as indicated by increased body weight over time since induction of diabetes. Increased kidney weight to body weight ratios have been previously described in diabetic rats [6] as has increased absolute kidney size in animals [6, 19, 20] and in type I diabetes in man [1]. However, accelerated renal growth antedates measureable renal hemodynamic changes following induction of diabetes in rats [21] and occurs with increased [2, 22], normal [6] or decreased [6] GFR and RBF. Baseline arterial blood pressures were lower in the severely diabetic rats in both studies, this confirming observations we have made with another strain of diabetic rats [6].

The two models of diabetes, subtotal pancreatectomy in which islet mass is markedly reduced and streptozotocin induced diabetes in which pancreatic islet cells are spared but for specific β-cell destruction [23], resulted in different renal hemodynamic outcomes. Thus, pancreatectomized rats, a model which in our hands produces moderate hyperglycemia, had increased renal blood flow at baseline renal perfusion pressure. In contrast, streptozotocin diabetic rats had lower baseline renal blood flows and glomerular filtration rates than controls, although insulin treated compared to untreated streptozotocin diabetic rats had higher values for these parameters. These animals had higher plasma glucose levels than generally seen in our pancreatectomy model. Similar results for baseline renal hemodynamic measures have been reported by several groups of investigators [2, 4, 7, 8, 24–26]. Generally, moderately diabetic rats with lower values for plasma glucose levels than achieved in the insulin-treated diabetic rats in the current study have increased renal blood flow and GFR. Some [2, 26] but not all [4, 24] strains of moderately diabetic rats also have increased glomerular capillary pressures. Thus, the severity of the diabetic state in the insulin-treated streptozotocin animals in the present studies may explain why renal hyperperfusion and hyperfiltration were not seen. It is also possible that the differences in baseline renal blood flow in the pancreatectomy versus streptozotocin diabetic models represents residual nephrotoxic effects of streptozotocin. However, these influences have been reported to be short lived [27]. Attributing these differences in renal hemodynamics to depletion or preservation of pancreatic islet cell types other than β-cells or to differences in pancreatic exocrine function would be highly speculative but would be deserving of further study if the observations reported here can be confirmed.

However, regardless of whether the baseline levels of RBF in the various groups of diabetic rats were increased or decreased, the responses to graded reductions in RPP were consistent

between the groups and strikingly different from the responses of the control rats. While all groups of rats evidenced autoregulation of blood flow at RPPs above 100 to 110 mm Hg, further reductions in RPP resulted in greater decreases in RBF in the control as compared to the diabetic groups. Conversely, whether the baseline measures of RVRs were higher (pancreatectomy model) or lower (streptozotocin model) than the respective controls, the patterns of change in RVR with progressive lowering of RPP were similar between the diabetic groups and different from the controls. The control groups had an initial fall in RVR as RPP was reduced towards 100 mm Hg. RVR then became level between RPPs of 110 to 80 mm Hg and rose thereafter. The RVRs in the three diabetic groups became level below 70 mm Hg and did not rise. We can conclude that whatever factors determined baseline RVR in the diabetic animals, the presence of or induction of strong vasodilatory forces or the inability to generate strong vasoconstrictive forces, or both, resulted in maintenance of RBF in these rats at extremely low perfusion pressures. Also, diabetic animals with increased baseline RBF based on decreased RVR can further decrease RVR and thus do not have maximal renal vasodilatation at baseline.

Several factors known to influence renal vascular tone or responsiveness may be altered in diabetic rats. Firstly, food and thus protein intake was increased by about 66% when measured in the streptozotocin diabetic rats in these studies as we have previously shown [6]. Increased protein intake is known to increase RBF in normal and diabetic rats [6, 7], probably through reductions in both afferent and efferent glomerular arteriolar resistances [6, 7]. The mechanism by which protein intake influences renal vascular resistance is not entirely understood. There are data consistent with increased glomerular production of vasodilatory prostaglandins in intact and subtotally nephrectomized rats maintained on increased (50%) compared to reduced (6%) dietary protein intake [28]. Further, Paller and Hostetter [29] and Murray [30] have shown that rats fed 50% as compared to 6% dietary protein have diminished systemic and renal vasoconstrictor responses to infused angiotensin II, and that these vasoconstrictor responses could be normalized by prostaglandin synthesis inhibitors but not by angiotensin converting enzyme inhibitors. However, it is unclear whether the 60% increase in protein intake in diabetic compared to control rats in the present studies could have effected the differences related to more than eight fold difference in dietary protein in the above cited experiments [28–30].

Both increased dietary protein intake and diabetes are associated with renal hypertrophy in rats [6, 19, 20]. However, renal hypertrophy, per se, is unlikely to explain the results of the current study since unilateral nephrectomy in adult rats produced no changes in the shape of the autoregulatory curve or in the autoregulation factor [12]. In young intact or uninephrectomized rats prostaglandin inhibition resulted in progressive decrements in RVR in both the normal and hypertrophied kidneys at RPP below the normal range, this resulting in autoregulation curves remarkably similar to those seen in our diabetic rats [16]. Chevalier, Carey and Kaiser concluded from these results that prostaglandin-dependent renin release is important in increasing RVR at RPP below 70 mm Hg in these young rats [16]. In older rats, however, inhibition of prostaglandins did not influence the autoregulatory response [16]. Whatever the mechanism(s) whereby dietary protein intake might influence autoregulatory responses, studies in diabetic and control rats which control for dietary protein intake need to be done.

As suggested above, alterations in the prostaglandin-renin angiotensin system could influence renal autoregulation. Indomethacin infusion caused increased afferent and efferent glomerular arteriolar resistance and decreased transcapillary hydraulic pressure and single nephron glomerular filtration rate in streptozotocin diabetic rats but had no effect on glomerular hemodynamics in control rats [25]. Increased perfusate glucose levels caused a dose dependent vasodilatation and increase in glomerular filtration rate in isolated perfused kidneys from both diabetic and normal rats, and these increases could be partially prevented by prostaglandin synthetase inhibitors [31]. Also, vasodilatory prostaglandin, but not thromboxane B_2, production was increased in glomeruli isolated from untreated streptozotocin diabetic rats but not from diabetic rats rendered normoglycemic by insulin therapy [32]. Similar findings in aorta of streptozotocin diabetic rats have been reported [33]. These alterations in prostaglandin metabolism could act directly on glomerular arteriolar tone or indirectly through modulation of renin release from juxtaglomerular cells [34]. In this regard, despite lower plasma renin concentrations Ballermann, Skorecki and Brenner have reported lower glomerular angiotensin receptor concentrations at low, intermediate or high salt intakes in untreated diabetic Munich-Wistar rats [35]. These receptor abnormalities were normalized by insulin treatment which moderated but did not normalize the hyperglycemia [35]. On the other hand, Bank et al found that low salt intake normalized the elevated GFR and RBF of untreated streptozotocin diabetic, Sprague-Dawley rats and that the effect of this diet could be reversed by administration of an angiotensin converting enzyme inhibitor [36]. Thus, the role of alterations in glomerular angiotensin receptors in influencing glomerular hemodynamics in diabetic rats is unclear.

Reductions have also been described in the renal synthesis of kallikrein in diabetic rats [37] and the renal kinins can stimulate glomerular mesangial cell [38] and arteriolar [39] eicosinoid production. Insulin-treated diabetic rats with hyperfiltration have increased renal and urinary active kallikrein while insulin untreated, severely diabetic rats with reduced GFR and RBF have reduced renal and urinary kallikreins [40, 41].

Bank et al also suggested that defective transmembrane calcium flux across vascular smooth muscle cells could be responsible for decreased renal vascular resistance in streptozotocin diabetic rats based on findings of reduced GFR and RBF in hyperfiltering kidneys of these animals infused with Ca^{++} and insulin and the abrogation of this effect by simultaneous infusion of the calcium channel blocker verapamil [42]. Hyperglycemia per se could lead to abnormalities of intracellular calcium transport through stimulation of the aldose reductase pathway and myo-inositol depletion [43]. Increased plasma levels of atrial natiuretic peptide have also been implicated in the hemodynamic abnormalities of insulin-treated diabetic Munich-Wistar rats [44], but it is difficult to understand how graded reduction in RPP would influence the blood levels of this peptide hormone. Blantz and Konnen have shown, in microperfusion studies, that the addition of glucose to late proximal tubular perfusion fluid blunts the normal single nephron filtra-

tion rate response to increased distal perfusion rate [45]. Blantz et al also demonstrated that microperfusion of glucose-free fluid in hydropenic rats with hyperglycemia induced by glucose administration reduced single nephron glomerular filtration rate (SNGFR) while similarly infused glucose containing proximal tubular fluid from a hyperglycemic rat failed to change SNGFR [46]. Thus, glucose can have local effects on glomerular tubular feedback through mechanisms independent of systemic circulatory alterations. A decrease in the sensitivity of the tubuloglomerular feedback mechanism was confirmed by Jensen et al in studies of moderately hyperglycemic streptozotocin diabetic Wistar rats [47], while Rasch and Holck demonstrated ultrastructural abnormalities of the macula densa of this rat model [48], perhaps providing a structural basis for these functional abnormalities. Woods, Mizelle and Hall showed that glucose infused into the kidney of normal dogs results in increased GFR and RBF and decreased RVR at normal RPP. However, reductions in RPP caused more rapid declines in RBF in these kidneys than in the normoglycemic controls [49]. These latter studies suggest that hyperglycemia, per se, may not be an adequate explanation for the results obtained in our studies. Finally, as reviewed by Ditzel [50], it has been hypothesized that abnormalities in tissue oxygenation in diabetes may underlie disturbances in microvasculatory autoregulation.

It is apparent that there are complex alterations in the factors regulating renal vascular tone in the diabetic rat model. These alterations might be influenced by the mode of induction of diabetes, the strain of rat studied, the severity of the diabetic state and the administration of insulin. The basic abnormalities in renal vascular resistance described in the present studies occurred with different methods of diabetes induction, whether or not the animals had increased or decreased RBF at baseline and whether or not insulin treatment was given. This suggests that further study of autoregulation in this model could uncover factors influencing renal vascular tone in diabetic rats that would be helpful in the understanding of the glomerular hemodynamic perturbations of diabetes.

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