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Renal vessel changes in diabetic KK-mice

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Summary. Glomerular hyperfiltration is thought to be of pathogenic importance in the structural abnormalities seen in diabetic nephropathy but its cause is not known. It has been suggested that the changes in the preglomerular vascular system may lead to a disturbance of glomerular blood flow in diabetes. We therefore examined the potential role of changes in the vascular system supplying the glomerulus in diabetic mice. The kidneys of 15 diabetic KK-mice (aged 2, 5 and 12 months) were studied and compared with those of 15 non-diabetic NMRI-mice. We determined vessel cross-sectional, wall and lumen areas of 408 small intrarenal arteries, 5,140 arterioles and 518 preglomerular afferent arterioles using a morphometric method. At 2 months, diabetic arteries and arterioles were considerably smaller than the controls, while preglomerular afferent arterioles were the same size. At 12 months, however, all diabetic vessels measured were much larger than the controls. This was chiefly due to an excessive increase in lumen area: in the diabetic arteries the mean ($\pm$SEM) lumen area at 12 months was $1{,}057 \pm 142$ vs 616 ± 72 sqμ in controls ($P < 0.001$), in arterioles 176 ± 7 vs 115 ± 4 sqμ ($P < 0.001$) and in preglomerular afferent arterioles (at 5 months) 131 ± 8 vs 95 ± 7 sqμ ($P < 0.001$). The dilatation of small intrarenal arteries and arterioles in diabetic mice may result from progressive impairment of vasoconstriction and may be a cause of the glomerular hyperfiltration in diabetes.

Key words: Blood flow autoregulation – Glomerular hyperfiltration – Diabetic nephropathy – KK-mice – Morphometry

Introduction

Glomerular hyperfiltration is characteristic of early diabetes in man and has also been demonstrated in streptozotocin-diabetic rats and genetically

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diabetic mice (Brenner et al. 1981; Brown et al. 1982; Davies et al. 1985; Gaertner 1978; Hamuro 1971; Hasslacher and Ritz 1985; Jensen et al. 1981; Mathiesen et al. 1984).

This early fuctional abnormality is thought to be of great importance for the pathogenesis of the diabetic glomerulosclerosis but its cause is uncertain (Brenner et al. 1981; Brown et al. 1982; Hostetter et al. 1982; Mauer et al. 1981; Mogensen et al. 1979; Mogensen 1982; Mogensen and Christensen 1984). In the normal kidney glomerular hyperfiltration does not occur. Autoregulation of both renal blood flow and glomerular filtration rate (GFR) is present and total blood flow through the kidney and GFR remain constant at perfusion levels between approximately 80 and 250 mmHg. The glomerulus is protected against overperfusion and thus hyperfiltration is prevented (Detweiler 1980; Witzleb 1977).

There is strong experimental and clinical evidence that the autoregulatory mechanism which guarantees normofiltration resides within the preglomerular vessels: in response to an increase in intravascular pressure the smooth muscle cells of these vessels react by shortening, thereby constricting the vessels, so that a new higher resistance is established. This myogenic autoregulation appears to be the main mechanism controlling glomerular inflow (Detweiler 1980; Witzleb 1977; Parving et al. 1984). It has been suggested that failure of the vascular system supplying the glomerulus to contract adequately in response to an increase in intraluminal pressure may lead to disturbance of glomerular blood flow in diabetes (McMillan 1981) but a systematic morphometric study of these preglomerular vessels has never been carried out.

The aim of this study therefore was to examine the preglomerular vessels in diabetic KK-mice morphometrically. These animals represent an excellent model for studies on diabetic nephropathy as they develop human-like glomerulosclerosis within the first year of life (Ehrenreich et al. 1973; Oppermann et al. 1973; Wehner et al. 1972).

Material and methods

Animals

Diabetic KK-mice. We studied 15 genetically diabetic KK-mice aged 2 ($n=5$), 5 ($n=5$) and 12 months ($n=5$) respectively. The animals were all male and weighed between 20.9 ± 0.6 g (2 mo.) and 30.1 ± 0.7 g (12 mo.). The kidney weights were 0.225 ± 0.011 g (2 mo.) and 0.3503 ± 0.011 g (12 mo.). Their fasting blood glucose levels were determined by the glucose UV-test using the Hexokinase method (Boehringer, Mannheim, FRG). The levels were 141 ± 25 mg/100 ml at 2 mo., 164 ± 20 mg/100 ml at 5 mo. and 200 ± 10 mg/100 ml at 12 months. The animals are almost fully in correspondence with the statements of Nakamura and Yamada (1967).

Controls. A non-diabetic KK-variant does not exist. We therefore used 15 age- and sex-matched NMRI-mice (Ivanovas, Kisslegg, FRG) as non-diabetic controls. Body and kidney weights of KK and NMRI mice were similar in all age groups. The blood glucose levels of the animals were between 125 ± 3.3 mg/100 ml (2 mo.) and 96 ± 15 mg/100 ml (12 mo.).

The animals (KK and NMRI) received the same food (Ssniff mixed mouse food, Intermast GmbH, Bockum-Hoevel, FRG) and had free access to water.

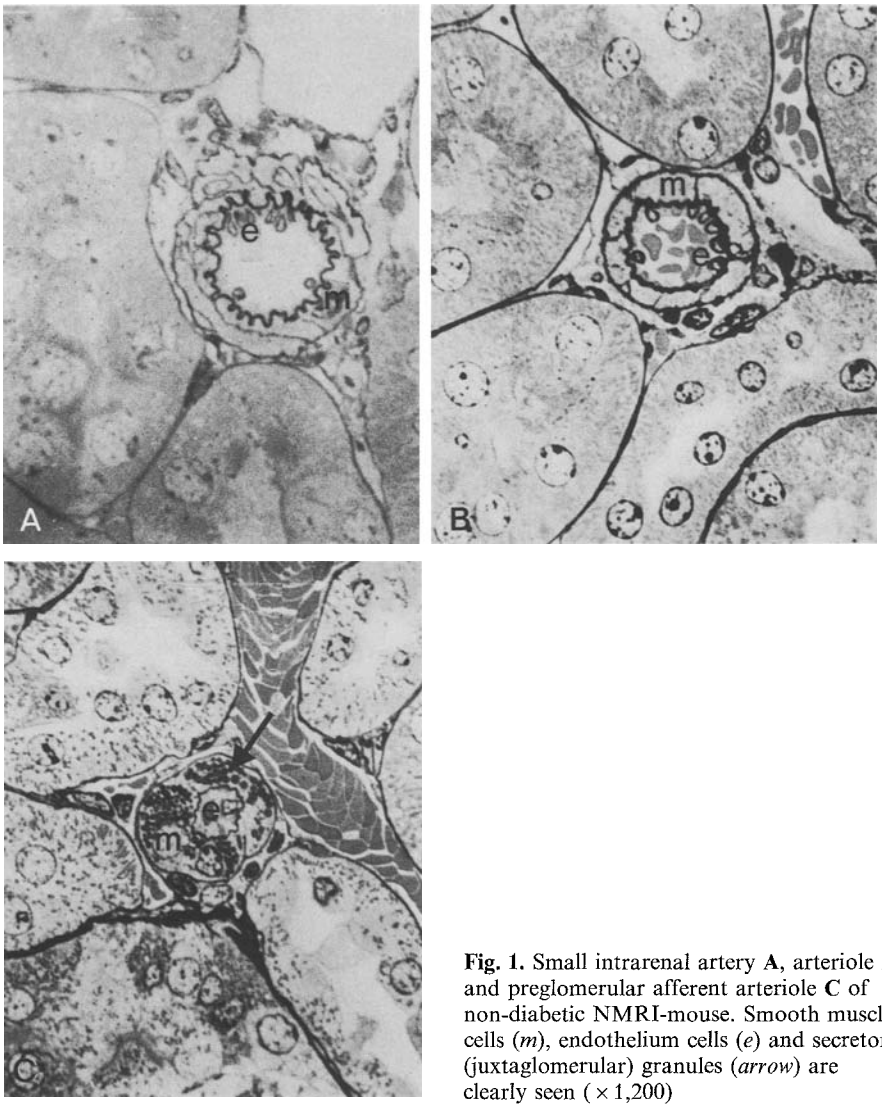

Fig. 1. Small intrarenal artery **A**, arteriole **B** and preglomerular afferent arteriole **C** of non-diabetic NMRI-mouse. Smooth muscle cells (*m*), endothelium cells (*e*) and secretory (juxtaglomerular) granules (*arrow*) are clearly seen (× 1,200)

Histological method. The mice were killed at either 2, 5 or 12 months and the kidneys removed immediately. These were then fixed in phosphate-buffered formalin (4%), postfixed in osmic acid (3%) and finally embedded in methylated acrylate. Using an ultramicrotome we prepared sections of 0.5–1.0 μ thickness of the renal cortex at various sites, which were then stained with silver methenamine.

Morphometric method. We examined 1) small intrarenal arteries (ART) (Fig. 1 A), 2) arterioles (ALE) (Fig. 1 B) and 3) preglomerular afferent arterioles (PAA) (Fig. 1 C). The vessel types were indentified using the following criteria:

1. the group of small arteries was confined to vessels with a wall consisting of 2 or at a maximum, 3 layers of smooth muscle cells

Table 1. Number of vessels studied in controls (C) and diabetic mice (D)

Age (months)	Arteries		Arterioles		PAA*	
	C	D	C	D	C	D
2	75	64	838	893	145	115
5	25	132	761	1,255	106	128
12	78	34	627	766	24	–**

* preglomerular afferent arterioles
** a sufficient number of PAA could not be found in this group

2. arterioles were defined as vessels with a single layer of smooth muscle cells
3. preglomerular afferent arterioles were identified by their secretory granules.

Morphometric measurements were only carried out on clearly perpendicular sections, using the point counting method (Wehner et al. 1972) in which a grid was applied to the screen of a Reichert Visopan microscope (objective 63/0.75, 160/0.17) at an image scale of 800:1 (distance between the points 3.75 μm). Using this method we determined 1) vessel cross-sectional area, 2) vessel lumen area and 3) vessel wall area in all normal and diabetic vessels. A total of 408 small intrarenal arteries, 5,140 arterioles and 518 preglomerular afferent arterioles were studied (Table 1).

All sections were coded and the measurements were carried out under blind conditions, i.e., the histological preparations were allocated to the various experimental groups only after analysis was completed.

Statistical method

Results are expressed as mean ±SEM. The significance of differences was assessed using Student's *t*-test. The limit for the error probability was 2 $P<0.05$.

Results

Vessel cross-sectional area

At the age of 2 months the diabetic arteries (ART) and arterioles (ALE) had significantly smaller cross-sectional areas than the controls (1.59 ± 0.11 vs 2.28 ± 0.24 mm^2 $P<0.025$, and 298.80 ± 6.85 vs 384.12 ± 9.18 sqμ, $P<0.001$, respectively) whereas normal and diabetic preglomerular afferent arterioles (PAA) had the same size (Table 2). With age all vessels examined showed an increase in cross-sectional area with the exception of the non-diabetic ART. This increase, however, was much greater in the diabetic mice and this applies to all vessel types studied. At the age of 12 months the diabetic ART and ALE had significantly greater cross-sectional areas than the non-diabetic controls (2.89 ± 0.29 vs 2.13 ± 0.17 mm^2, $P<0.025$, and 520.92 ± 15.66 vs 447.48 ± 12.88 sqμ, $P<0.001$, respectively). The difference between the diabetic and the normal PAA at 5 months was not statistically significant.

Vessel lumen area

At the age of 2 months the diabetic ART and ALE had significantly smaller lumen areas than the controls (360.00 ± 34.88 vs 512.52 ± 69.29 sqμ, $P<0.01$,

Table 2. Vessel cross-sectional area of arteries (ART), arterioles (ALE) and preglomerular afferent arterioles (PAA) in non-diabetic NMRI-mice (N) and diabetic KK-mice (D)

Age (months)	ART (mm^2)		ALE (sqμ)		PAA (sqμ)	
	N	D	N	D	N	D
2	2.28±0.24	1.59±0.11*	384.12±9.18	298.80±6.85**	780.12±25.66	797.04±29.25
5	2.22±0.13	1.84±0.11*	477.72±11.39	351.00±7.22**	905.76±36.76	970.56±35.31
12	2.13±0.17	2.89±0.29*	447.48±12.88	520.92±25.60**	981.00±58.02	

Mean values±SEM. *P*-values indicate significant difference from normal control: * = $P<0.025$, ** = $P<0.001$

Table 3. Vessel lumen area of arteries (ART), arterioles (ALE) and preglomerular afferent arterioles (PAA) in non-diabetic NMRI-mice (N) and diabetic KK-mice (D)

Age (months)	ART (sqμ)		ALE (sqμ)		PAA (sqμ)	
	N	D	N	D	N	D
2	515.52±69.29	360.00±34.48*	94.32±2.80	70.92±1.78**	68.76±4.01	68.76±6.23
5	594.72±49.42	462.96±36.80	113.76±3.75	102.34±2.29**	95.04±7.31	131.76±8.53**
12	616.68±72.99	1,057.68±142.46**	115.56±4.55	176.04±7.36**	91.44±9.48	–

Mean values±SEM. *P*-values indicate significant difference from normal control: * = $P<0.01$, ** = $P<0.001$

Table 4. Mean percentage change in lumen area in arteries (ART), arterioles (ALE) and preglomerular afferent arterioles (PAA) in non-diabetic NMRI-mice (N) and diabetic KK-mice (D)

	N	D
ART (at 12 months)	+20% (n.s.)	+194% ($P<0.001$)
ALE (at 12 months)	+23% ($P<0.001$)	+148% ($P<0.001$)
PAA (at 5 months)	+38% ($P<0.001$)	+ 92% ($P<0.001$)

The significances refer to the change at 12 and 5 months respectively compared to the values at 2 months

Table 5. Vessel wall area of arteries (ART), arterioles (ALE) and preglomerular afferent arterioles (PAA) in non-diabetic NMRI-mice (N) and diabetic KK-mice (D)

Age (months)	ART (mm^2)		ALE (sqμ)		PAA (sqμ)	
	N	D	N	D	N	D
2	1.76±0.18	1.23±0.08*	289.63±7.32	227.93±5.96**	713.29±24.86	728.13±27.35
5	1.62±0.10	1.38±0.08	363.45±8.44	248.58±5.59**	810.67±33.26	835.87±32.10
12	1.52±0.11	1.83±0.16	331.17±9.61	344.16±9.64	889.49±55.53	–

Mean values±SEM. P values indicate significant difference from normal control: * = $P<0.025$, ** = $P<0.001$

and 70.92±1.78 vs 94.32±2.80 sqμ, $P<0.001$, respectively) (Table 3). At 2 months no difference was found between the normal and the diabetic PAA. With increasing age all vessels examined showed an increase in lumen area. This increase, however, was again much greater in the diabetic mice and this applies to all vessel types studied. At the age of 12 months the diabetic ART and ALE had significantly greater lumen areas than the normal controls (1,057.68±142.46 vs 616.68±72.92 sqμ, $P<0.001$, and 176.04±7.36 vs 115.56±4.55 sqμ, $P<0.001$, respectively). The same was found for the diabetic PAA at 5 months (131.76±8.53 vs 95.05±7.31 sqμ, $P<0.001$). The percentage increase in lumen area from the second to the twelfth and fifth month respectively ranged from +92 to +194% in the diabetic vessels whereas in the normal controls this increase ranged only from +20 to +38% (Table 4).

Vessel wall area

At the age of 2 months the diabetic ART and ALE had significantly smaller wall areas than the controls (1.23±0.08 vs 1.76±0.18 mm^2, $P<0.025$, and 227.93±5.96 vs 289.63±7.32 sqμ, $P<0.001$, respectively) (Table 5). The difference in wall area between the normal and the diabetic PAA was not statistically significant. With increasing age all vessels examined showed a substantial increase in wall area with the exception of the normal ART. This increase was again much greater in the diabetic mice and this applies

Table 6. Mean percentage change in wall area in arteries (ART), arterioles (ALE) and preglomerular afferent arterioles (PAA) in non-diabetic NMRI-mice (N) and diabetic KK-mice (D)

	N	D
ART (at 12 months)	−14% (n.s.)	+48% ($P<0.001$)
ALE (at 12 months)	+14% ($P<0.001$)	+51% ($P<0.001$)
PAA (at 5 months)	+14% ($P<0.001$)	+15% ($P<0.001$)

The significances refer to the change at 12 and 5 months respectively compared to the values at 2 months

to all vessel types studied. At 12 months, however, the arithmetical difference between the values for the normal and the diabetic ART and ALE was not statistically significant (the same is true for the normal and the diabetic PAA at 5 months). The percentage increase in wall area from the second to the twelfth and fifth month respectively ranged from +15 to +51% in the diabetic vessels whereas in the normal controls this increase ranged only from −14 to +14% (Table 6).

Discussion

The main finding of this study was that the small intrarenal arteries (ART), arterioles (ALE) and preglomerular afferent arterioles (PAA) of the diabetic KK-mice dilate considerably with increasing duration of diabetes. Similar changes were not found in the non-diabetic control mice although we can not exclude the theoretical possibility that differences between the diabetic and non-diabetic strains may also, at least partly, be due to their genetic heterogenicity.

There is no congenic control for the KK-mouse. Cross-breeding with other mice which correspond to the usual in-bred stocks can be used as a normal control, according Coleman (1982). In other comparable investigations by different authors non-diabetic mice were used such as C 57 BL-mouse (Nakamura and Yamada 1967), ICR-mouse (Matsuo et al. 1971) and Swiss albino (SA)-mouse (Reddi et al. 1975).

The results indicate that true vessel dilatation is occuring in the diabetic mice, not vessel hypertrophy alone, since the increase in lumen area clearly exceeds the concomitant increase in wall area. These findings suggest that in the preglomerular vascular system of the diabetic mice the ability to maintain an adequate vessel tone is increasingly impaired with increasing duration of the disease. Inability to maintain an adequate vessel tone represents failure of the autoregulatory mechanism which controls glomerular inflow and may be a causal factor for the glomerular hyperfiltration in diabetes.

The autoregulation of renal blood flow and GFR depends primarily on the ability of the smooth muscle cells to contract in response to an increase in intraluminal pressure. It has been suggested recently that the vascular (arteriolar) mechanism underlying the normal autoregulation is

defective in patients with diabetic nephropathy (Parving et al. 1984) and there is strong evidence that this myogenic mechanism is affected by structural and metabolic abnormalities occuring in diabetes (Hostetter et al. 1982). Early hyalinosis of the afferent arteriole is a typical finding in the diabetic kidney (Bader and Meyer 1977; Mauer et al. 1976; McMillan 1981) and is very likely to affect vessel contractility and myogenic autoregulation. Furthermore, degeneration of arterial and arteriolar wall materials, such as smooth muscle cells, and metabolic alterations in smooth muscle metabolism have repeatedly been reported in diabetes (Bohlen and Niggl 1979, 1980a; Mauer et al. 1981; Wolinsky et al. 1978) and there is considerable evidence that normal vessel growth ist impaired in early diabetes (Bohlen and Niggl 1980a, Bohlen and Hankins 1982).

We could show that the arteries and arterioles of the young, 2 months old diabetic KK-mice had markedly reduced wall areas and were significantly smaller than the non-diabetic controls and it is indeed possible that early degeneration of the smooth muscle cells and a depression of normal vessel maturation early in the course of the disease are responsible for these findings.

Degeneration of the smooth muscle cells is probably the main factor responsible for the impairment of the myogenic autoregulation in the preglomerular vessels of the diabetic KK-mice. However, other (metabolic) abnormalities occuring in diabetes like an impairment of oxygen release and tissue oxygenation (Colwell et al. 1979; Ditzel 1975; 1977; McMillan 1975) and changes in the renin-angiotensin system (Mauer et al. 1981; McMillan 1975; Tomita et al. 1982) may contribute to the failure of this mechanism in these diabetic vessels.

This is the first time that a systematic morphometric examination of the preglomerular vessels has been carried out and that progressive dilatation of these vessels has been demonstrated in a diabetic kidney. Our results indicate that the ability to maintain an adequate vessel tone is progressively impaired in the preglomerular vessels of diabetic KK-mice. This suggests severe damage of the blood flow autoregulation in these vessels. Failure of this crucial mechanism which under physiological conditions prevents excessive glomerular blood flow in the normal kidney may be one factor responsible for the glomerular hyperfiltration in diabetes.

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