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Morphometric investigations on intrarenal vessels of streptozotocin-diabetic rats

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Summary. We have investigated 975 different grazing sections of vessels in kidney preparations of 20 rats of the Wistar strain. Half of these genetically identical animals had an insulin-deficiency diabetes induced by injection of streptozocin. The kidneys were removed for investigation after 2 and 12 weeks duration of diabetes. The vessel cross-section, wall, lumen and endothelial surface area were determined in renal arteries, arterioles and preglomerular afferent arterioles in a blind experiment. Statistically detectable changes were found in the diabetic vessels in the early stage of the diabetes. Preglomerular afferent arterioles showed a highly significant and increasing lumen dilatation commencing after 2 weeks. Diabetic arteries and arterioles developed narrower lumina. A significant thickening of the endothelium took place at the same time in both vessel types. All three vessel regions became smaller and had thinner walls than healthy vessels as the diabetes progressed. The findings on the afferent vessels indicate that haemodynamic effects on the glomerulus are to be expected. Familial diabetic gloermulopathy begins with a reversible hyperfiltration. However, the mechanism has not been clarified in the context of the diabetic metabolic disorder, and this change is probably the haemodynamic consequence of the substantial dilatation of the preglomerular afferent arterioles. With their renin-positive segment, these arterioles are the centre of intrarenal regulation. The increase of the capillary glomerular pressure associated with the dilatation of the preglomerular afferent arterioles is a crucial factor in the development of diabetic glomerulopathy.

Key words. Kidney – Diabetes – Arterioles – Autoregulation – Morphometry

Introduction

Microangiopathy is the main cause of the morbidity and mortality of diabetic patients. The progressive destruction of the renal microcirculation ultimately leads to terminal renal failure in numerous diabetics. In this process, haemodynamic factors have a crucial effect. The early phase of diabetes mellitus (stage I) is characterized functionally by glomerular hyperfiltration with a raised glomerular filtration rate (GFR). This can be demonstrated both clinically and experimentally. Furthermore, it is shown that patients with high GFR values in the early phase of the disease are more likely to develop a manifest glomerulopathy (Hostetter et al. 1982; Brunetti and Bueti 1985; Blumberg 1986; Brenner et al. 1986; Dunn et al. 1986; Anderson et al. 1989). Since the intraglomerular pressure is determined by the transmission of the systemic blood pressure and the resistance of the preglomerular and postglomerular vessels, this system of vessels becomes the focus of interest (Kimura et al. 1989, 1990; Harvey et al. 1990). Particular attention must be paid to the preglomerular afferent arterioles, since they are an important vascular segment of the autoregulation of the renal circulation (Steinhausen et al. 1989; Stein 1990).

Our own morphometric investigations on the renal vascular system of KK mice, which are spontaneously diabetic for genetic reasons, have shown that the preglomerular arterioles in particular display a marked dilatation in relatively early stages of the disease (Volkmann and Wehner 1986; Wehner and Volkmann 1987). Since there were genetic differences between the diabetic mice and the control animals in this experimental model, we carried out a morphometric investigation of the renal vascular system on streptozotocin-diabetic rats. The sole difference between experimental and control animals in these investigations was the blood sugar levels.

Materials and methods

A total of 20 male Wistar rats was investigated. These were 8 weeks old and between 170 and 190 g in weight at the beginning of the experiment. In 10 of these animals, diabetes mellitus was induced by i.v. injection of 50 mg streptozotocin/kg body weight into the tail vein. The 10 non-diabetic animals served as control group. Two and 12 weeks after the induction of hyperglycaemia,

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Table 1. Number of grazing sections of vessels investigated

	Arteries	Arterioles	Preglomerular afferent arterioles
Normal ($n=10$)	147	250	72
Diabetic ($n=10$)	169	287	50

5 animals and their controls were killed and the kidneys were removed (Rudas 1972; Ganda 1976; Wehner et al. 1980; Wehner and Petri 1983).

Investigation of the blood sugar level and urinary glucose excretion was carried out with glucose tests based on the hexokinase method (Boehringer, Mannheim, FRG). Proteinuria was measured as a 24-h-value with the Biuret method.

The kidneys were removed immediately after killing the animals and fixed in buffered 4% formalin (immersion fixation; Tracy et al. 1986, 1990; Yoshida et al. 1989; Miller and Meyer 1990). Cortical tissues obtained from locations near to the surface were postfixed for 2 h in 2% osmic acid and embedded in Plexiglas. Sections of this material 0.5–1 μm thick were prepared and silver-stained according to Movat (Wehner 1970). Semithin sections, 14–20 per animal, were mounted on one slide. The preparations were coded and only assigned to the individual experimental groups after the morphometric analysis and completion of the computational part of the investigations.

In the morphometric studies, the following vessels were investigated under a Visopan projection microscope (Reichert) at a magnification of 1:1000 (oil immersion):

1. Renal arteries with at least two layers of smooth muscle cells.
2. Renal arterioles with only one layer of muscle cells (Mulvany and Aalkjaer 1990).
3. Preglomerular afferent arterioles without muscle cells and with granulated epithelioid cells adjoining the epithelium. These vessels can be demonstrated in the immediate vicinity of the glomeruli.

Only approximately orthograde grazing sections of blood vessels which could be identified precisely were evaluated. The two cross-sectional parameters were permitted to deviate by a maximum of only 15% from the mean cross-section. Altogether, 975 different grazing sections distributed over the individual investigation groups as shown in Table 1 were evaluated. The vessels were evaluated using the point counting method with a point distance of 6 μm and scanned area of 36 μm^2 (Wehner 1974). In this way, the following vessel parameters were determined: total vessel cross-section (i.e. area of the entire grazing section of the vessel), vessel lumen (i.e. area of the internal lumen), total wall surface (i.e. area of the wall including the endothelium) and the area of the endothelial layer.

The results are specified as mean values ($\bar{x}$) with a standard deviation ($\pm$SD). The significance of differences was assessed using Student's t-test. The limit for the probability of error was 2 $P<0.05$, $P=0.05$ (*), $P=0.01$ (**). The asterisks in the tables designate the significant differences between normal and diabetic animals in the respective age group.

Results

There was a pronounced hyperglycaemia in the streptozotocin-diabetic rats over the entire duration of the experiment. Furthermore, substantial glucosuria and progressive proteinuria could be demonstrated when compared with the non-diabetic animals (Table 2). Light microscopic investigation of the preparations did not show any manifest differences between the diabetic and the non-diabetic animals.

Morphometrically, there were significant differences in the endothelial surface area of the arteries, which was

Table 2. Clinical test values of the experimental and control animals

	Normal		Diabetic	
	2 weeks ($n=5$)	12 weeks ($n=5$)	2 weeks ($n=5$)	12 weeks ($n=5$)
Blood sugar (mg/100 ml)	90.5±36.4	89.4±20.9	292.1±46.3**	288.2±43.2**
Glycosuria (g/24 h)	0.008±0.012	0.006±0.006	3.144±1.577**	1.695±0.598**
Proteinuria (mg/24 h)	15.35±8.3	11.85±6.45	55.5±22.4**	100.3±126.3**

* $P=0.05$
** $P=0.01$

Table 3. Renal arterial surface ($\mu m^2 \pm$SD) of streptozotocin-diabetic and non-diabetic rats with varying durations of diabetes

	Normal		Diabetic	
	2 weeks ($n=5$)	12 weeks ($n=5$)	2 weeks ($n=5$)	12 weeks ($n=5$)
Cross-section	1332±161	1231±201	1119±215	1148±225
Lumen	92.3±29.8	101.3±34.8	82±44.5	70.5±26.4
Wall	1237±151	1127±170	1037±192	1077±218
Endothelial	173.8±17.8	136.4±25.9	143.6±28.3*	160±25.4*

* $P=0.05$

Table 4. Areas of the renal arterioles ($\mu m^2 \pm SD$) of streptozotocin-diabetic and non-diabetic rats with varying duration of diabetes

	Normal		Diabetic	
	2 weeks ($n=5$)	12 weeks ($n=5$)	2 weeks ($n=5$)	12 weeks ($n=5$)
Cross-section	421.9±16.7	430.7±14	443.1±73.9	418.5±25.9
Lumen	27.8±11.8	36±8.6	33±8.4	30.7±7.3
Wall	394±13	394±11.8	409.6±65	387.7±29
Endothelial	65.7±10	56.3±5	71.6±9.5*	68.6±8.6

* $P=0.05$

Table 5. Areas of the preglomerular afferent arterioles ($\mu m^2 \pm SD$) of streptozotocin-diabetic and non-diabetic rats with different duration of diabetes ($1=P<0.01$)

	Normal		Diabetic	
	2 weeks ($n=5$)	12 weeks ($n=5$)	2 weeks ($n=5$)	12 weeks ($n=5$)
Cross-section	607±55.8	675.4±126	625±140	631±99
Lumen	31.6±5.7	41.1±12.6	36.1±15*	64.9±20** [1]
Wall	574.9±55.8	634.2±125.2	581.4±132.1	566.2±218
Endothelial	60.6±14.4	62.9±12.6	65±16.9	57.8±6.1

* $P=0.05$
** $P=0.01$

smaller in diabetic animals (143 μm^2) than in non-diabetic animals (173 μm^2) after 2 weeks. After a duration of diabetes of 12 weeks, there were no appreciable changes in the remaining parameters, and there was a significant increase only of the endothelial surface area in diabetic animals. These now showed a very much greater endothelial surface area (160 μm^2) than the non-diabetic animals (136 μm^2). There were no significant differences in any of the other parameters (Table 3). After 2 weeks, there were only inappreciable changes in the arteriolar cross-sectional lumen and wall surface area. The endothelial surface area showed a significant increase from 65.7 μm^2 to 71.6 μm^2 in diabetic vessels. This was also demonstrated after 12 weeks. The endothelial surface area was very much larger in the diabetic vessels (68.6 μm^2) than in the controls (56.3 μm^2). It was also shown for the arterioles that the diabetic vessels were smaller and more constricted after a duration of diabetes of 12 weeks than the vessels of the non-diabetic controls (Table 4).

With regard to the preglomerular afferent arterioles, there was a slightly greater cross-sectional area (625 μm^2) in the diabetic vessels after 2 weeks than in the controls (607 μm^2), a somewhat greater wall area (581 μm^2 in the diabetic vessels as compared to 574 μm^2 in the control vessels) and also a somewhat greater endothelial surface area (65 μm^2 as compared to 60.6 μm^2). The lumen area of the diabetic vessels was strikingly and significantly changed after 2 weeks (36.1 μm^2 and 31.6 μm^2 respectively). This phenomenon also continued after a duration of diabetes of 12 weeks. Compared to the non-diabetic controls (41.1 μm^2), the lumen area of the diabetic vessels (64.9 μm^2) was significantly enlarged by about 90% in excess of normal. This significant enlargement of the lumen of the preglomerular afferent arterioles was also shown in relation to the values after a diabetes duration of 2 weeks (36.1 μm^2) and 12 weeks (64.9 μm^2) (Table 5).

Discussion

All three vessel regions in the diabetic animals become smaller on the whole with the duration of hyperglycaemia when compared with the non-diabetic control animals. They are also more constricted in the region of the arteries and arterioles. The following statistically verifiable results are evident:

1. After 12 weeks diabetes, the endothelial surface area of the arteries and arterioles is very much greater than in controls.
2. After 2 weeks, there is a highly significant increase in the lumen of the preglomerular afferent arterioles which was more marked after 12 weeks when compared with the non-diabetic controls (90%).
3. These alterations, which are evidently specific to diabetes, are manifest to a different extent in the three vessel regions investigated. A marked proliferation of the endothelium is most prominent in the diabetic arteries and arterioles. In contrast to this, there is an excessive dilatation of the preglomerular afferent arterioles without an appreciable endothelial reaction.

The results show that specific alterations occur on apparently healthy renal vessels even after short duration of diabetes (2 weeks). This is also shown by investigations of Bohlen and Niggl (1979a, b), who were able

to demonstrate that the vessels were also smaller after 4–5 weeks in streptozotocin-diabetic mice, as well as from our own investigations on spontaneously diabetic KK mice (Volkmann and Wehner 1986; Wehner and Volkmann 1987). The available empirical data rule out a genetic cause of the vascular hypoplasia which was assumed by Gattone et al. (1983) in spontaneously hypertensive rats. The findings also show that a true vascular dilatation is evidently present in the diabetic preglomerular afferent arteriole and is not a vascular hypertrophy, as is also shown by our own investigations on KK mice (Wehner and Volkmann 1987). This also applies to the cerebral arterioles of diabetic rats, which have a larger internal diameter than in normal animals (Rubin and Bohlen 1985). Micropuncture studies show that the plasma flow rate in the glomeruli rises in rats with chemically induced diabetes mellitus. This is associated with vasodilatation of the afferent arterioles and with raised transcapillary hydraulic pressure gradient which results in a raised GFR (Michels et al. 1980; Hostetter et al. 1981; Jensen et al. 1981). The opposite finding of a reduced GFR in the first weeks of untreated streptozotocin diabetes in rats was reported by Allen et al. (1990). Investigations on the hydronephrotic rat kidney show that vascular dilatation or change in diameter is an important factor in autoregulation, especially in afferent arterioles (Steinhausen et al. 1989). Recent investigations on microvascular cast preparations confirm this for spontaneous and experimentally hypertensive rats (Kimura et al. 1989, Tojo et al. 1990). The investigations of Kimura et al. (1990) on the vessel preparations of streptozotocin-diabetic Wistar rats are of particular interest: in agreement with results obtained with our morphometric methods, they show that the afferent arterioles are significantly dilated in diabetic animals with normal blood pressure and a pronounced proteinura. In the authors' view, this dilatation leads to an elevation of glomerular pressure and thus to glomerular damage: glomerulosclerosis. The proliferation of the endothelium we have demonstrated is consistent with the findings of other authors, who have also demonstrated alterations in the endothelium of diabetic arterioles. These structures are either involved in the process of autoregulation via polyene metabolism or via the vasoconstrictor endothelin (Porta et al. 1987; Murakawa et al. 1990). A disorder of the mechanosensors of the vessel wall between the endothelium and the smooth musculature is also conceivable with disturbance of the release either of the vasoconstrictor endothelin or the vasodilator endothelium-derived relaxing factor (Fajardo 1989).

Hormonal and myogenic factors must thus be discussed as causes for the dilatation of the afferent arterioles and thus for the disturbance of the autoregulation of renal haemodynamics (Dunn et al. 1986; Harvey et al. 1990; Morff 1990). The investigation of Steinhausen et al. (1989) on hydronephrotic kidneys shows that the distal afferent arterioles react in different ways to different stimuli, so that both myogenic reactivity and the pressure sensor function for renin release may be disturbed in these arterioles (Steinhausen et al. 1990a). In addition, there are evidently differences between the cortical and juxtamedullary vessels with regard to their autoregulatory characteristics. These differences are either due to a high prostaglandin content or a high prostaglandin sensitivity (especially of the juxtamedullary vessels) (Steinhausen et al. 1990b). The renin-positive segment of the preglomerular afferent arteriole is evidently the centre of intrarenal regulation and control of glomerular blood flow and filtration as well as the location of the main effector of the tubulo-glomerular feedback mechanism (Moore et al. 1990, Sadayoshi and Carretero 1990). This is also indicated by our findings in the preglomerular afferent arterioles containing renin granules and the investigation of Moore and Casellas (1990). In this region, the autoregulation is strictly calcium-dependent, as shown by experiments with calcium-channel blockades (Moore et al. 1990).

Other authors consider that the hyperglycaemia per se is responsible for the alteration (Hostetter et al. 1982) or that it is due to the hyperglycaemia-induced volume expansion with release of atrial natriuretic peptide and subsequent hyperfiltration (Anderson and Brenner 1988). The vasodilatory prostaglandins of the smooth muscle cells play a major role in this system of vasodilatation (Stahl 1986; Stahl and Thaiss 1987). Moreover, the induction of diabetes is associated with high renin and angiotensin II levels, which fall to low values after 6 weeks (Kikkawa et al. 1986). The regulation of the renal circulation thus depends on autoregulation, which is evidently associated with the resistance of the afferent arterioles (intrinsic myogenic mechanism) and the activity of numerous vasoactive agents (Stein 1990).

Our results are consistent with the opinion of most investigators that glomerular hypertension plays a key role in the initiation and progression of diabetic glomerulopathy (Hostetter et al. 1982; Wehner and Volkmann 1987; Anderson and Brenner 1988; Anderson et al. 1989; Björck and Aurell 1990; Kimura et al. 1990). Bank et al. (1987) do not rule out this possibility.

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