Chapter 2

Diabetes impairs the renal microvascular myogenic response more easily in rats from breeders selected for large litters

Marjon Roos
William van Rodijnen
Piet ter Wee
Geert Jan Tangelder

Submitted
Abstract

Of the type 1 diabetic patients 20-40% will develop nephropathy. The mechanisms initially involved are largely unknown, but a decreased preglomerular resistance may play a role. We investigated the reaction to changes in pressure (myogenic response) of distal interlobular arteries (ILAs) and afferent arterioles (AAs) in diabetic male Sprague-Dawley rats from 2 suppliers, one selecting breeders systematically for large litters (Charles-River) and the other at random (Harlan). In controls the reactivity to prostaglandin E₂ (PGE₂) was assessed, while in the latter substrain also determinants of the streptozotocin (STZ) induced diabetic model were studied. We employed for this the isolated perfused hydronephrotic kidney.

Diabetes significantly impaired the myogenic response of both ILAs and AAs in rats from breeders selected for large litters, but not in the other substrain. This was paralleled by an increased vasodilatation of these microvessels to PGE₂ in controls of the former substrain as compared to the latter. In rats from randomly selected breeders we also observed that increasing the severity of hyperglycemia (from approximately 18 to >33 mmol/L) by reducing or abolishing the partial substitution with human insulin, or elongating the diabetes duration from 4 to 6 weeks, did not impair the myogenic response. However, at a lower STZ-dose (30 instead of 60 mg/kg), and without insulin substitution, rats that developed moderate hyperglycemia (8-20 mmol/L) showed a clearly impaired myogenic response, in contrast to those which developed severe hyperglycemia (approximately 33 mol/L).

In conclusion, in diabetic rats from a substrain selected for large litters a decreased myogenic response of preglomerular arterioles occurs more easily than in off-spring from randomly selected breeders. These substrains also differed in their reaction to PGE₂, suggesting that a difference in PGE₂-signaling might be one of the possible factors determining whether a diabetic patient will develop nephropathy or not.
Introduction

Of the patients with type 1 diabetes mellitus 20 to 40% still develop nephropathy, despite an increased quality of metabolic control (13, 14). Diabetic nephropathy is initially characterized by microalbuminuria, and can lead via glomerulosclerosis to a complete loss of renal function. The mechanisms involved in the start of diabetic nephropathy are largely unknown (20). Several studies have shown that type 1 diabetes mellitus is characterized by glomerular hyperfiltration and an increased renal plasma flow prior to the onset of microalbuminuria, in patients as well as in animal models (20, 29). Furthermore, a decreased pregglomerular resistance has been indicated as one of the possible mechanisms involved (8, 10).

Several studies have established the importance of pregglomerular microvessels, i.e. interlobular arteries (ILAs) and afferent arterioles (AAs), for the regulation of glomerular capillary pressure (18, 29, 31). A decrease in their resistance will facilitate the relay of pressure to the glomerulus and may lead to hyperfiltration. To protect the glomerulus from changes in systemic blood pressure, the myogenic response of these vessels is an important regulating mechanism (15). It is the acute reaction of a blood vessel to an increase or decrease in transmural pressure (18). The small pregglomerular microvessels are difficult to isolate and cannot be studied directly in an intact kidney. The isolated perfused hydronephrotic rat kidney, however, allows their microscopic visualization and investigation of the myogenic response (27).

The present study was undertaken to establish in type 1 diabetic Sprague-Dawley rats possible determinants of the myogenic responsiveness related to this animal disease model, such as partial insulin substitution, level of hyperglycemia and commercial supplier. Regarding the latter, it has been reported that Sprague-Dawley rats sold by Charles-River are derived from breeders systematically selected for large litter size, while those purchased from Harlan come from randomly selected breeders (21). An increase in litter size may be associated with lower birthweight, based on less favorable nutrition in utero due to overcrowding (5, 26). The Barker hypothesis states that intrauterine malnutrition predisposes to vascular alterations leading among others to hypertension (2). In aboriginals it has been shown that lower birthweight disposes to albuminuria and an increased susceptibility to renal disease (9). Therefore, we postulated that selection for litter size might increase the risk for an impaired renal myogenic response in diabetes mellitus. The latter may involve prostaglandins (8), substances also important for ovulation, and hence, litter size (12). Since reduced ovulation and litter size has been found in mice lacking the
EP2-receptor for prostaglandin E2 (PGE2) (12), we also hypothesized that selection for litter size might be paralleled by a higher sensitivity to PGE2.
Materials and/or Methods

Male Sprague-Dawley rats were obtained from Harlan (n=48, Horst, The Netherlands) or Charles-River (n=12, Crl:CD®(SD)BR, Sulzfeld, Germany). The Sprague-Dawley rat originated in Madison, Wisconsin, in 1924 and was originally maintained and housed as an outbred strain, i.e. kept genetically heterogenous by minimal inbreeding. Subsequently, independent, isolated colonies have been established and maintained by different commercial vendors (6). Charles-River selected till recently their breeders based on litter size in an effort to maximize the number of pups, i.e. more than eight, obtained per breeding pair (3, 21). Harlan, on the other hand, takes breeders at random with no selection on he size of litter from which they were born (21). No cross-breeding has been reported between these vendors, and even not between different facilities of one vendor. All animals were housed and handled according to the guidelines of the Institutional Animal Care and Use Committee. They were fed standard chow (AM2, Hope farms, Woerden, The Netherlands) and had free access to water, both were available ad libitum.

Diabetic models

Two weeks after ligating the left ureter (see below), diabetes was induced by a single i.v. injection of 30 or 60 mg/kg of STZ (Sigma), dissolved in 0.02 mol/L sodium citrate buffer (pH=4.5); control animals received the buffer only. Blood glucose was determined at least ones a week in a blood sample taken from the tail tip, using a OneTouch Ultra meter (Lifescan, The Netherlands) in the animals that received partial insulin substitution (see below) and an Accutrend Alpha device (Boehringer Mannheim, Germany) in the others. Values reported are in rats with 4 weeks of diabetes the average of the last 3 weeks and in those with 6 weeks of diabetes that of the last 5 weeks.

Partial insulin substitution (human insulin, Insulatard, Novo Nordisk, The Netherlands) in rats which received the high STZ-dose was given to all rats from Charles-River and part of the animals from Harlan, daily. The amount of insulin given depended on measured blood glucose levels, aiming to keep them on average below 23.6 mmol/L (20), and on water intake, i.e. whether the latter declined to near normal levels. Control animals were daily subjected to the same accompanying handling.

Hydronephrotic kidney model

To induce unilateral hydronephrosis (at the left side), rats in the groups with partial insulin substitution were anesthetized with isoflurane (3%, Abbott), O₂
(0.76 L/min) en N₂O (1.2 L/min) and the others with Hypnorm (4 mg/kg fluanison and 0.126 mg/kg fentanyl, i.m.; Jansen Pharmaceutical, Beerse, Belgium) and diazepam (2 mg/kg, i.p.; Centrafarm, Etten-leur, The Netherlands). For pain relief before and after surgery all rats were injected subcutaneously with Temgesic (0.03 mg/kg Buprenorfine base; Schering-Plough B.V. Amstelveen, The Netherlands). Their left ureter was exposed through a small mid-abdominal incision and tied off with a suture. Six to 8 weeks later, tubular atrophy had advanced to a stage that allowed direct visualization of the individual renal microvasculature (27).

Isolation of the hydronephrotic kidney

All rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.; Sanofi Sante, Maassluis, The Netherlands) and ketamine (25 mg/kg, i.m.; Kombivet, Etten-Leur, The Netherlands) to obtain their hydronephrotic kidney for in vitro perfusion as described in detail elsewhere (23). First, blood was taken from the tail tip to assess their non-fasting glucose levels. Second, the hydronephrotic kidney was exposed through a wide abdominal incision. Its renal artery was cannulated via the abdominal aorta and perfusion was started in vivo. Perfusion medium consisted of Dulbecco’s Modified Eagle’s Medium Base (DMEM) supplemented with (in mmol/L) 23.8 bicarbonate, 5.5 D-glucose, 1 sodium pyruvate and 5.6 HEPES (all Sigma). The medium was equilibrated with 95% air - 5% CO₂ at 37°C. Under continuous single-pass perfusion, the hydronephrotic kidney was excised and moved to the stage of an inverted microscope (Axiovert 100; Zeiss, Weesp, The Netherlands) that was equipped with a thin glass viewing port on the bottom surface. Renal perfusion pressure was monitored at the level of the renal artery and kept constant at 80 mm Hg except when the myogenic response was performed (see below). Perfusion flow was measured with a transonic flowsensor (1 N, Transonic systems Inc, Ithaca, USA).

Microscopic observation of vessel diameters

A small region of the transparent cortex of the hydronephrotic kidney was immobilized and transilluminated with a light rod. ILAs and AAs were visualized with an objective lens (Zeiss, 40X, NA 0.60) and a CCD camera (7020/20; Philips, Eindhoven, The Netherlands), and recorded for off-line analysis using a VHS video recorder (RTV 825; Blaupunkt, Hildesheim, Germany). Images were digitized using a computer equipped with an acquisition board (model IVG-128; Datacube, Peabody, MA). Vessel diameters were measured with an automated custom designed program (16). In vessel segments of ~5 µm length the distance between vessel walls was scanned at ~1 Hz intervals for a period of
30 s. Measurements were obtained at each pixel and averaged. Each final value was derived from ~30 of these measurements. ILAs were determined just before the bifurcation of AAs and AAs just after branching from ILAs.

Experimental protocols
In all rats myogenic reactivity of ILAs and AAs was studied by elevating the renal perfusion pressure in 20-mm Hg steps from 80 to 180 mm Hg; each step being held for 2 minutes. Thirty seconds after each pressure increase, diameters were determined. Since, only preglomerular vessels are myogenically active (7), pressure-induced diameter changes were not determined in efferent arterioles. Multiple consecutive myogenic response curves were made in each kidney.

Subsequently, in a subgroup of controls of both substrains changes in renal perfusate flow and in diameter of ILAs and AAs to increasing PGE$_2$ concentrations were determined in hydronephrotic kidneys. The renal vascular bed was first pretreated with a cyclo-oxygenase 1 (COX-1) inhibitor at a relatively high dose to suppress intrinsic PGE$_2$ production. Then, 0.1 nmol/L angiotensin II (AngII) was added to the perfusate to preconstrict the vasculature and an increasing PGE$_2$ concentration was given (0.1 nmol/L - 1 µmol/L). For COX-1 inhibition ibuprofen or diclofenac were used in a concentration of 10 µmol/L and 1 µmol/L, respectively.

In a last series, the effect of increasing the glucose concentration in the perfusion medium on myogenic reactivity was studied in rats obtained from Harlan (n=4). In 2 experiments we started with 5 mmol/L glucose and after performing two myogenic response curves (see above) the glucose concentration in the perfusion medium was increased to 20 mmol/L for 1 hour and then two other pressure response curves were made. In 2 other experiments the glucose concentration in the perfusion medium was from the start 20 mmol/L, then two myogenic response curves were made as described above. Subsequently, the glucose concentration was decreased to 5 mmol/L for 1 hour whereafter another two myogenic response curves were made.

Analysis of data
All data are expressed as the mean ± standard error of the mean (sem). The n-value refers to the number of kidneys examined. Since multiple vessels were studied in a kidney, the mean values obtained for each vessel type per animal were used. ANOVA or a students t test with Bonferroni correction was performed on the raw data to analyze differences. From the PGE$_2$ curves the concentration at which half-maximal vasodilatation was observed was calculated. In the flow-induced changes a maximal diameter of 10 nmol/L was taken. All
analyses were performed using Graphpad Prism version 4.02 for Windows, (Graphpad Software, San Diego, California USA). $P<0.05$ was considered statistically significant.
Results

Comparison of rats from breeders selected for large litter size or at random

Table 2.1 shows that blood glucose levels did not differ between the control groups of these two substrains \( (p=0.23) \). In addition, their baseline diameters were similar for ILAs \( (p=0.52) \) and AAs \( (p=0.21; \text{table 2.1}) \). Figure 2.1 (left panel) shows that also the reaction of these microvessels to pressure did not significantly differ (ILAs \( p=0.51 \), AAs \( p=0.48 \)).

By contrast, in diabetic rats from breeders selected for large litters (figure 2.1, right panel) the myogenic response was significantly impaired, while

![Graph showing the reaction to stepwise elevation in renal perfusion pressure of interlobular arteries (ILAs) and afferent arterioles (AAs) in rats from breeders selected for large litter size (squares) or at random (triangles).](image)

Figure 2.1. Reaction to stepwise elevation in renal perfusion pressure of interlobular arteries (ILAs) and afferent arterioles (AAs) in rats from breeders selected for large litter size (squares) or at random (triangles). No difference between controls (closed symbols, left panel), while 4 weeks of diabetes mellitus (open symbols) induced an impaired response in rats from large litters (right panel), but not in offspring from randomly selected breeders (middle panel). Values are means±sem; for \( n \)-values see table 2.1. \( P \)-values for control vs diabetic.
this was not so in the other substrain (figure 2.1, middle panel). Table 2.1 shows that both diabetic groups had on average the same blood glucose levels ($p=0.94$) and received a similar dose for partial insulin substitution ($p=0.44$).

Table 2.1. Comparison of blood glucose levels and baseline diameters of distal interlobular arteries (ILAs) and afferent arterioles (AAs) in control (C) and diabetic (DM) Sprague-Dawley rats from breeders selected randomly (Harlan) or for large litter size (Charles-River). All diabetic rats received 60 mg/kg streptozotocin; Insulin dose used for partial substitution is presented as well. Vessel diameters were investigated at a diabetes duration of 4 weeks and with a renal perfusion pressure of 80 mm Hg.

<table>
<thead>
<tr>
<th>Breeding selection</th>
<th>Randomly</th>
<th>For large litter size</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C (n=14)</td>
<td>p*</td>
</tr>
<tr>
<td>Blood glucose mmol/L</td>
<td>7.8±0.4</td>
<td>0.0002</td>
</tr>
<tr>
<td>Insulin U/day</td>
<td>–</td>
<td>4.3±0.4</td>
</tr>
<tr>
<td>ILAs µm</td>
<td>27.8±0.7</td>
<td>0.03</td>
</tr>
<tr>
<td>AAs µm</td>
<td>20.2±0.8</td>
<td>0.12</td>
</tr>
</tbody>
</table>

*, $p$-value diabetic vs. control.

Interestingly, diabetes induced in off-spring from randomly selected breeders showed a tendency towards smaller basal microvascular diameters as compared to their concomitant controls, while it did not so in rats from breeders selected for large litter size. Figure 2.2 shows that renal microvessels of control rats from breeders...

Figure 2.2. Sensitivity to prostaglandin E\(_2\) (PGE\(_2\)) in hydronephrotic kidneys of control rats from breeders selected for large litters (square, Charles-River, n=6) or at random (triangle, Harlan, n=5). Changes in perfusion flow (left panel) and in diameter of distal interlobular arteries (ILAs, middle panel) and afferent arterioles (AAs, right panel) as obtained under COX-1 inhibition and preconstriction of vessels with angiotensin II (AngII). Values are means±sem. Numbers in the graph (left panel) refer to $p$-values for diabetic vs control rats at the corresponding time point.

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selected for large litter size (square) are more sensitive to PGE2, as indicated by the leftward shift of their curves, than those in animals from smaller litters (triangle). Lower doses of PGE2 (<10^{-8} mol/L) dilated the preconstricted ILAs (middle panel) and AAs (right panel), which is reflected in an increase in renal perfusion (left panel). The PGE2-doses eliciting halve of this flow increase (IC50 in negative log) were 9.9±0.2 for rats from large litters and 9.2±0.1 for the others (p=0.004). In ILAs the IC50-values were also significantly different (9.8±0.2 vs 9.2±0.1, p=0.03), but not in AAs (9.8±0.2 vs 9.6±0.1, p=0.3). Higher PGE2 doses (>10^{-8} mol/L) constricted renal vessels other than ILAs and AAs (middle and right panel) as is indicated by the decrease in organ perfusion (left panel).

Effect of changing the diabetic model parameters in rats from randomly selected breeders

Table 2.2. Other diabetic protocols with 4 weeks duration investigated in Sprague-Dawley rats from randomly selected breeders (Harlan). Values of blood glucose and baseline diameters of distal interlobular arteries (ILAs) and afferent arterioles (AAs) are presented, and compared (p-value) to the concomitant control (see table 2.1, first column). Streptozotocin (STZ) and/or partial insulin substitution doses used are indicated; the last group was subdivided based on the threshold regarded to separate moderate from severe hyperglycemia (i.e. 23.6 mmol/L; (20)). Vessel diameters were determined at a renal perfusion pressure of 80 mm Hg.

<table>
<thead>
<tr>
<th>STZ  mg/kg</th>
<th>Insulin U/day</th>
<th>Blood Glucose mmol/L</th>
<th>ILAs µm</th>
<th>AAs µm</th>
</tr>
</thead>
<tbody>
<tr>
<td>60</td>
<td>3.2±0.3</td>
<td>26.6±0.7</td>
<td>22.7±0.5</td>
<td>18.5±1.2</td>
</tr>
<tr>
<td>60</td>
<td>0</td>
<td>&gt;33.3*</td>
<td>24.6±1.0</td>
<td>17.4±0.6</td>
</tr>
<tr>
<td>30</td>
<td>0</td>
<td>32.6±0.7</td>
<td>25.2±0.9</td>
<td>18.8±1.7</td>
</tr>
</tbody>
</table>

*, all values higher then the value indicated.
Table 2.2 shows the other 3 diabetic protocols investigated with 4 weeks duration in these rats; it also presents the subdivision of the last group (STZ 30 mg/kg, no insulin) based on blood glucose values being higher or lower than 23.6 mmol/L, i.e. the threshold regarded to separate moderate from severe hyperglycemia (20). The tendency in diabetic rats from randomly selected breeders towards smaller basal microvascular diameters is again apparent, especially in ILAs. Figure 2.3 shows that the first diabetic group in table 2.2 (left column), i.e. less partial insulin substitution and higher blood glucose levels than in table 2.1, still had a myogenic response in both ILAs and AAs.

Figure 2.4, left panel, shows that even an absence of insulin supplementation in 4 week diabetic rats from randomly selected breeders (Harlan; left panel; see table 2.2 second column) or acutely increasing the glucose concentration in the perfusion medium (middle panel; n=4, mean blood glucose 21.5±5.3 mmol/L) had no effect on pressure induced changes in the diameter of interlobular arteries (ILAs, upper panel) and afferent arterioles (AAs, lower panel), nor did a diabetic duration of 6 weeks (right panel; n=7, Harlan). Controls designated by closed symbol and diabetic animals by open symbols, with diamonds for high glucose in the perfusion medium (middle panel). Values are means±sem.

Figure 2.4, right panel, shows that increasing the diabetes duration from 4 to 6 weeks (60 mg/kg STZ, no insulin, blood glucose...
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> 33.3 mmol/L, n=7) also did not impair the myogenic response. However, in contrast to 4 weeks duration of diabetes (table 2.2, second column), basal diameters of ILAs and AAs at 6 weeks diabetes duration, being 26.4±0.9 (p=0.27) and 19.6±0.5 µm (p=0.60), respectively, were not different from control (see table 2.1, first column).

Interestingly, when the STZ-dose was halved (without insulin substitution), the animals which developed diabetes with moderate hyperglycemia (range 8-20 mmol/L; see table 2.2, fourth column) did develop a clearly impaired myogenic response, as is shown in figure 2.5. In contrast, those that developed severe hyperglycemia with the same STZ dose (table 2.2, third column) did not.

![Figure 2.5](image_url)

**Figure 2.5.** Difference in myogenic response of interlobular arteries (ILAs) and afferent arterioles (AAs) in 4 week diabetic rats from randomly selected breeders (Harlan), that received half the streptozotocin dose (30 mg/kg) without insulin substitution (see table 2.2): attenuation in those with moderate hyperglycemia (open circles), but not in severe hyperglycemia (open triangles). Values are means±sem. P-values indicate significant difference from controls (closed symbols; see table 2.1).
Discussion

The present study shows that in type 1 diabetic rats the myogenic response of small preglomerular arterioles is more easily impaired in off-spring from breeders selected for large litter size as compared to those from randomly selected parents. Control rats from the two substrains did not differ in their renal microvascular responsiveness to pressure, but off-spring from breeders selected for large litters had an increased sensitivity to PGE\(_2\). In rats from randomly selected breeders, we also observed that increasing the severity of hyperglycemia, by reducing or abolishing the partial insulin substitution, or elongating the diabetes duration from 4 to 6 weeks, did not induce an impaired myogenic response in renal arterioles.

In Sprague-Dawley rats we found that the impact of diabetes mellitus on renal microvessels differed between rats coming from randomly selected breeders or those selected for large litters. In the latter, diabetes attenuated the vasoconstrictive response to an increase in pressure, but not in the others under the same conditions. One reason might be a difference between these substrains in the balance between vasoconstrictive and vasodilating influences. This is suggested by our finding that in control animals these substrains differ in the vasodilating reaction of their renal microvessels to PGE\(_2\). Another difference between these two substrains as described in the literature is related to nitric oxide (NO) (3, 21). Under chronic inhibition of NO-production, arterial blood pressure increases in both substrains, but much less in the rats from breeders selected for large litter size (3, 21), indicating a lower basal NO-production in these animals. This makes it unlikely that the impaired renal myogenic response during diabetes in our rats from large litters was caused by an exaggerated production of NO and/or sensitivity to NO.

Our findings show that rats obtained from breeders selected for large litters have renal arterioles which are more sensitive to the vasodilatory effects of PGE\(_2\). It seems that in this substrain the relative contribution of PGE\(_2\) to the balance between vasodilation and/or vasoconstriction has increased as compared to other vasodilators such as NO (see above), in contrast to rats from smaller litters. A relationship between smaller litter size and less effectiveness of PGE\(_2\) as a vasodilator, is indicated by the report that mice with targeted disruption of the EP\(_4\) PGE\(_2\) receptor gene have a reduced litter size, due to an impaired ovulation (12); of the four PGE\(_2\) receptors EP\(_2\) and EP\(_4\) generally mediate smooth muscle relaxation and, hence, vasodilatation (28). In afferent arterioles of rat kidneys PGE\(_2\) elicits vasodilatation mainly by activating EP\(_4\) receptors (28). Various studies indicate that diabetes mellitus may increase the production of prostaglandins (11, 24). In animals with an increased sensitivity for the
vasodilating effects of PGE$_2$, this would lead to an impairment of vasoconstriction, as observed in the present study regarding the renal myogenic response. Indeed, Hayashi et al. have shown that the impaired response to pressure in afferent arterioles of diabetic rats could be restored by COX-inhibition (8).

Increasing in diabetic rats the severity of hyperglycemia from a level of about 18 mmol/L onwards, did in our study not lead to an altered myogenic responsiveness in preglomerular arterioles. Findings from Michels and coworkers indicate that severe hyperglycemia in diabetic rats (> 23.6 mmol/L) is accompanied by a sharp decline in single nephron glomerular filtration rate and glomerular pressure (17). They suggest that in the case of severe hyperglycemia these declines do not relate to changes induced by the diabetes, but rather by water deprivation and subsequent extracellular volume contraction (17). Volume contraction is known to produce higher arteriolar resistances in rats (1). Interestingly, to obtain in our study, a mean blood glucose level below 23.6 mmol/L, we had to give an amount of partial insulin substitution that at the same time caused the daily water intake to decline to near normal levels. In other experiments we found for that situation a normal plasma sodium concentration, indicating no dehydration (22). A decrease in glomerular filtration rate in the presence of high blood glucose levels seems in accordance with the trend observed in the present study that baseline arteriolar diameters were smaller in most cases for the diabetic groups of rats from randomly selected breeders with glucose levels above 23.6 mmol/L.

Partial insulin substitution with two different doses yielded quantitatively a normal myogenic response in diabetic kidneys of rats from randomly selected breeders, of whom the beta cells were destroyed by a high dose of STZ. By contrast, injection of a low concentration of STZ did lead to an attenuated myogenic response in part of these animals. Given their relatively low blood glucose (see table 2.2, right column), an appreciable amount of insulin must have been present in this subgroup. Why partial beta cell destruction caused an attenuated arteriolar myogenic response while total destruction with partial insulin substitution did not, may be related to a possible effect of STZ on renal arterioles. However, Hayashi et al. have shown that rats injected with STZ but who did not develop overt diabetes have a normal reaction to pressure (8). Secondly, it might have been caused by a difference in type of insulin. In the present study we used for substitution an ultralente insulin derived from recombinant human sources. The amino acid sequences of animal insulins are very similar to the human form, with 98% homology. However, it can not be
excluded that the human insulin had a different effect in the strains derived from the two vendors, given the observed difference in myogenic response.

Pressure generated constriction of arteriolar vascular smooth muscle cells is among others dependent upon reactive oxygen species (ROS) (19). Moreover, ROS have been implicated in the processes leading to diabetic nephropathy. In afferent arterioles it has been shown that acetylcholine-induced vasodilatation is impaired in diabetic rats and that ROS contribute to this impaired response (25). Furthermore, in human endothelial cells high glucose induced the generation of ROS and subsequently an altered prostanoid profile (4). In a pilot experiment we studied the effect of inhibiting the generation of ROS on the myogenic response in 4 week diabetic rats from randomly selected breeders with a normal quantitative response to pressure. We found no clear effect of ROS inhibition (data not shown), indicating that increased ROS production was not a compensating mechanism to normalize the myogenic response.

Our data indicate that PGE$_2$ at concentrations lower than $10^{-8}$ mol/L dilates distal interlobular arteries and afferent arterioles which were preconstricted with angiotensin II, and that their vasodilatation is paralleled by an increase in renal perfusion flow. Increasing the PGE$_2$ concentration (>10$^{-8}$ mol/L) did not further dilate or constrict these small microvessels, but total renal flow decreased significantly. This apparent vasoconstrictor action of PGE$_2$ in the kidney can not be caused by the small microvessels, since they were dilated, but must have been caused by the larger preglomerular arteries. In this respect, van Rodijnen and coworkers have shown that PGE$_2$ caused in interlobular arteries with diameters between 30-70 µm vasodilation at lower but vasoconstriction at higher concentrations, while in even larger ones it caused only vasoconstriction (30).

In conclusion, in diabetic Sprague-Dawley rats from a substrain selected for large litter size a decreased myogenic response occurs more easily than in diabetic rats from randomly selected breeders. A decreased preglomerular reaction to increases in systemic arterial pressure might in diabetic subjects lead to a higher chance to develop nephropathy. Our study suggests that a difference in PGE$_2$-signaling might be one of the possible factors involved in determining whether a diabetic individual will develop nephropathy or not.

**Acknowledgments**

This study was supported by a grant of the Dutch Kidney Foundation, C99.1808. The authors would like to thank Wim Gerrissen for taking care of the animals.
References