Impairment of Afferent Arteriolar Myogenic Responsiveness in the Galactose-Fed Rat

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HAYLEY G. FORSTER, PIETER M. TER WEE, TSUNEO TAKENAKA, THOMAS C. HOHMAN*, AND MURRAY EPSTEIN[1]

Nephrology Section, Department of Veterans Affairs Medical Center and Division of Nephrology, University of Miami School of Medicine, Miami, Florida 33125

Abstract. **Previous studies from our laboratory have demonstrated impaired afferent arteriolar responsiveness to pressure in rats 4–6 weeks after the induction of diabetes mellitus. Although the responsible mechanisms mediating this renal autoregulatory defect have not been fully defined, increased polyol metabolism has been implicated as a possible factor involved in the pathogenesis of diabetic complications. We therefore investigated the possible role of this metabolic disturbance in renal autoregulation using the galactose-fed rat, a model characterized by increased polyol pathway activity independent of hyperglycemia or insulin deficiency. Hydronephrosis was induced to permit direct visualization of renal microvessels. Pressure-induced vasoconstriction of afferent arterioles was assessed by quantitating vessel diameter following stepwise increments of renal perfusion pressure (RAP; from 80 to 180 mm Hg) in the hydronephrotic kidneys from control rats and rats fed a 50% galactose diet for 2 or 4 weeks. Vessel diameters were measured from video images by computer-assisted image processing. Control rats exhibited progressive afferent arteriolar vasoconstriction when RAP was increased from 80 to 180 mm Hg (−17.3% ± 1.0%; $P <$ 0.001). In contrast, myogenic responses to increases in pressure were absent in the afferent arterioles of rats fed a 50% galactose diet for either 2 (−4.1% ± 1.9%; not significant) or 4 weeks (−2.9 ± 3.4%; not significant). Our demonstration that the impairment of afferent arteriolar responsiveness to increasing RAP in the normoglycemic galactose-fed rat was identical to that observed in the STZ-diabetic rat suggests that increased polyol accumulation may contribute to the impairment of renal autoregulation in the diabetic rat.** [P.S.E.B.M. 1994, Vol 206]

Several lines of evidence indicate that renal hemodynamics are altered early in the course of diabetes mellitus (1–2). Studies in experimental diabetes have described a decrease in afferent arteriolar resistance accompanied by an increase in glomerular capillary pressure and flow (3–5). The resultant glomerular hyperfiltration that follows these microcirculatory alterations has been implicated as an important determinant in the development of glomerulosclerosis (3, 4, 6).

The afferent arteriole performs a pivotal role in regulating glomerular capillary pressure in the kidney. Utilizing a video-microscopic technique, we have recently demonstrated that in contrast to normal kidneys, the afferent arterioles of diabetic kidneys exhibit an impaired response to increases in renal arterial pressure (7). The determinants of this autoregulatory derangement have not been fully established. Increased polyol metabolism, however, has been implicated as one of the possible factors involved in the development of the functional and structural derangements that occur in the diabetic state.

The present studies were undertaken to determine whether increased polyol formation contributes to the impairment in autoregulation documented in experi-

[1] To whom requests for reprints should be addressed at Nephrology Section, Veterans Affairs Medical Center, 1201 NW 16th Street, Miami, FL 33125.

* Present address: Wyeth-Ayerst Research, CN 80000. Princeton, NJ 08543.

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mental diabetes. The galactose-fed rat constitutes an ideal model in which to study the relative participation of the polyol pathway in renal hemodynamics. Galactose is rapidly absorbed, producing systemic sugar concentrations that are proportional to the amount of galactose ingested (8). Independent of insulin, this hexose readily enters tissues where it can be reduced by aldose reductase to galactitol, at the expense of the cofactor NADPH (β-nicotinamide adenine dinucleotide phosphate, reduced form). The flux of substrate through this first step of the polyol pathway may be much greater with galactose feeding than in diabetes because aldose reductase has a 4-fold greater affinity for galactose than for glucose (9). The major advantage of this model is that the formation of polyol and all of its sequelae occur in the presence of insulin, making it possible to dissociate the effects of exaggerated polyol pathway activity from those due to insulin deficiency (10). Our findings indicate that galactose feeding results in impaired afferent arteriolar responsiveness to increases in renal arterial pressure similar to that found in streptozotocin (STZ)-induced diabetes mellitus.

Materials and Methods

Preparation of the Donor Animals. Isolated perfused hydronephrotic rat kidneys were utilized to determine the effects of galactose feeding on afferent arteriolar responsiveness to increasing renal arterial pressure. Unilateral hydronephrosis was established in 6-week-old, male Sprague-Dawley rats (Charles River, MA) by ligation of the right ureter under methoxyflurane anesthesia (11, 12) (Metofane; Pittman-Moore, Mundelein, IL). After 4–6 weeks, the hydronephrotic kidneys were excised and perfused *in vitro* as described below. At this point, renal tubular atrophy had progressed to a stage that allowed direct microscopic visualization of the renal microvessels (11, 13).

For the perfusion studies, three groups of animals were prepared. The control group (n = 17 rats) was fed a standard rat chow diet *ad libitum*. The galactose-fed rats were placed in metabolic cages and were allowed free access to a powdered 50% galactose diet (Harlan-Teklad, Madison, WI) for either 2 weeks (n = 6; GAL-2) or 4 weeks (n = 6; GAL-4) before the perfusion studies.

Perfusion of Hydronephrotic Kidneys. For perfusion of the hydronephrotic kidneys, the rats were anesthetized with methoxyflurane. The kidneys were exposed through a midline incision and the renal artery of the hydronephrotic kidney was cannulated by introducing the perfusion cannula through the mesenteric artery and across the aorta. Perfusion with warm oxygenated media (pH 7.4) was initiated during the cannulation procedure to avoid ischemia of the perfused kidney. The hydronephrotic kidney was then excised and placed on the stage of an inverted microscope (Model K, Nikon), modified to accommodate a heated chamber equipped with a thin glass viewing port on the bottom surface (11).

The perfusion media consisted of a Krebs-Ringer bicarbonate buffer containing 6.5% bovine serum albumin (Bovuminar; Intergen Co., Purchase, N.Y.), 5 m*M* D-glucose, and a complement of amino acids as detailed previously (12). Perfusate was provided to the kidney at a constant pressure from a pressurized media reservoir. The reservoir pressure was maintained by the inflow of warm, hydrated gas mixture of 95% O_2 and 5% CO_2, which exited through an adjustable back pressure regulator (model 10BP; Fairchild Industrial Products, Winston-Salem, NC). Perfusion pressure was monitored at the level of the renal artery and kept constant at 80 mm Hg for equilibration for at least 60 min. Perfusion pressure was adjusted by the same back pressure regulator that controlled the exit of gas from the media reservoir. The effluent was returned to the pressurized chamber by two rolling pumps (Masterflex, Chicago, IL).

Determination of Vessel Diameter. Video images of the renal microvessels were obtained utilizing an Ikegami video camera (model ITC-47; Tokyo, Japan) and recorded by a video cassette recorder. Vessels were selected for study on the basis of adequate flow as estimated by the response to a temporary (approximately 2 sec) occlusion of the perfusion line. To determine vessel diameters, the video recording was transmitted to an IBM-AT computer equipped with a video acquisition board (IVG-128; Datacube, Peabody, MA). Vessel diameters were estimated with an automated program, custom designed to determine the mean distance between parallel edges. A segment of the afferent arteriole approximately 10 μm in length was scanned at 2- to 5-sec intervals. Each value of vessel diameter was calculated by averaging all measurements obtained during the plateau of the response and thus represents the mean of 20–40 individual determinations.

Perfusion Experimental Protocols. *Pressure-induced vasoconstriction.* Afferent arteriolar responses to increases in pressure were assessed in the kidneys from each group. Initially, RAP was maintained at 80 mm Hg. Thereafter, RAP was elevated in a stepwise fashion by 20 mm Hg increments to a maximum of 180 mm Hg. Afferent arterioles were scanned for at least 1 min at each level of RAP.

Effects of cyclooxygenase inhibition. In a previous study, we demonstrated that the addition of ibuprofen to the perfusate media restored pressure-induced afferent arteriolar vasoconstriction in 4–6 week diabetic rat kidneys (7). We therefore undertook an identical treatment protocol with the control and

GAL-4 rat kidneys. Afferent arteriolar diameters were determined over the range of RAP described above. Subsequently, 100 μM ibuprofen was added to the media. After a 30-min equilibration period, pressure responses of the same vessels were reevaluated.

Biochemical Determination of Polyol Accumulation in the Kidney. To correlate the extent of polyol accumulation in renal tissue as a function of galactose feeding, we quantitated galactitol accumulation in the cortex and medulla of rats fed diets of 20%, 30%, 40%, and 50% galactose (Bioserve, Springfield, NY) for up to 28 days. For this experiment, male Sprague-Dawley rats (Charles River, MA) weighing 50–60 g were randomized into groups of equal body weight with six animals per group. The control group had free access to Purina rat chow with 20% glucose.

On the day of tissue harvest, the animals were anesthetized with halothane and the kidneys were perfused with saline at a flow rate of 5 ml/min. For the perfusion, a catheter was inserted into the renal artery and the vena cava was bisected. Kidneys that were not properly perfused (blanched) were discarded. The perfusions were performed to remove erythrocytes, since these cells contain high levels of aldose reductase and accumulate considerable concentrations of polyol.

The capsules were removed from the perfused kidneys and the cortex was carefully dissected from the medulla. Cortical and medullary tissues from both kidneys of each animal were separated, weighed and frozen on porcelain plates cooled with dry ice. These tissues were stored at −20°C prior to galactitol analysis.

Tissue galactitol was quantitated by high-pressure liquid chromatography using a pulsed amphometric detector linked to Carbopac-PA anion exchange column and a Carbopac-PA guard column fitted with an eluent degassing module (Dionex, Sunnyvale, CA). Cortical and medullary tissues were homogenized in 0.3 M $ZnSO_4$ at a dilution of about 25 mg of tissue per ml. The homogenate was neutralized with an equal volume of 0.3 M $Ba(OH_2)$ and centrifuged at 100g for 10 min. Following the addition of 25 μg of perseitol, an internal standard, to the supernatant fraction, a 50-μl aliquot was injected onto the chromatographic column. Galactitol was eluted with a 0 to 15 mM gradient of NaOH. This method has a limit of detection of 0.5 μg/ml of galactitol and a linear response for concentrations ranging between 0.5 and 300 μg/ml.

Analysis of Data. All data are expressed as the mean ± standard error. Unless otherwise stated, the n values refer to the number of afferent arterioles studied. Data were analyzed by one way analysis of variance followed by t test. Changes within experimental groups were subjected to a paired analysis, a P value <0.01 was used as level of significance after correction by the Bonferroni method. Differences between groups were assessed by unpaired analysis, a value of $3P < 0.05$ was taken as the level for statistical significance.

Results

Mean blood glucose values on the day of study did not differ between the three groups (85 ± 5 in GAL-4, 81 ± 3 in GAL-2, and 108 ± 9 mg/dl in control). All GAL-4 rats developed cataracts that became evident by 27 ± 2 days. Daily urinary output in both galactose fed groups was markedly increased compared with control; on the day before study, urine volume averaged 128 ± 6 in GAL-4 and 128 ± 5 in GAL-2 versus 23 ± 3 ml/24 hr in control animals ($P < 0.001$).

Characterization of Pressure-Induced Vasoconstriction. Feeding of the 50% galactose diet resulted in an abolition of afferent arteriolar responsiveness to stepwise elevations of renal arteriolar pressure (RAP). Figure 1 depicts representative tracings of af-

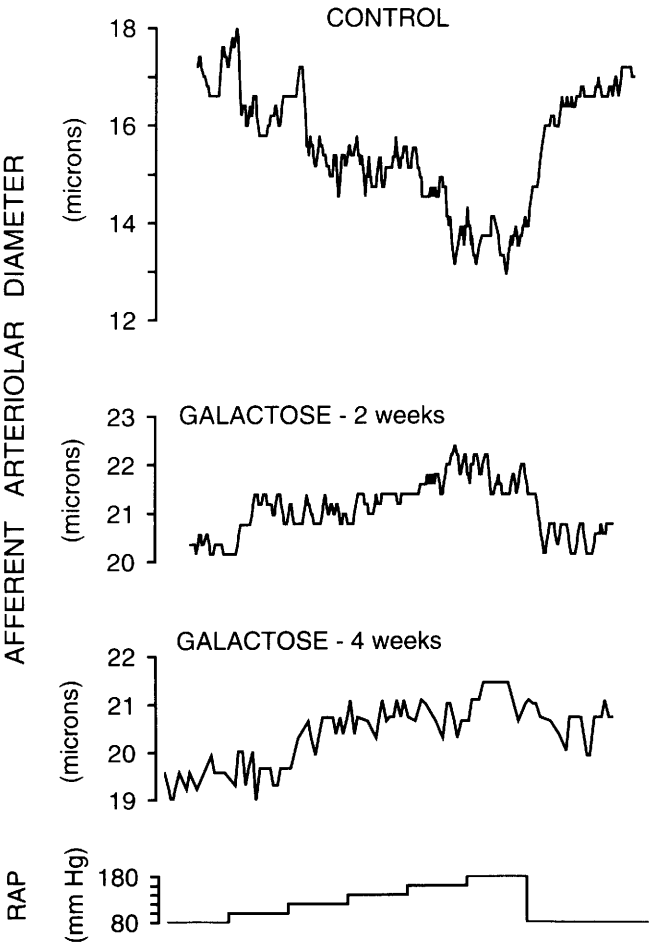

Figure 1. Representative tracings illustrating pressure responses of afferent arterioles in kidneys isolated from a control rat and 2- and 4-week galactose-fed rats. Increasing renal arterial pressure (RAP) from 80 to 180 mm Hg elicited pressure-dependent decreases in diameter of arterioles from normal kidneys, whereas, in the galactose-fed rat kidneys this response was greatly attenuated.

ferent arteriolar responses to pressure from all three groups. Increasing pressure from 80 to 180 mm Hg elicited progressive afferent arteriolar vasoconstriction in kidneys from control rats. In contrast, afferent arterioles from both the 4-week and 2-week galactose-fed rats manifested an abolished response to increases in RAP.

Mean baseline diameter in the GAL-4 arterioles was significantly larger than that of control (20.2 ± 1.0 μm vs 17.9 ± 0.5 μm; $P < 0.001$), whereas GAL-2 mean basal diameters tended to be higher than control (19.6 ± 0.6 μm; not significant vs control and GAL-4). Figure 2 summarizes the pressure-induced responses of all three groups. To facilitate comparison between the groups, data are depicted as percent change from basal (80 mm Hg) diameter. Afferent arterioles from the control rats demonstrated a progressive vasoconstriction to stepwise elevation of RAP. A significant reduction in diameter was first observed at 100 mm Hg (from 17.9 ± 0.5 to 17.3 ± 0.5 μm; $P < 0.01$; $n = 35$) with a maximal constriction of −17.3% ± 1.0% observed at 180 mm Hg (from 17.9 ± 0.5 to 14.8 ± 0.4 μm; $P < 0.01$; $n = 35$). The subsequent decrease in RAP to 80 mm Hg resulted in a prompt increase in afferent arteriolar diameter back to baseline values (17.7 ± 0.5 μm; not significant vs baseline). In contrast to the controls, the galactose-fed rat afferent arterioles exhibited an impairment of constrictor responses to stepwise increases in RAP (Fig. 2). In the GAL-4 group, elevation of RAP to 180 mm Hg failed to significantly alter afferent arteriolar diameter (from 20.2 ± 1.0 to 19.6 ± 1.2 μm; $n = 18$). Likewise, in the GAL-2 kidneys, afferent arteriolar responses to increasing pressure were similarly abolished (from 19.6 ± 0.6 at 80 mm Hg to 18.8 ± 0.7 μm at 180 mm Hg; $n = 29$).

Effects of Cyclooxygenase Inhibition on Pressure-Induced Vasoconstriction. Figure 3 summarizes the effects of ibuprofen on pressure induced vasoconstriction in the control and GAL-4 kidneys. The administration of 100 μ*M* ibuprofen to the media decreased basal afferent arteriolar diameters in the control kidneys (from 20.6 ± 0.7 to 17.5 ± 0.8 μm, $P < 0.005$) but did not substantively alter basal afferent arteriolar diameters in the GAL-4 kidneys (from 19.5 ± 1.2 μm to 19.0 ± 1.2 μm; $P > 0.05$; $n = 16$). The addition of ibuprofen, however, did not alter the myogenic afferent arteriolar vasoconstrictor responses to increasing pressure in the control kidneys. Thus, in the absence of ibuprofen (Fig. 3; filled circles), elevations in RAP elicited stepwise reductions in afferent arteriolar diameter (from 20.6 ± 0.7 to 16.65 ± 0.7, $P < 0.001$, $n = 9$) corresponding to a 19.7% ± 2.3% decrement at 180 mm Hg. Following treatment with ibuprofen (open circles), pressure-induced vasoconstrictor responses were nearly identical to those observed in the absence of ibuprofen. At 180 mm Hg, a 21.8% ± 1.9% decrement in vessel diameter was obtained (from 17.5 ± 0.9 to 13.7 ± 0.8 μm, $P < 0.001$, and $P > 0.5$ versus the response in the absence of ibuprofen).

In contrast to the afferent arteriolar responses in the control kidneys, ibuprofen substantively enhanced

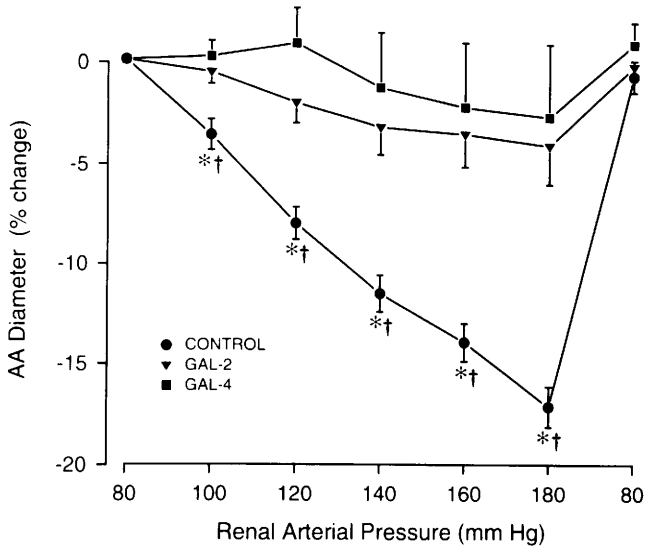

Figure 2. Data summarizing afferent arteriolar responses to an elevation in renal arterial pressure (RAP) in kidneys from control rats and rats fed a 50% galactose diet for 2 (GAL-2) and 4 (GAL-4) weeks. Pressure-induced vasoconstriction was abolished in both galactose-fed groups as compared with the controls. Results are mean ± SEM. *$P < 0.01$ vs 80 mm Hg; †3$P < 0.05$ vs control.

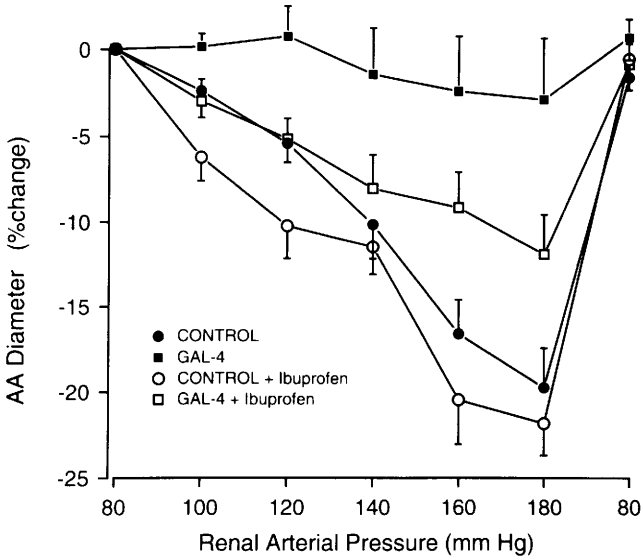

Figure 3. Effects of ibuprofen on pressure-induced vasoconstriction of afferent arterioles in kidneys from control rats and rats fed a 50% galactose diet for 4 weeks. The addition of ibuprofen (100 μ*M*) to the perfusate markedly improved the afferent arteriolar vasoconstrictor responses to increases in renal arterial pressure (RAP) in the galactose-fed rats. The addition of ibuprofen had no effect on the unimpaired afferent arteriolar constrictor responses from the control kidneys. Responses are expressed as percent changes from basal diameter (i.e., 80 mm Hg). Results are mean ± SEM.

the pressure-induced afferent arteriolar vasoconstriction in the GAL-4 kidneys. Prior to the administration of ibuprofen (filled squares), afferent arterioles from the GAL-4 kidneys failed to manifest vasoconstrictor responses to stepwise increases in RAP from 80 to 180 mm Hg (i.e., from 19.5 ± 1.2 to 18.8 ± 1.3 μm; n = 16). Following the treatment with 100 μM ibuprofen (open squares), however, pressure-induced afferent arteriolar vasoconstrictor responsiveness was markedly improved. Thus, an elevation in RAP from 80 to 100 mm Hg elicited significant vasoconstriction (from 19.0 ± 1.2 to 18.5 ± 1.2 μm; $P < 0.01$; n = 16). Further increases in RAP produced progressive reductions in arteriolar diameter. At 180 mm Hg, afferent arteriolar diameter was vasoconstricted by −11.6% ± 2.4% (i.e., from 19.0 ± 1.2 to 16.8 ± 1.2 μm; $P < 0.001$), a constriction which was significantly different from that observed in the absence of ibuprofen.

Dose-Related Accumulation of Galactitol in Renal Tissue. To correlate the extent of polyol accumulation in renal tissue as a function of varying galactose feeding, we determined galactitol accumulation in the cortex and medulla of rats fed diets of 20% glucose and 20%, 30%, 40%, and 50% galactose for 7, 14, 21, and 28 days, respectively. Values for galactitol accumulation in the renal cortex are depicted in Figure 4.

Compared with the results with animals fed a 20% glucose diet, galactose feeding led to significantly higher cortical galactitol levels for all investigated galactose diets. In rats fed a 50% galactose diet, average cortical levels of galactitol were 1.01 ± 0.09 nmol/mg tissue at 1 week, 1.42 ± 0.18 at 2 weeks, 1.76 ± 0.19 at 3 weeks, and 1.87 ± 0.16 at 4 weeks. Galactitol levels at Week 3 and 4 were significantly higher than those at Week 1, $P < 0.05$ and <0.01 respectively.

Similar patterns and levels of galactitol accumulation were observed in the cortex of animals fed the 40% galactose diet. In contrast, when animals were fed a 20% galactose diet, cortical galactitol levels did not increase with prolonged feeding beyond those observed on Day 7 of the diet.

Whereas the levels of galactitol accumulation in the renal medulla were approximately four times greater than that observed in the cortex, the patterns of galactitol accumulation were similar for both tissues (Fig. 5).

Discussion

Patients with insulin-dependent diabetes who manifest an elevated glomerular filtration rate (GFR) are at risk for developing diabetic nephropathy (14, 15). In STZ-diabetic rats, glomerular hyperfiltration provides early evidence of functional damage. Micropuncture studies in the STZ-diabetic rat have demonstrated that the attendant glomerular hyperfiltration is based on increases in glomerular capillary pressure and plasma flow as a consequence of decreased afferent arteriolar resistance (3–5). In accord with these findings, we have recently demonstrated that pressure-induced vasoconstriction of the afferent arteriole was abolished in rats 4–6 weeks after the induction of diabetes mellitus by administration of streptozotocin (7). The mechanisms mediating the altered myogenic responsiveness of the afferent arteriole in diabetes, however, have not been fully elucidated.

In tissues where glucose uptake is independent of insulin (i.e., the lens, retina, peripheral nerves, and kidney), diabetic hyperglycemia results in increased tissue levels of glucose. This excess glucose is reduced to sorbitol by the enzyme aldose reductase. The increased formation and accumulation of sorbitol in

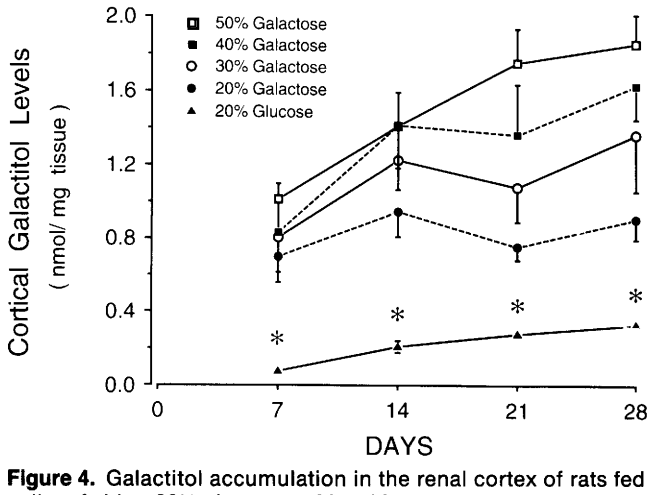

Figure 4. Galactitol accumulation in the renal cortex of rats fed a diet of either 20% glucose or 20%, 30%, 40%, or 50% galactose for 7, 14, 21, or 28 days. Compared with animals fed a 20% glucose diet, all levels of galactose feeding produced significantly higher cortical galactitol levels. Results are mean ± SEM. *$P < 0.01$ for all levels of galactose vs 20% glucose.

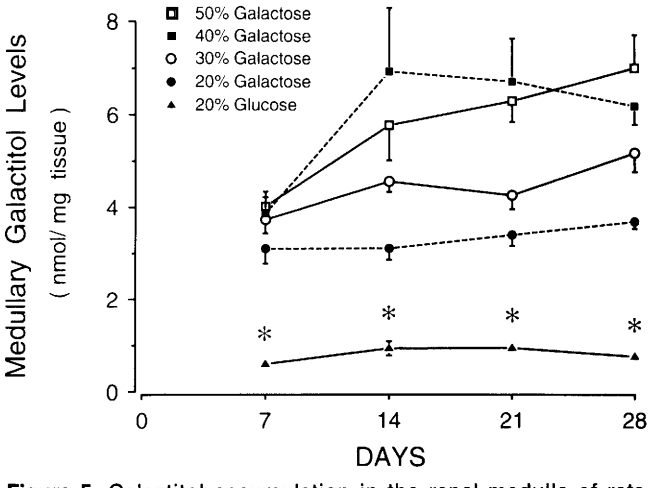

Figure 5. Galactitol accumulation in the renal medulla of rats fed a diet of either 20% glucose or 20%, 30%, 40%, or 50% galactose for 7, 14, 21, or 28 days. Compared with animals fed a 20% glucose diet, all levels of galactose feeding produced significantly higher medullary galactitol levels. Results are mean ± SEM. *$P < 0.01$ for all levels of galactose vs 20% glucose.

these tissues is accompanied by a depletion of free myo-inositol, loss of Na^+, K^+-ATPase activity, and increased consumption of the enzyme cofactors NADPH and NAD^+, leading to changes in cellular redox potential (16–20). These metabolic derangements have been postulated to result in cellular dysfunction and, ultimately, the morphological lesions that characterize diabetic neuropathy, nephropathy, retinopathy, and cataract formation (16, 17, 19, 21–23). Indeed, the prevention of sugar cataracts, the preservation of retinal and neural ultrastructure, as well as the prevention and reversal of nerve condition abnormalities in animal models of diabetes by aldose reductase inhibitors has been firmly established (24–29). In analogy, it is tempting to speculate that aldose reductase inhibitors may confer a similar salutary effect on an additional target organ—the kidney.

Accumulating experimental evidence has implicated polyol pathway activity in the loss of renal vascular tone in early experimental diabetes. Several investigators have demonstrated that treatment with aldose reductase inhibitors prevented glomerular hyperfiltration in diabetic rats (30–35). In contrast, Daniels *et al.* (36) reported that STZ-diabetic rats treated for 4 weeks with the aldose reductase inhibitor, statil, maintained elevated GFR values compared with control rats, although the intracellular accumulation of sorbitol was normalized. Therefore, in order to investigate further the relative contribution of the polyol pathway to the impairment of afferent arteriolar tone seen in early diabetes, we studied galactose-fed rats, an experimental model of increase polyol pathway metabolism without hyperglycemia and insulin deficiency.

In the present study, we have demonstrated that afferent arteriolar vasoconstrictor responses to increases in renal perfusion pressure were abolished in the kidneys of 2- and 4-week galactose-fed rats (Fig. 2). The abolition of myogenic responsiveness observed in the galactose-fed rats in this study was identical to that documented previously in 4- to 6-week STZ-diabetic rats (7). Furthermore, our present results are in accord with the demonstration of several renal functional derangements observed in the galactose-fed rat: utilizing micropuncture techniques, Banks *et al.* reported significant increases in single nephron GFR and flow, and decreases in afferent resistance in rats fed a 50% galactose diet for 10–14 days (37). These results were similar to data derived from their studies in the STZ-diabetic rat (34, 38). Moreover, Lorentz *et al.* reported that increases in creatinine clearance, microalbuminuria, and polyol accumulation in rats fed a 30% galactose diet were similar to those observed in STZ-diabetic rats (39). Finally, Pugliese *et al.* demonstrated that galactose feeding induced increments in GFR similar to those observed in STZ-diabetic rats, and that these increases were prevented by aldose reductase inhibition (35). In concert, these observations support a potential role of the polyol pathway in contributing to the impaired myogenic responsiveness of the afferent arteriole.

In the present studies from our laboratory, animals fed a 50% galactose diet for 4 weeks developed mature cataracts. The role of polyol accumulation in the etiology of galactosemic or diabetic cataract formation has been well characterized. Studies attempting to establish the relationship between cataract formation and the accumulation of polyol in the lens have demonstrated that lenticular polyol levels must be determined before the functional integrity of the lens is lost and polyol levels fall (40). To determine whether the loss of renal microvascular responsiveness is also accompanied by a loss of renal cell functional integrity and consequent loss of polyol, we quantitated cortical polyol levels in animals fed galactose for 7–28 days. Whereas galactitol levels increased progressively with the duration of time in the rats fed a 50% galactose diet, substantive galactitol accumulation occurred after only 2 weeks of galactose feeding (Fig. 4). These observations provided a basis for conducting the present studies following two weeks of galactose feeding. The results of our study with the GAL-2 rats and the biochemical findings indicate that the loss of renal microvascular responsiveness is a very early change resulting from increased polyol metabolism. Indeed, a comparison of the GAL-2 rat data to those of the GAL-4 disclosed an identical impairment of afferent arteriolar constrictor responses to increases in renal perfusion pressure. Of interest, galactitol levels were at least four times higher in the medulla than in the cortex at all galactose dietary concentrations and timed intervals used (Fig. 5). This might be attributable to the fact that although aldose reductase has been localized throughout the renal tissue, its highest distribution and activity has been demonstrated in the inner medulla (41, 42).

The possibility that alterations in renal eicosanoids may also contribute to impaired myogenic responsiveness merits consideration. Enhanced renal prostaglandin synthesis has been readily demonstrated in renal tissues from rats rendered diabetic by administration of STZ (43–47). In our previous studies of the 4- to 6-week STZ-diabetic hydronephrotic kidneys, we demonstrated that the loss of myogenic responsiveness in the afferent arteriole could be restored acutely with cyclooxygenase inhibition (7). The results of recent studies suggest a possible role for polyols in prostaglandin-mediated hyperfiltration in early diabetes. For example, Frey *et al.* (48) have shown that the aldose reductase inhibitor sorbinil prevented the increase in urinary PGE_2 production from STZ-diabetic rats of 2-week duration. Additionally, Craven *et al.*

(31) demonstrated that aldose reductase inhibition normalized the production of vasodilatory prostaglandins in isolated glomeruli and prevented hyperfiltration in moderately hyperglycemic STZ-diabetic rats. In the present study, the addition of the cyclooxygenase inhibitor, ibuprofen, induced a marked restoration of constrictor ability in the GAL-4 afferent arterioles that were nonresponsive previously. Because our hydronephrotic kidney model is devoid of circulating hormones and sympathetic innervation, it seems reasonable to speculate that prostanoid-mediated local vasodilation of smooth muscle could contribute, at least partially, for the loss in pressure-induced vasoconstriction observed in the galactose-fed rats.

The mechanism whereby increased prostaglandins are generated in the galactose-fed rat is unclear. Unfortunately, there is a paucity of experimental information relating to alterations in renal cyclooxygenase products from rats fed a galactose diet or in tissues incubated with galactose supplemented media. In a preliminary report, Zager *et al.* (49) demonstrated that 24-hr urinary excretion of PGE_2 and 6-keto-$PGF_{1\alpha}$ between 151 and 240 days was elevated in rats fed a 30% galactose diet. These increases, which were prevented by treatment with the aldose reductase inhibitor sorbinil, suggest that the increased flux of galactose through the polyol pathway may contribute to the modulation of renal eicosanoid production. However, the conditions whereby these studies were performed were not fully specified, and important variables including renal blood flow (RBF) and glomerular filtration rate were not reported. Consequently, it is not possible to infer the specific cellular contributions to or mechanisms whereby enhanced prostaglandin synthesis is produced in the kidney. In cell culture experiments, Zager and colleagues (50) have demonstrated that aldose reductase expression and PGE_2 production in rat mesangial cells are regulated in concert. Recent observations from a study using a model of mouse cerebral microvessel endothelial cells are at variance with those from the investigations of Zager *et al.* Yorek *et al.* (51) demonstrated that sorbitol accumulation was only mildly increased whereas galactitol levels were dramatically increased in response to incubation with glucose or galactose, respectively. Nevertheless, only chronic exposure to glucose was associated with increases in the vasodilatory prostaglandin E_2 in response to stimulation by A23187. It should be noted however, that glucose metabolism in brain endothelium differs considerably compared to that of other vessels predisposed to diabetic complications.

Finally, it has been suggested that the alterations in glomerular eicosanoid synthesis are linked to glucose-induced activation of glomerular protein kinase C (PKC) in STZ-diabetic rats and to glomeruli and mesangial cells incubated with glucose (52). This increase in PKC activity correlated with an increase of PLA_2, suggesting that the former enhances the release of membrane bound arachidonate for action by cyclooxygenase. The results of additional studies by the same investigators (53) suggest that PKC activation is linked to a down-regulation of receptor sites for the vasoconstrictor prostanoid thromboxane A_2. Thus PKC activation might contribute to an apparent dominant effect of the vasodilatory prostaglandins in the glomerular hyperfiltration of early diabetes. Nevertheless, because the galactose-fed rat is not hyperglycemic, glucose-induced increases in free arachidonate via activation of PKC could not account for the increases in renal eicosanoid synthesis. It would be of interest, however, to ascertain if galactose elicits the same effects from membrane phospholipids at the cellular level. Additional studies delineating renal tissue prostaglandin production in the galactose-fed rat are clearly indicated.

The demonstration that cyclooxygenase inhibition in the GAL-4 rats partially restores vasoconstrictor responses to increases in perfusion pressure merits comment. To ascertain whether the effects of ibuprofen constitute a nonspecific effect or is specific to the galactose-fed rat, we carried out a series of additional studies in control rats. As noted previously (see Fig. 3), the afferent arteriolar vasoconstrictor responses in ibuprofen-treated control kidneys were indistinguishable from those of the same vessels before the addition of the cyclooxygenase inhibitor. These observations underscore the specificity of cyclooxygenase inhibition in improving pressure-induced vasoconstrictor responsiveness in the galactose-fed rat.

It is conceivable that increased polyol pathway metabolism may contribute to the impaired myogenic responsiveness in the galactose-fed rat by alterations of endothelial cell function. Constricting and relaxing factors such as endothelin and nitric oxide, released by endothelial cells might influence vascular tone by exerting their actions locally on underlying smooth muscle cells. Recent studies have focused on the role of endothelial cell abnormalities in contributing to the pathophysiologic disarray of diabetes mellitus. Tolins *et al.* (54) demonstrated that STZ-diabetic rats maintained a persistent vasodilation at reduced perfusion pressure. Infusion of the nitric oxide synthase inhibitor, L-NAME, normalized the diabetic autoregulatory defect and also abrogated the attendant hyperfiltration in the diabetic rats. Additional evidence for a role of nitric oxide in diabetic hyperfiltration was provided by Cooper *et al.* (55). They demonstrated that a bolus infusion of L-NAME decreased botn the GFR and RBF in conscious, unrestrained, STZ-diabetic rats but decreased only RBF in the control with concomitant increases in systolic blood pressure from both groups. The demonstration that aldose reductase inhibition re-

stored PGI_2 synthesis and prevented defects in endothelium-dependent relaxation in response to acetylcholine and Ca^{++} ionophore A23187 in aortae of STZ-diabetic rats (56, 57) suggests a possible role for the polyol pathway in contributing to endothelial cell defects. This possibility is consistent with the observation that the key enzyme in the polyol pathway, aldose-reductase, is present in the endothelium of rat arteries (58). In the kidney, enzymatic studies of glomerular homogenates (41) have also demonstrated the presence of aldose reductase. Conceivably, further studies with L-NAME in diabetic and galactose-fed rats may help to delineate whether the polyol pathway mediates altered NO synthesis in the autoregulatory defect seen in the present study.

Finally, the loss of arteriolar vasoconstrictor responses in the galactose-fed rat may also result from the dysfunction of smooth muscle cells due to increased polyol formation. Impaired contraction has been linked to increased polyol pathway activity in cardiac and skeletal muscles from STZ-diabetic and galactose-fed rats (59–61). Several possible mechanisms may underlie these contractile deficits, including defective Ca^{++} handling by the sarcoplasmic reticulum, a reduction of spontaneous pacemaker activity in the myocardium, and degeneration of muscle fiber in diabetic and galactose-fed rats. The possibility that increased polyol accumulation could mediate these adverse effects in the cells by intracellular osmotic disruption appears unlikely, however, since galactitol accumulation was 12 times higher than that of sorbitol in the studies by Cotter and Cameron (59, 60). Thus, if the defect was due only to osmotic stress, one would have anticipated a much greater contractile defect in the galactose-fed rat. In analogy, we did not observe differences of impaired myogenic responsiveness between the STZ-diabetic (7) and the galactose-fed rats; therefore, it appears more likely that the polyol flux, rather than an accumulation of polyol pathway metabolites mediates these contractile changes. Finally, Kikawa *et al.* demonstrated significant levels of intracellular polyol accumulation in mesangial cells incubated with either galactose or glucose that were accompanied by a loss of contractility (62). Collectively, these data suggest that increased polyol pathway metabolism may exert similar effects on smooth muscle cell function, thereby contributing to the vascular complications seen in diabetes.

In conclusion, we have demonstrated that galactose feeding for 2 and 4 weeks produces a marked impairment of afferent arteriolar responsiveness to increases in perfusion pressure. This impairment of myogenic responsiveness may have several functional consequences. To the extent that a loss of afferent arteriolar responsiveness might result in the unbuffered transmission of systemic pressure to the glomerular capillary bed, the present studies in the galactose-fed rat suggest a possible role for alterations in the polyol pathway in the pathogenesis of glomerular capillary hypertension and hyperfiltration present in diabetes.

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