Effect of K$_{\text{ATP}}$ Channel Blocker U37883A on Renal Function in Experimental Diabetes Mellitus in Rats$^1$

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ABSTRACT
An increase in glomerular filtration rate (GFR) in early diabetes mellitus is considered a risk factor for the development of diabetic nephropathy. Insulin deficiency may increase the activity of ATP-sensitive potassium channels (K$_{\text{ATP}}$), which could promote afferent arteriolar vasodilation and thus contribute to glomerular hyperfiltration in early diabetes mellitus. To further elucidate this hypothesis we performed renal clearance experiments in anesthetized rats at 2 and 6 weeks after onset of streptozotocin-induced insulin-treated diabetes mellitus and studied the acute effect of the putative K$_{\text{ATP}}$ channel blocker 4-morpholinecarboximidine-N-1-adamantyl-N-$\beta$-cyclohexylhydrochloride (U37883A) on renal function. In control rats, application of U37883A (1.5 mg/kg i.v. bolus plus 1.5 mg/kg/hr) induced a significant reduction in heart rate, but did not affect or even slightly increased mean arterial blood pressure. Furthermore, U37883A did not significantly affect renal vascular resistance, renal blood flow or GFR, but caused an eukalemic diuresis and natriuresis and lowered plasma renin activity. Diabetic rats at both 2 or 6 weeks after streptozotocin exhibited essentially an identical response to U37883A; in particular, RVR and glomerular hyperfiltration remained unchanged. These results show that in both control and diabetic rats, the renal excretory function, renin secretion and pace setting in the heart were less sensitive to U37883A, implying a functional contribution of K$_{\text{ATP}}$ channel activity. However, in both control and diabetic rats, renal vascular resistance, renal blood flow, or GFR were not altered by U37883A. These results argue against a substantial role for K$_{\text{ATP}}$ channels in the basal control of renal hemodynamics in both nondiabetic and diabetic rats.

The pathogenesis of diabetic nephropathy is poorly understood, but glomerular injury has been ascribed to glomerular capillary hypertension and hyperfiltration, which occur early in the course of the disease (Hostetter et al., 1981; Mogensen and Christensen, 1984; Mogensen, 1986). The described changes in renal function in early diabetes are thought to be the consequence of afferent arteriolar vasodilation or an imbalance between afferent and efferent arteriolar resistance (Hostetter et al., 1981).

ATP-sensitive K$^+$ channels (K$_{\text{ATP}}$, channels) link the metabolic state of the cell (phosphorylation potential: [ATP]/[ADP][Pi]) to the permeability of the cell membrane for K$^+$, the latter being a major determinant of cell membrane potential. Activation of K$_{\text{ATP}}$ channels in vascular smooth muscle in many organs causes vasodilation, whereas K$_{\text{ATP}}$ channel inhibition, provided the channels are initially open, exerts vasoconstriction (Quayle et al., 1997).

A reduction of insulin availability and glucose uptake as found in insulin-dependent diabetes mellitus could lower the phosphorylation potential in smooth muscle cells. The resulting activation of K$_{\text{ATP}}$ channels could lead to afferent arteriolar vasodilation and thereby contribute to glomerular hyperfiltration in diabetes mellitus. Indeed, preliminary experiments employing the in vitro blood perfused juxtamedullary nephron technique suggested that K$_{\text{ATP}}$ channels in the afferent arteriole could play a role in diabetic glomerular hyperfiltration: It was observed that K$_{\text{ATP}}$ channels are normally expressed in rat afferent arteriole smooth muscle, but do not contribute significantly to basal tone (Ikenaga et al., 1996). However, both the functional availability and basal activation of K$_{\text{ATP}}$ channels appeared to be increased in the afferent arteriole during the early stage of diabetes mellitus in the rat (Ikenaga et al., 1996). Therefore, to further elucidate a potential role of K$_{\text{ATP}}$ channels in diabetic glomerular hyperfiltration, we studied the acute effect of the putative K$_{\text{ATP}}$ channel blocker U37883A on renal hemodynamics in experimental diabetes mellitus in vivo. Like glibenclamide, U37883A is an effective inhibitor of in vitro relaxation as well as $^{42}$K$^+$ efflux induced by K$_{\text{ATP}}$ channel openers and is an

ABBREVIATIONS: BGL, blood glucose level; FF, filtration fraction; GFR, glomerular filtration rate; Hct, arterial hematocrit; HR, heart rate; [K$^+$], plasma plasma potassium concentration; MAP, mean systemic arterial blood pressure; [Na$^+$], plasma plasma sodium concentration; PRA, plasma renin activity; RBF, renal blood flow; RNaR, renal sodium reabsorption; RVR, renal vascular resistance; TGF, tubuloglomerular feedback; UV, urinary flow rate; UKV, urinary potassium excretion; UNaV, urinary sodium excretion.

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effective blocker of in vivo hypotension produced by various K\textsubscript{ATP} channel openers (Meisheri et al., 1993a; Ohrnberger et al., 1993; Meisheri et al., 1993b). In contrast to glibenclamide, however, U37883A was found not to affect insulin secretion (Guillemare et al., 1994; Meisheri et al., 1993a) and therefore appears to be selective for vascular over pancreatic K\textsubscript{ATP} channels.

Beside a pressure-induced (myogenic) vasomotion, afferent arteriolar tone is regulated by the TGF. The TGF refers to the inverse relationship between the electrolyte concentration at the macula densa and the single nephron glomerular filtration rate, the latter being altered predominantly through changes in afferent arteriolar tone. Studies in humans and rats suggested that fractional reabsorption in the proximal tubule and loop of Henle is increased in the hyperglycemic and rats suggested that fractional reabsorption in the proximal tubule and loop of Henle is increased in the hyperglycemic state of diabetes mellitus (Hannenouche et al., 1990; Bank and Aynedjian, 1990; Vallon et al., 1995b). Thus, by lowering the luminal electrolyte concentration at the macula densa, this increase in tubular reabsorption may contribute to glomerular hyperfiltration (Vallon et al., 1995b).

K\textsubscript{ATP} channels play a role not only in systemic hemodynamics, but also contribute to fluid and electrolyte reabsorption in the kidney (Quast, 1996). In the proximal tubule Na\textsuperscript{+} is transported out of the cell through the basolateral membrane by Na\textsuperscript{+}-K\textsuperscript{+}-ATPase at the expense of K\textsuperscript{+} entry. K\textsuperscript{+} entering the cell by Na\textsuperscript{+}-K\textsuperscript{+}-ATPase leaves the cell again via an ATP-regulated K\textsuperscript{+} conductance in the basolateral membrane. An increase in Na\textsuperscript{+} entry across the luminal membrane will stimulate basolateral Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity, which by reducing the cytosolic [ATP]/[ADP] ratio increases the basolateral K\textsuperscript{+} conductance (pump-leak coupling) (Beck et al., 1994; Welling, 1995; Tsuchiya et al., 1992). In the thick ascending limb of Henle's loop (TALH), reabsorption of Na\textsuperscript{+} and Cl\textsuperscript{-} is carried out by Na\textsuperscript{+}-2Cl\textsuperscript{-}-K\textsuperscript{+} cotransport and therefore requires a minimum concentration of luminal K\textsuperscript{+} (Greger and Schlatter, 1981; Greger, 1985). Because of the discrepant amount of Na\textsuperscript{+} and K\textsuperscript{+} delivered from glomerular filtrate, in TALH most of the luminal K\textsuperscript{+} is derived by recycling from the tubular epithelium (Greger and Schlatter, 1981; Greger, 1985). It has recently been proposed that blockade of K\textsubscript{ATP} channels by glibenclamide or U37883A inhibits K\textsuperscript{+} recycling and reabsorption in TALH thereby contributing to the described diuretic and natriuretic response (Wang et al., 1995a, 1995b). The unchanged K\textsuperscript{+} excretion in spite of a significant natriuresis observed in response to glibenclamide or U37883A (Clark et al., 1993; Wang et al., 1995a; Wang et al., 1995b; Ludens et al., 1995) has been linked to inhibition of K\textsubscript{ATP}-mediated K\textsuperscript{+} secretion in the distal nephron (Wang et al., 1995a, 1995b).

Thus, an increased activity of tubular K\textsubscript{ATP} channels may be a permissive factor for the rise in reabsorption in proximal tubule and TALH in diabetes mellitus. To elucidate the possibility that inhibition of tubular reabsorption by K\textsubscript{ATP} channel blockade could affect glomerular hyperfiltration in diabetics mellitus through activating TGF, we also studied the effect of U37883A on renal excretion rates and plasma renin activity, the latter being also regulated at least in part by the luminal electrolyte concentration at the macula densa. Since there is evidence that different mechanisms may contribute to the alterations in renal function in early and established experimental diabetes mellitus (Kikkawa et al., 1986; Vallon et al., 1997a), the effect of U37883A was studied at 2 and 6 weeks after induction of diabetes.

**Methods**

All animal experimentation described here was conducted in accord with the NIH Guide for the Care and Use of Laboratory Animals and the German Law on the Protection of Animals. Male Sprague-Dawley rats weighing 200 to 250 g were made diabetic by STZ (65 mg/kg i.p.; Sigma Chemical, St. Louis, MO) dissolved in sodium citrate buffer (pH 4.2). One day later, the glucose concentration was determined in tail blood samples, and only those animals with blood glucose levels >300 mg/100 ml were included in further experiments. Diabetic rats were treated daily with Ultralente insulin (0.5-1.5 IU s.c. in late afternoon, Novo Industry, Copenhagen, Denmark) to adjust blood glucose levels at ~300 to 350 mg/dl. Blood glucose concentration was determined in tail blood samples twice a week. The animals were allowed free access to a regular rat pellet diet and tap water. Vehicle-injected non-diabetic rats fed the same diet served as controls.

**Measurement of Renal Hemodynamics and Renal Excretion Rates**

Two weeks (early diabetes) or 6 weeks (established diabetes) after STZ injection, the diabetic rats and the respective control rats were anesthetized with Inactin (100 mg/kg i.p.) and prepared for clearance experiments as previously described (Vallon et al., 1995a). Briefly, the animals were placed on a servo-controlled heating table to maintain body temperature at 37°C. A tracheostomy was performed to facilitate free breathing. The left femoral artery was cannulated to obtain blood samples and monitor arterial pressure (Statham P23Db transducer). The right jugular vein was cannulated for infusion of 0.85% saline (1.5 ml/hr) containing [3H]inulin (100 μCi/dl) as a marker of glomerular filtration rate and PAH (1 g/dl) to determine renal plasma flow. In addition, Ringer's saline (in mM: 30 NaHCO\textsubscript{3}, 4.7 KCl, 111 NaCl) was infused at 0.7% body wt/hr in control rats and 1.0% body wt/hr in diabetic rats. The bladder was cannulated for urine collection. After completion of the surgical preparation, the animals were allowed to stabilize for 120 min before starting the measurements.

Two-period renal clearance experiments were carried out, each period lasting 40 min. After finishing the first period (base-line measurements), an i.v. bolus injection of U37883A or vehicle, respectively, was performed (total volume of 250 μl over a 5-min time interval). Thereafter, the same dose was applied by continuous infusion per hour. Doses of 1.5 and 7.5 mg/kg of U37883A were applied in the series on early diabetes and 1.5 mg/kg in the series on established diabetes. Ten min after finishing the bolus injection, the second period was started. Arterial blood samples (160 μl each) were withdrawn in the middle of the two timed urine collection periods and were analyzed for hematocrit, [3H]inulin, PAH and Na\textsuperscript{+} and K\textsuperscript{+} concentration. Urinary flow rate was determined gravimetrically and urine was analyzed for [3H]inulin, PAH, and Na\textsuperscript{+} and K\textsuperscript{+} concentration. Glomerular filtration rate was calculated by the inulin clearance method. Renal plasma flow (RPF) was determined from the clearance of PAH as described previously (Tucker et al., 1993) including acid hydrolysis prior to assay to recover free PAH in glycine urine (Dalton et al., 1988). A PAH extraction ratio of 0.85 was used (Tucker et al., 1993). Renal filtration fraction (FF) was calculated according to the following equation: FF = GFR/RPF.

Renal blood flow (RBF) and renal vascular resistance (RVR) were calculated as follows: RBF = RPF/(1 - Hct) and RVR = MAP/RBF, where Hct is arterial hematocrit and MAP is mean systemic arterial blood pressure.

**Measurement of PRA**

After finishing the clearance experiments, arterial blood was drawn for measurement of PRA in vehicle-treated rats as well as in
rats treated with 1.5 mg/kg U37883A as previously described (Osswald et al., 1978). Briefly, from the catheter in the femoral artery, 200 μl of blood was drawn and added to 200 μl of precooled Hepes-Tris buffer containing 2 mg/ml Na₃EDTA (pH 7.4). After spinning at 6000 × g for 1 min, the supernatant was transferred to a precooled vial and stored at −80°C until further analysis. PRA was determined from the synthesis of angiotensin I, which was measured by standard radioimmunoassay as outlined below.

At the end of the clearance experiments, the two kidneys were excised, decapsulated, placed in an oven at 50°C and after drying overnight, the kidney dry weight was determined.

Materials and Analytic Methods

U37883A was kindly provided by Upjohn (Kalamazoo, MI). Urinary and serum concentrations of sodium and potassium were measured by flame photometry (ELEX 6361, Eppendorf). Concentration of ³H-inulin in plasma and urine was measured by liquid scintillation counting.

Measurement of plasma renin activity. Renin activity was determined by its capacity to generate angiotensin I, the latter being detected using a specific radioimmunoassay for angiotensin I. The detection limit of the assay was 5 pg Company) with 82% efficiency. Renin activity was calculated using ³H-inulin in plasma and urine was measured by liquid phase scintillation counting.

Plasma of bilaterally nephrectomized rats was used as renin substrate. Renin substrate (4 μl) and 10 μl enzyme inhibitors (2,3-dimercapto-propanol 80 μl/100 ml plus 8-hydroxyquinoline 132 mg/100 ml) were added to 10 μl of each sample. After incubating the mixture for 1.5 hr at 37°C the reaction was stopped on ice. Then, 80 μl (−2 nCi) of ¹²⁵I-labeled angiotensin I exhibiting a specific activity of 2200 Ci mmol⁻¹ (NEN Life Science Products, Boston, MA) plus 80 μl of a specific polyclonal rat angiotensin I antibody from rabbit (gift from E. Hackenthal, Heidelberg, Germany) were added and thoroughly mixed. After equilibration for 18 hr at 4°C, free and bound ¹²⁵I-angiotensin I were separated with bovine β-globulin (Cohn Fraction IV-1, Sigma Chemical)-coated charcoal (Norit A, Serva, Heidelberg, Germany) and were added and thoroughly mixed. 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**Results**

**Basal renal function in control and diabetic rats.** As depicted in table 1, rats with early and established diabetes mellitus exhibited moderate hyperglycemia, a modest retardation in body weight, but an increase in kidney weight compared with control rats. MAP was slightly lower in diabetic rats, reaching a significant difference in the series on established diabetes. HR diminished in both early and established diabetes by 10%. Arterial hematocrit was not different between control and diabetic rats. RNAr was significantly increased in both early and established diabetes. As shown in figure 1, glomerular hyperfiltration was evident in rats with early or established diabetes mellitus. In early diabetes, the increase in GFR was associated with a fall in RVR and a rise in RBF. In established diabetes, however, glomerular hyperfiltration occurred with no change in RVR or RBF, thus filtration fraction was increased.

**Effect of U37883A on renal function in control and diabetic rats.** As shown in figure 2, application of U37883A to control rats induced a dose-dependent reduction in HR, but did not affect (“early” controls) or even slightly increased (“established” controls) MAP. U37883A did not significantly affect RVR, RBF, or GFR (figs. 3 and 4), but caused a dose-dependent eu kaliuretic diuresis and natriuresis (figs. 4 and 5). Rats with both early or established diabetes mellitus exhibited essentially an identical response to U37883A (figs. 2–5). Particularly, RVR and glomerular hyperfiltration remained unchanged.

**Plasma renin activity in control and diabetic rats:**

**Effect of U37883A.** As compared with control rats, plasma renin activity (PRA) was increased by about 50–60% in both early and established diabetes mellitus. Application of U37883A lowered PRA in both “early” and “established” groups of control and diabetic rats. Since both basal values of PRA and the response in PRA to U37883A were not significantly different when comparing rats from the “early” and “established” series, values for control and diabetic rats from both series were pooled in figure 6.

**Discussion**

We hypothesized that an increase in Kₐ₅₃₆ channel activity could contribute to diabetic glomerular hyperfiltration i) through direct afferent arteriolar vasodilation and ii) through a permissive role for the increase in tubular reabsorption which by lowering the luminal signal of the TGF at
the macula densa reduces afferent arteriolar tone. To elucidate a potential role of K\textsubscript{ATP} channel activation in diabetic glomerular hyperfiltration, we studied the effect of the putative K\textsubscript{ATP} channel blocker U37883A on renal function in diabetic rats.

We observed that in control rats, application of U37883A induced a significant reduction in heart rate and did not affect (“early” control group) or slightly increased (“established” control group) mean arterial blood pressure (fig. 2). Since a fall in heart rate may reflect a decrease rather than an increase in cardiac output, the response in blood pressure may reflect a distinct increase in peripheral vascular resistance. Since K\textsubscript{ATP} channels in vascular smooth muscle are in general closed under physiological conditions, no substantial effect of K\textsubscript{ATP} channel blockade on peripheral vascular resistance is expected. As previously described in anesthetized and conscious rats (Wang et al., 1995b; Ludens et al., 1995), U37883A caused an eukaliuric diuresis and natriuresis (figs. 4 and 5). This response is in accordance with the described role of K\textsubscript{ATP} channels in tubular function and, in contrast to vascular smooth muscle, points to the high open probability of these channels in the tubular system under physiological conditions which makes them susceptible to pharmacological channel blockade. The effects of U37883A on tubular reabsorption occurred without a significant change in RVR, RBF or GFR (figs. 3 and 4). Thus, the K\textsubscript{ATP} channels on renal resistance vessels appeared to be predominantly closed under physiological conditions. These results suggest that in control rats, K\textsubscript{ATP} channel activity contributed to tubular reabsorption, but not to the basal control of renal hemodynamics.

Glomerular hyperfiltration was evident in rats with early or established insulin-treated diabetes mellitus. In early diabetes, the increase in GFR was associated with a fall in RVR and an increase in RBF, glomerular hyperfiltration in established diabetes was associated with an increase in FF.
thus FF was increased (fig. 1). The underlying mechanism of this increase in FF cannot be determined from the present study, but in order for both GFR and FF to increase, either effective filtration pressure and/or glomerular ultrafiltration coefficient must increase. While some studies reported that the increase in GFR in diabetic patients is associated with an increase in FF (Mogensen and Andersen, 1973; Ditzel and Junker, 1972), other studies in diabetic patients (Christiansen et al., 1981) and in early experimental diabetes mellitus (Hostetter et al., 1981) suggested that glomerular hyperfiltration is the consequence of an increase in RBF. Although the reasons for these different findings remain unclear, the present experiments suggest that the duration of diabetes mellitus could play a role.

Rats with early or established diabetes mellitus exhibited essentially an identical response to U37883A as compared with control rats (figs. 2–5): it caused bradycardia and slightly increased blood pressure in the established diabetes group, and induced a comparable diuresis and natriuresis without significantly altering renal potassium excretion. Furthermore, U37883A did not affect RVR, RBF or GFR in diabetic rats. Thus, if K<sub>ATP</sub> channels are present in the renal resistance vessels of diabetic rats, they appeared to be predominantly closed. Like in control rats, these results suggest that K<sub>ATP</sub> channel activity contributed to tubular reabsorption, but not to the basal control of renal hemodynamics in early or established insulin-treated diabetes mellitus in rats.

We initially hypothesized that an increased activity of K<sub>ATP</sub> channels in proximal tubule and loop of Henle could play a permissive role for the described increase in reabsorption at these tubular sites in diabetes mellitus, which by lowering the luminal electrolyte concentration at the macula densa could contribute to glomerular hyperfiltration. We observed that in both control and diabetic rats, application of U37883A induced a diuresis and natriuresis, but did not affect RVR, RBF, or GFR. Based on previous studies which
proposed that inhibition of reabsorption in TALH contributes to the natriuretic effect of U37883A (Wang et al., 1995b; Ludens et al., 1995), it appears unexpected that the resulting increase in the luminal electrolyte concentration at the macula densa through activation of TGF did not cause an increase in RVR or a fall in GFR. Since lowering reabsorption upstream to the macula densa will lower GFR only when the TGF mechanism remains intact, it could be inferred that U37883A must have desensitized the macula densa to changes in luminal electrolyte concentration. Since i) the TGF response depends on tubular transport across the macula densa, and ii) this transport occurs in analogy to the TALH through a “potassium-dependent” Na\(^{-}\text{-}2\text{-Cl}^{-}\text{-K}^{+}\)-cotransporter, U37883A by inhibiting potassium recycling and therefore luminal potassium availability may have inhibited the reabsorption not only in TALH, but also in the macula densa. Indeed, it has been shown recently that luminal potassium is required for full activation of the TGF response (Vallon et al., 1997b). Such a proposed effect of U37883A on TALH and TGF would be similar to the effect of the loop diuretic furosemide, which inhibits the Na\(^{-}\text{-}2\text{-Cl}^{-}\text{-K}^{+}\)-cotransporter directly in both TALH and macula densa, and therefore increases the luminal TGF signal without lowering GFR.

Beside controlling the vascular tone of the afferent arteriole, sensing of the luminal electrolyte concentration at the macula densa also contributes to the control of renin secretion. We observed that in both control and diabetic rats, application of U37883A significantly lowered PRA (fig. 6). This response was most likely not pressure-induced since blood pressure was not consistently increased in response to U37883A. The decrease in PRA in response to U37883A, however, is in contrast to the response to furosemide which through inhibition of macula densa transport is known to increase renin secretion. This implies that either U37883A did not desensitize the TGF response (which would leave the unchanged GFR unexplained) and/or U37883A elicited a predominant direct inhibitory effect on renin secreting juxtaglomerular cells. Supporting a direct role of K\(_{\text{ATP}}\) channels on juxtaglomerular cells in renin release, previous studies showed that the K\(_{\text{ATP}}\) channel opener cromakalim stimulates whereas the K\(_{\text{ATP}}\) channel blocker glibenclamide inhibits renin release from cultured juxtaglomerular cells (Ferrier et al., 1989; Linseman et al., 1995). Furthermore, application of U37883A also lowers plasma renin activity under conditions where the diuretic and natriuretic effect and therefore supposedly the increase in the luminal TGF signal is absent, as found under conditions of dietary potassium restriction (Vallon et al., 1998).

In summary, the present study shows that in both control and diabetic rats, the renal excretory function, the renin secretion, the pace setting in the heart, and probably the peripheral vascular resistance were sensitiv to U37883A, implying a functional contribution of K\(_{\text{ATP}}\) channel activity. However, in both control and diabetic rats, the renal vascular resistance, renal blood flow or glomerular filtration rate were not altered by U37883A. These results argue against a substantial role for K\(_{\text{ATP}}\) channels in the basal control of renal hemodynamics in both nondiabetic and diabetic rats.

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