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Eukaliuric diuresis and natriuresis in response to the K_{ATP} channel blocker U37883A: Micropuncture studies on the tubular site of action

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abstract
1 *Systemic* application of U37883A, a blocker of ATP sensitive potassium (K_{ATP}) channels, elicits diuresis and natriuresis without significantly altering urinary potassium excretion.

2 To elucidate tubular sites of action upstream to the distal nephron, micropuncture experiments were performed in nephrons with superficial glomeruli of anaesthetized Munich-Wistar-Frömter rats during *systemic* application of U37883A (1, 5 or 15 mg kg^{-1} i.v.).

3 The observed eukaliuric diuresis and natriuresis in response to U37883A at 15 mg kg^{-1} was accompanied by an increase in early distal tubular flow rate (V_{ED}) from 10–18 nl min^{-1} reflecting a reduction in fractional reabsorption of fluid up to this site (FR-fluid) of 13%. The latter proposed an effect on water-permeable segments such as the proximal tubule which could fully account for the observed reduction in fractional reabsorption of Na$^+$ up to the early distal tubule (FR-Na$^+$) of 8% and the increase in early distal tubular Na$^+$ concentration ([Na$^+$]$_{ED}$) from 35–51 mM whereas [K$^+$]$_{ED}$ was left unaltered.

4 In comparison, furosemide (3 mg kg^{-1} i.v.), which acts in the water-impermeable thick ascending limb, elicited diuresis, natriuresis and kaliuresis which were associated with a fall in FR-Na$^+$ of 10% with no change in FR-fluid, and a rise in [Na$^+$]$_{ED}$ from 42–117 mM and [K$^+$]$_{ED}$ from 1.2–5.7 mM with no change in V_{ED}.

5 Direct late proximal tubular fluid collections confirmed a significant inhibition of fluid reabsorption in proximal convoluted tubule in response to *systemic* application of U37883A.

6 These findings suggest that the diuretic and natriuretic effect upstream to the distal tubule in response to *systemic* application of U37883A involves actions on water-permeable segments such as the proximal convoluted tubule.

Keywords: Micropuncture; U37883A; thick ascending limb; reabsorption; Munich-Wistar-Frömter rats; K_{ATP} channels; proximal tubule

Abbreviations: Δ AR-fluid, change in absolute fluid reabsorption up to the late proximal convoluted tubule; ATF, artificial tubular fluid; EXP, experimental period; FR-fluid, FR-Na$^+$, FR-K$^+$, fractional reabsorption of fluid, sodium and potassium ion up to the early distal tubule; Δ FR-fluid, fractional fluid reabsorption up to the late proximal convoluted tubule; GFR; glomerular filtration rate; K_{ATP} channels: ATP sensitive potassium channels; [K$^+$]$_{ED}$, early distal tubular K$^+$ concentration; K$^+_{urine}$, urinary K$^+$ excretion; LH, loop of Henle; MAP, mean arterial blood pressure; MWF, Munich-Wistar-Frömter; [Na$^+$]$_{ED}$, early distal tubular Na$^+$ concentration; Na$^+_{urine}$, urinary Na$^+$ excretion; PCT, proximal convoluted tubule; SD, Sprague-Dawley; SNGFR: single nephron glomerular filtration rate; Δ SNGFR, change in SNGFR; TALH: thick ascending limb of Henle's loop; V_{ED}, early distal tubular flow rate; Δ V_{LP}, change in late proximal tubular flow rate; V_{urine}, urinary flow rate

Introduction

There is evidence that ATP sensitive potassium (K_{ATP}) channels play a role in tubular reabsorption in the proximal tubule and thick ascending limb of Henle's loop (TALH) as well as in reabsorption and K$^+$ secretion in both distal tubule and cortical collecting duct (for review see Quast, 1996). Indeed, *systemic* application of the K_{ATP} channel blockers U37883A (4-morpholinecarboximidine-N-1-adamantyl-N′-cyclohexyl-hydrochloride) or glibenclamide has been shown to induce diuresis and natriuresis without eliciting a pronounced increase in urinary potassium ion secretion or a change in glomerular filtration rate (Clark *et al.*, 1993; Ludens *et al.*, 1995a; Wang *et al.*, 1995a,b; Vallon *et al.*, 1998a,b). Furthermore, *systemic* application of U37883A has been shown to lower renin secretion or plasma renin activity (Linseman *et al.*, 1995; Vallon *et al.*, 1998a,b), a feature which

is uncommon to other diuretics and which is in accordance with studies showing that the K_{ATP} channel opener cromakalim stimulates whereas the K_{ATP} channel blocker glibenclamide inhibits renin release from cultured juxtaglomerular cells (Ferrier *et al.*, 1989; Linseman *et al.*, 1995). In contrast to glibenclamide, U37883A was found not to affect insulin secretion (Guillemare *et al.*, 1994; Meisheri *et al.*, 1993a) and therefore appears to be selective for vascular and tubular over pancreatic K_{ATP} channels.

Although availability and concentrations in the tubular lumen of U37883A achieved by *systemic* application are not known, Wang *et al.* (1995b) employing *intratubular* application of U37883A in micropuncture studies proposed that the diuresis and natriuresis in response to U37883A may be the result of inhibiting K_{ATP} channels and thus impairing reabsorption in the water-impermeable thick ascending limb of Henle's loop (TALH). In addition, blockade of K_{ATP} channels in the distal tubule and cortical collecting duct was

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proposed to suppress K^+ secretion which prevents kaliuresis, but further increases Na^+ excretion (Wang *et al.*, 1995b).

The present study aimed to elucidate which tubular segment upstream to the distal tubule contributes to the diuresis and natriuresis in response to *systemic* application of U37883A. U37883A was applied at doses of 1, 5 and 15 mg kg^{-1}, which represents the dose range previously employed to elucidate the diuresis and natriuresis as well as the inhibition of plasma renin activity in response to U37883A (Ludens *et al.*, 1995a; Wang *et al.*, 1995b; Vallon *et al.*, 1998a,b). These doses were also shown previously to inhibit the hypotensive response to application of various K$_{ATP}$ channel openers (Ludens *et al.*, 1995b; Meisheri *et al.*, 1993b). In a first series of experiments, late proximal tubular flow rate or early distal tubular flow rate and Na^+ and K^+ concentration were measured concomitantly with urinary excretion during *systemic* application of U37883A. For comparison, the response to furosemide which is known to inhibit TALH reabsorption was assessed. Furthermore, these experiments were performed in Munich-Wistar-Frömter (MWF) rats which have multiple nephrons with superficial glomeruli which allow to collect tubular fluid from the early distal tubule relatively close to the macula densa. This can help to minimize the input from drug effects on the distal tubule during early distal tubular fluid collection. The latter could be of importance for being able to ascribe drug actions to the TALH, if the drug tested, like U37883A, also acts on the distal tubule. Because experiments with *systemic* application of U37883A revealed a predominant effect on the proximal convoluted tubule rather than TALH, additional studies were performed with *intratubular* application of the drug to test for potential different effects of *systemic* and *intratubular* application of U37883A.

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. Experiments were predominantly performed in male Munich-Wistar-Frömter (MWF) rats weighing between 200 and 400 g. In the microperfusion series with U37883A in Henle's loop, both male MWF and Sprague-Dawley (SD) rats (220–300 g) were used to assess potential differences between rats strains.

Surgical preparation

Surgical preparation was performed as described before (Huang *et al.*, 1998). Briefly, rats were anaesthetized with thiobutabarbital (Inactin$^®$ 120 mg kg^{-1} i.p.). Animals were then placed on a temperature-controlled operating table to keep rectal temperature at 37°C. After tracheostomy (PE-200), a catheter (PE-50) was inserted into the right jugular vein for infusion of Ringer saline (mmol l^{-1}: NaCl 111, KCl 4.7, NaHCO$_3$ 30) at a rate of 1.3% of body weight h^{-1}. For assessment of whole kidney and single nephron glomerular filtration rate (GFR, SNGFR) [^{3}H]-inulin was added to Ringer saline to deliver 100 μCi h^{-1}. Arterial blood pressure was recorded *via* a catheter (PE-50) which was inserted in the left femoral artery and connected to a pressure transducer (P23dB, Gould-Statham, Oxnard, CA, U.S.A.). The left kidney was exposed by flank incision, carefully freed of perirenal fat and immobilized in a lucite cup. The kidney was covered with prewarmed paraffin oil. Both the urinary bladder and the left ureter were cannulated (PE-50) to maintain free urine drain.

After completion of the surgical preparation, the animals were allowed to stabilize for 90 min before starting functional studies.

Series 1: Effect of systemic application of U37883A on reabsorption (i) of the whole kidney, (ii) up to the early distal tubule, and (iii) up to the late proximal convoluted tubule
After starting a timed urine collection from the left kidney, distal or proximal tubular configuration of nephrons with superficial glomeruli were identified by dye injection into Bowman space using a micropipette (1–3 μm tip) filled with coloured artificial tubular fluid (ATF; see Series 3). In one
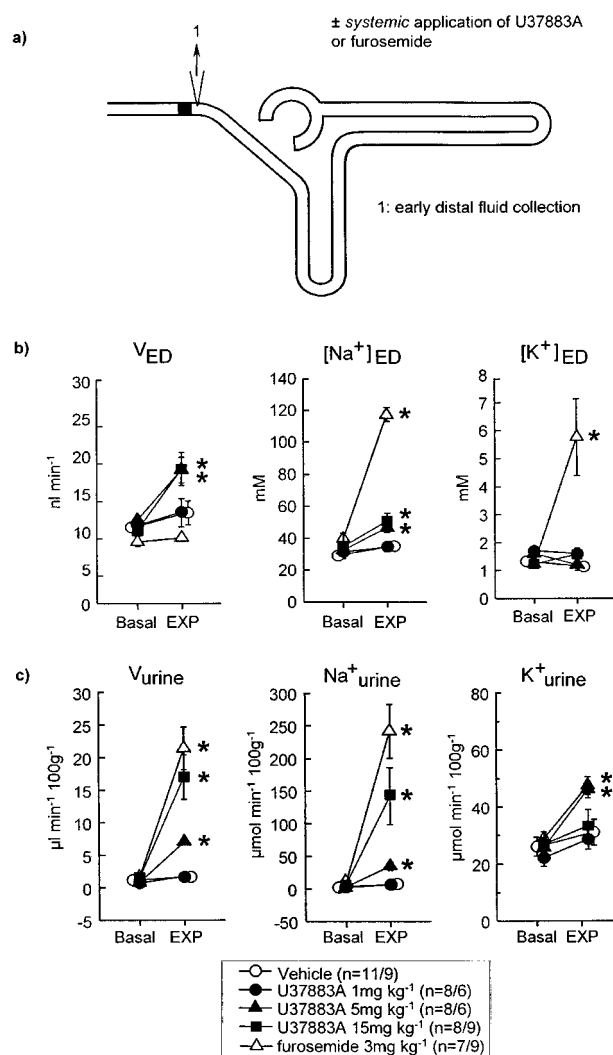

Figure 1 Effect of *systemic* application of U37883A or furosemide on (i) early distal tubular flow rate and sodium and potassium ion concentration (V_{ED}, [Na$^+$]$_{ED}$, [K$^+$]$_{ED}$) as well as (ii) urinary fluid, sodium ion and potassium ion excretion (V_{urine}, Na$^+$$_{urine}$, K$^+$$_{urine}$). (a) Schematic illustration of the nephron (with glomerulus, proximal tubule, loop of Henle and distal tubule) and of micropuncture manoeuvres. (b and c) Depicted is the effect of the experimental manoeuvre (EXP: i.v. administration of Vehicle, U37883A, or furosemide on the respective parameters as compared to basal measurement (Basal). Mean±s.e.mean *n* is given as number of nephrons/number of rats; *$P < 0.05$ vs basal. Notice that the diuresis and natriuresis in response to U37883A at 15 mg kg^{-1} was associated with an increase in [Na$^+$]$_{ED}$ and V_{ED}. In comparison, the diuresis, natriuresis and kaliuresis in response to furosemide, which acts in the water-impermeable TALH, was associated with a substantial rise in [Na$^+$]$_{ED}$ and [K$^+$]$_{ED}$ without altering V_{ED}. U37883A at 5 mg kg^{-1} elicited a similar response up to early distal tubule as 15 mg kg^{-1} (as indicated by similar rises in V_{ED} and [Na$^+$]$_{ED}$), which, however, was associated with an attenuated diuresis and natriuresis and the presence of kaliuresis.

series, a collecting pipette (7–9 μm tip) was inserted into the first accessible distal tubular loop to perform a timed collection of tubular fluid (at least 2½ min in duration) under free-flow conditions, utilizing a short mineral oil block, to obtain 'Basal' measurements (see Figure 1a). In another series, timed tubular fluid collections were performed in the last accessible loop of the proximal convoluted tubule (10–12 μm tip) (see Figure 2a). Following these collections, tubular fluid was allowed to escape from the collection hole or, after pushing away the short mineral oil block, escaped downstream. Thereafter, the timed urine collection from the left kidney was stopped. Subsequently, U37883A (1, 5 or 15 mg kg^{-1}) or Vehicle (Ringer saline) were applied i.v. as a slow bolus injection. In the series with early distal tubular collection, additional studies were performed with bolus injection of furosemide (3 mg kg^{-1} i.v.). Ten minutes after completing the bolus injection, a second urine collection from the left kidney was started and at the same time the early distal or late proximal tubular sites were punctured for paired recollection (experimental, 'EXP' in Figure 1). GFR, urinary flow rate and whole kidney sodium and potassium ion excretion as well as SNGFR, early distal flow rate and sodium and potassium ion concentration, and late proximal tubular flow rate were determined as described below.
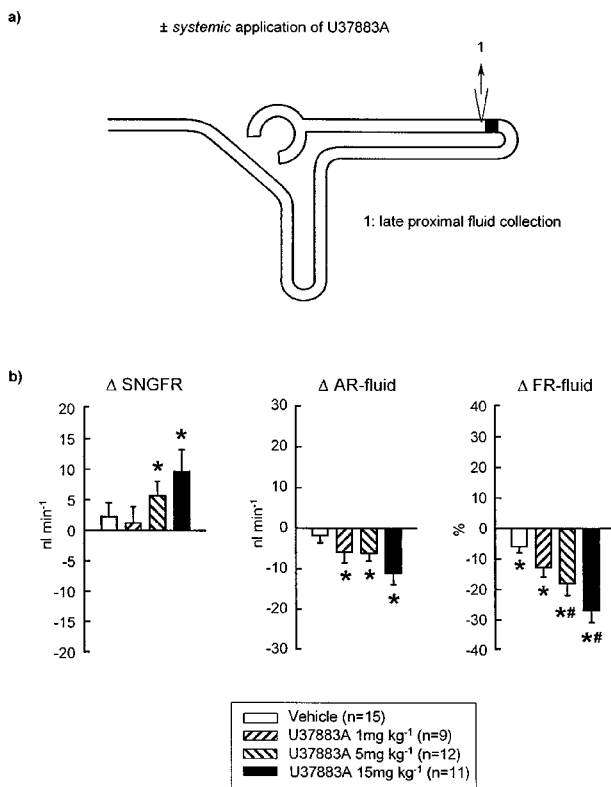
Series 2: Effect of systemic application of U37883A on proximal convoluted tubule reabsorption during stopped-flow perfusion This series aimed to elucidate whether U37883A has to be filtered in the glomerulus in order to affect proximal tubular reabsorption. After identification of proximal nephron configuration, a perfusion pipette directly attached to a calibrated Hampel microperfusion pump and filled with the appropriate type of artificial tubular fluid (ATF, see Series 3) was inserted into the first surface loop of the proximal convoluted tubule (see Figure 3a). A wax block was introduced immediately upstream to the perfusion pipette and venting the tubular system upstream to the wax block allowed the filtrate to escape freely onto the surface. Three to five minutes elapsed at an initial perfusion with ATF (40 nl min^{-1}) before a collecting pipette (9–11 μm tip) was inserted into the last surface loop of the proximal convoluted tubule to perform a timed collection of tubular fluid (at least 2½ min in duration) utilizing a short mineral oil block, and obtain 'basal' measurements. Subsequently, U37883A (15 mg kg^{-1}) or Vehicle (Ringer saline) were applied i.v. as a slow bolus injection. Ten minutes after completing the bolus injection of U37883A or Vehicle, the late proximal tubular sites were punctured for paired recollection. Late proximal tubular flow rate was determined as described below.
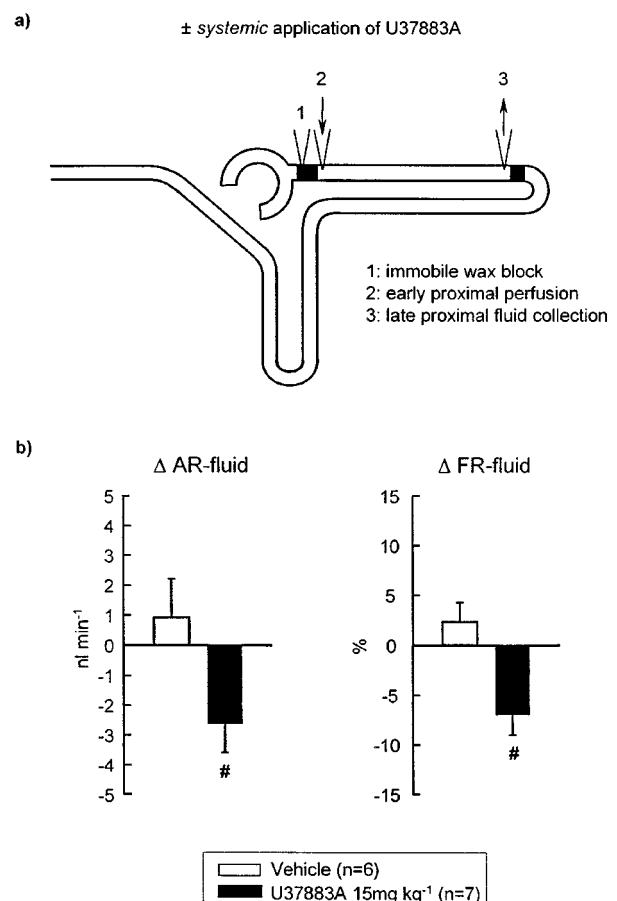

Figure 2 Effect of *systemic* application of U37883A on single nephron filtration rate (SNGFR) and proximal convoluted tubule fluid reabsorption as determined by free-flow collection from late proximal tubular site. (a) Schematic illustration of micropuncture manoeuvres. (b) Depicted are the changes in SNGFR (Δ SNGFR), and absolute (Δ AR-fluid) and fractional (Δ FR-fluid) reabsorption of fluid in the proximal convoluted tubule in response to *systemic* application of U37883A or Vehicle as compared to basal measurements. Mean $\pm$ s.e.mean. *$P < 0.05$ vs basal; Δ FR-fluid: #$P < 0.05$ vs vehicle. Notice that systemic application of U37883A caused a significant inhibition of fluid reabsorption in the proximal convoluted tubule. Whereas during fluid collection from the early distal tubular site, systemic application of U37883A left the SNGFR unaltered (Table 1), an increase in SNGFR was observed during fluid collection from the late proximal tubular site.

Figure 3 Effect of *systemic* application of U37883A on proximal convoluted tubule fluid reabsorption as determined by stopped-flow perfusion. (a) Schematic illustration of micropuncture manoeuvres. (b) Depicted are the changes in absolute (Δ AR-fluid) and fractional (Δ FR-fluid) reabsorption of fluid in the proximal convoluted tubule in response to *systemic* application of U37883A as compared to Vehicle treatment. Mean $\pm$ s.e.mean. #$P < 0.05$ vs vehicle. Notice that systemic application of U37883A significantly reduced fluid reabsorption in proximal convoluted tubule during stopped-flow perfusion.

Series 3: Effect of intratubular application of U37883A on proximal convoluted tubule or loop of Henle reabsorption during stopped-flow perfusion Reabsorption in the proximal convoluted tubule and in the loop of Henle were studied during orthograde microperfusion of the respective tubular segment with U37883A added to the perfusate. Different from previous reports (Wang *et al.*, 1995b) intratubular application of U37883A in the present study was found not to alter loop of Henle reabsorption. Therefore, additional experiments were performed to study (i) the effect of U37883A on loop of Henle reabsorption in SD rats in order to exclude potential differences between rats strains, and (ii) the effect on loop of Henle reabsorption of glibenclamide, another K$_{ATP}$ channel blocker, as well as its two major metabolites found in urine namely W039580A and W039109B (Heptner *et al.*, 1969). Concentrations of U37883A (10–100 μM) or glibenclamide (250 μM) employed were those used in previous studies (Wang *et al.*, 1995a,b). Concentrations for the two metabolites of glibenclamide were set at a somewhat lower concentration than glibenclamide, namely 100 μM. Tubular reabsorption was studied during microperfusion of functionally isolated (i) proximal convoluted tubule (PCT, see Figure 4a) or (ii) loop

of Henle (LH, see Figures 5a). After proximal and distal nephron configuration were identified, a perfusion pipette directly attached to a calibrated Hampel microperfusion pump and filled with the appropriate type of ATF (see below) was inserted into (i) the first, or (ii) the last surface loop of the PCT. A wax block was introduced immediately upstream to the perfusion pipette and venting the tubular system upstream to the wax block allowed the filtrate to escape freely onto the surface. Three to five minutes elapsed at an initial perfusion with ATF (40 nl min^{-1} for PCT, 20 or 25 nl min^{-1} for LH) before a collecting pipette (7–9 for LH or 9–11 μm tip for PCT) was inserted into (i) the last surface loop of the PCT, or (ii) the first accessible distal tubular loop and a timed collection of tubular fluid was obtained. Subsequently, a second perfusion pipette connected to a second Hampel pump (pump 2) and filled with ATF containing the respective drug or Vehicle was inserted in close proximity to the first pump. Pump 1 was switched off and pump 2 was started. After a time period of 10 min, a second tubular collection was performed.

The following types of ATF were employed: For identifier and PCT (in mM): NaCl 113, NaHCO$_3$ 25, KCl 4, MgSO$_4$ 1, CaCl$_2$ 2, Na$_2$HPO$_4$ 1, Glucose 5, urea 5, 0.075% FD&C green, pH 7.4. For LH (in mM): NaCl 130, NaHCO$_3$ 10, KCl 4, CaCl$_2$ 2, urea 7.5, 0.075% FD&C green, pH 7.4.
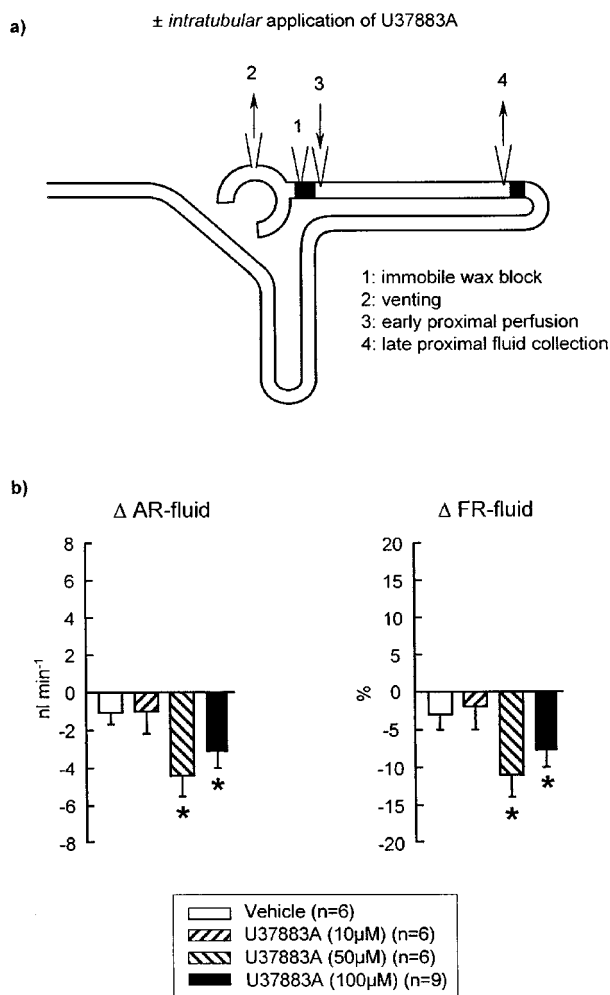

Figure 4 Effect of *intratubular* application of U37883A on proximal convoluted tubule fluid reabsorption during stopped-flow perfusion. (a) Schematic illustration of micropuncture manoeuvres. (b) Depicted are the changes in absolute (Δ AR-fluid) and fractional (Δ FR-fluid) reabsorption of fluid in the proximal convoluted tubule in response to *intratubular* application of U37883A or vehicle as compared to basal measurements. Mean $\pm$ s.e.mean. *$P < 0.05$ vs basal. Notice that intratubular application of U37883A significantly inhibited fluid reabsorption in the proximal convoluted tubule.
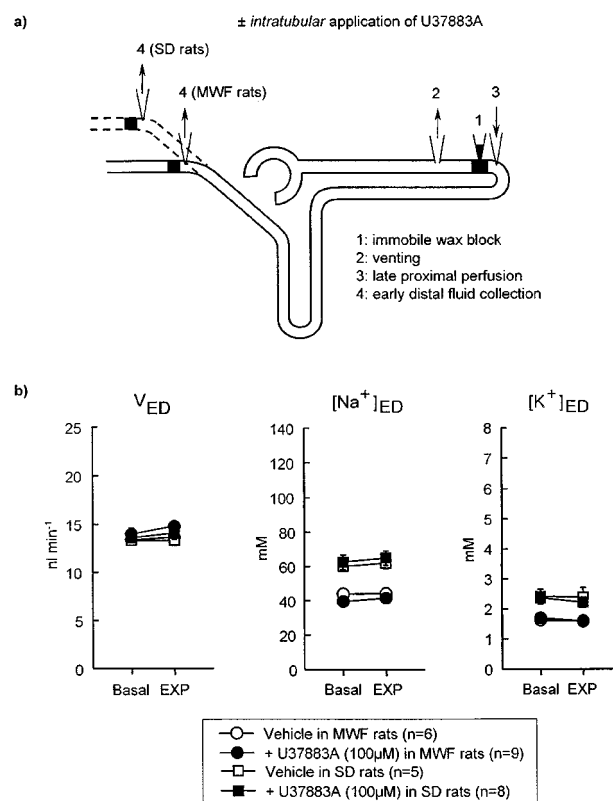
Figure 5 Effect of *intratubular* application of U37883A on loop of Henle reabsorption during stopped-flow perfusion. (a) Schematic illustration of micropuncture manoeuvres. (b) Depicted is the effect of the experimental manoeuvre (EXP: *intratubular* application of Vehicle or U37883A) on early distal tubular flow rate (V$_{ED}$) and sodium and potassium ion concentration ([Na$^+$]$_{ED}$, [K$^+$]$_{ED}$) as compared to basal measurement (Basal). Experiments were performed in Munich-Wistar-Frömter (MWF) and Sprague-Dawley (SD) rats. In contrast to SD rats, MWF rats have nephrons with superficial glomeruli which allow to puncture the early distal tubule closer to the macula densa. Mean $\pm$ s.e.mean. *$P < 0.05$ vs basal. Notice that *intratubular* application of U37883A did not significantly alter V$_{ED}$ [Na$^+$]$_{ED}$, or [K$^+$]$_{ED}$ in MWF or SD rats.

Materials

The following drugs were used: U37883A was kindly provided by Pharmacia & Upjohn, (Michigan, U.S.A.). Glibenclamide and furosemide were purchased from Sigma (St. Louis, MO, U.S.A.). The metabolites of glibenclamide, W039580A and W039109B, were kindly provided by Dr H. Englert (Hoechst AG, Frankfurt, Germany). Glibenclamide and its two metabolites were firstly dissolved in ethanol/DMSO (1:1) to obtain stock solutions and were then further diluted in ATF to achieve final concentrations. For experiments with glibenclamide or its metabolites, ATF plus the respective concentration of ethanol/DMSO was used as 'Vehicle'. U37883A and furosemide were dissolved in Ringer solution (for *systemic* application) or ATF (for *intratubular* application) using an ultrasound bath as performed previously (Vallon *et al.*, 1997a; Huang *et al.*, 1998).

Analytic methods and statistics

Urinary flow rate was determined gravimetrically and plasma and urine were analysed for sodium and potassium ion concentration by flame photometry (ELEX 6361, Eppendorf, Germany). Late proximal and early distal tubular flow rate were measured by transferring the collected fluid sample to a constant bore capillary. Sodium and potassium concentration in the distal tubular fluid were determined as previously described (Vallon *et al.*, 1997b; Huang *et al.*, 1998) with a micro flame photometer which was developed and built by Rolf Englert and Klaus Stieler (Department of Pharmacology, University of Tübingen, Germany) based on the original conception by Wolfgang Hampel (Wissenschaftlicher Gerätebau, Frankfurt/Main, Germany). Concentration of ^{3}H-inulin in plasma, late proximal and early distal tubular fluid, and urine were measured by liquid phase scintillation counting. Paired or unpaired Student's *t*-test were performed to analyse the data. $P < 0.05$ was considered to be statistically significant.

Results

Series 1: Effect of systemic application of U37883A on reabsorption (i) of the whole kidney, (ii) up to the early distal tubule, and (iii) up to the late proximal convoluted tubule

Systemic application of Vehicle did not significantly alter mean arterial blood pressure (MAP: 110 ± 1 vs 110 ± 1 mmHg, NS), GFR (0.37 ± 0.07 vs 0.34 ± 0.02 ml min^{-1} 100 g body wt^{-1} NS), urinary flow rate or the excretion of sodium or potassium ion (see Figure 1c). Likewise, SNGFR, early distal

tubular flow rate (V_{ED}) and early distal tubular sodium or potassium ion concentration ($[Na^+]_{ED}$, $[K^+]_{ED}$) as well as the fractional reabsorption of fluid or sodium or potassium ion upstream to the early distal tubule (FR-fluid, FR-Na$^+$, FR-K$^+$) were not significantly altered in response to Vehicle application (see Figure 1b and Table 1). *Systemic* application of U37883A at 1, 5 or 15 mg kg^{-1} did not significantly alter MAP or GFR as compared to basal measurements (MAP: 114 ± 7 vs 121 ± 4 mmHg, 113 ± 3 vs 124 ± 4 mmHg or 111 ± 7 vs 111 ± 4 mmHg, NS; GFR: 0.40 ± 0.12 vs 0.46 ± 0.18 ml min^{-1} 100 g body wt^{-1}, 0.28 ± 0.03 vs 0.33 ± 0.03 ml min^{-1} 100 g body wt^{-1} or 0.39 ± 0.07 vs 0.32 ± 0.04 ml min^{-1} 100 g body wt^{-1} NS). Application of U37883A at 15 mg kg^{-1}, however, elicited a diuresis and natriuresis without significantly altering urinary potassium excretion (see Figure 1c). The latter response was accompanied by an increase in V_{ED} and $[Na^+]_{ED}$ (see Figure 1b) and a decrease in FR-fluid, FR-Na$^+$ and FR-K$^+$ without a change in SNGFR (see Table 1). In comparison, *systemic* application of furosemide elicited a diuresis and natriuresis which was accompanied by a substantial kaliuresis (see Figure 1c). Furthermore, furosemide caused a significant increase in $[Na^+]_{ED}$ and $[K^+]_{ED}$ without altering V_{ED} (see Figure 1b) or SNGFR (see Table 1). The latter changes were associated with a fall in FR-Na$^+$ and FR-K$^+$ but not in FR-fluid. Application of furosemide did not significantly alter MAP (112 ± 3 vs 112 ± 4 mmHg, NS) or GFR (0.25 ± 0.02 vs 0.28 ± 0.02 ml min^{-1} 100 g body wt^{-1}, NS).

Although *systemic* application of U37883A at a dose of 5 mg kg^{-1} elicited almost similar rises in V_{ED} and $[Na^+]_{ED}$ as well as decreases in FR-fluid and FR-Na$^+$ as 15 mg kg^{-1} (see Figure 1b and Table 1), which indicated a relatively similar response up to the early distal tubule, that dose caused a significantly lesser diuresis and natriuresis and induced a kaliuresis (see Figure 1c).

It was further observed that *systemic* application of U37883A at 5 or 15 mg kg^{-1} reduced absolute and fractional fluid reabsorption in the proximal convoluted tubule during free-flow collection from late proximal tubular site (see Figure 2b). In addition, U37883A increased SNGFR during late proximal tubular collection, a response which had not been observed during early distal tubular collection (see Figure 2b and Table 1).

Series 2: Effect of systemic application of U37883A on proximal convoluted tubule reabsorption during stopped-flow perfusion

As compared to Vehicle, *systemic* application of U37883A at 15 mg kg^{-1} significantly inhibited proximal tubular fluid reabsorption during stopped-flow perfusion (see Figure 3b).

Table 1 Effect of *systemic* application of U37883A or furosemide on single nephron glomerular filtration rate (SNGFR) and fractional reabsorption of fluid, and sodium and potassium ion upstream to the early distal tubule (FR-fluid, FR-Na$^+$, FR-K$^+$)

Group	n	SNGFR (nl min^{-1}) Basal	EXP	FR-fluid (%) Basal	EXP	FR-Na$^+$ (%) Basal	EXP	FR-K$^+$ (%) Basal	EXP
Vehicle	11	52 ± 6	53 ± 5	81 ± 2	78 ± 2	96 ± 1	95 ± 1	94 ± 1	93 ± 1
U37883A 1 mg kg^{-1} i.v.	8	51 ± 3	48 ± 3	79 ± 1	$75 \pm 2*$	96 ± 1	94 ± 1	93 ± 1	$91 \pm 1*$
U37883A 5 mg kg^{-1} i.v.	8	51 ± 3	56 ± 3	78 ± 1	$68 \pm 3*$	95 ± 1	$90 \pm 1*$	92 ± 1	90 ± 1
U37883A 15 mg kg^{-1} i.v.	8	57 ± 4	58 ± 6	81 ± 2	$68 \pm 4*$	95 ± 1	$87 \pm 2*$	93 ± 1	$83 \pm 3*$
Furosemide 3 mg kg^{-1} i.v.	7	49 ± 6	48 ± 7	84 ± 1	82 ± 1	95 ± 1	$85 \pm 1*$	96 ± 1	$77 \pm 5*$

Data are mean$\pm$s.e.mean. *n*: numbers of nephrons; Basal, basal measurements; EXP, experimental period (after administration of drug or Vehicle); $*P < 0.05$ vs Basal.

Series 3: Effect of intratubular application of U37883A on proximal convoluted tubule or loop of Henle reabsorption during stopped-flow perfusion

Fluid reabsorption in proximal convoluted tubule was significantly reduced during *intratubular* application of 50 or 100 μM U37883A (see Figure 4b). In comparison, *intratubular* U37883A (10, 50 or 100 μM) did not significantly alter the reabsorption of fluid, sodium or potassium ion in the loop of Henle (Figure 5b and Table 2). Similarly, loop of Henle reabsorption was unaffected by *intratubular* application of glibenclamide (250 μM) or its metabolites WO39580A or WO39109B (each 100 μM) (Table 2). All the above experiments have been performed in MWF rats. Assessing loop of Henle reabsorption in SD rats which do not have superficial glomeruli, it was found as expected that early distal tubular concentrations of sodium and potassium ion were significantly higher as compared to MWF rats (Figure 5b). Similar to MWF rats, however, *intratubular* application of U37883A (100 μM) did not alter the reabsorption of fluid, sodium or potassium ion in the loop of Henle in SD rats.

Discussion

The present study aimed to elucidate which tubular segment upstream to the distal tubule contributes to the diuresis and natriuresis in response to *systemic* application of the K$_{ATP}$ channel blocker U37883A. Confirming the observation that *systemic* application of U37883A elicits a diuresis and natriuresis, the present study further revealed that effects on water-permeable segments upstream to the distal tubule such as the proximal convoluted tubule contribute to this response.

Performing free-flow collections from the early distal tubular site in nephrons with superficial glomeruli, it was observed that the diuresis and natriuresis in response to *systemic* application of U37883A at 15 mg kg^{-1} was associated with an increase in V_{ED} from $10-18$ nl min^{-1} and in $[Na^+]_{ED}$ from $35-51$ mM, reflecting a decrease in fractional reabsorption of fluid and Na$^+$ up to the early distal tubule of 13 and 8%, respectively. These findings indicated an inhibition of reabsorption in water-permeable segments upstream to the early distal tubule such as the proximal tubule. Since the TALH is considered water-impermeable, this also indicated a substantial rise in the fluid and electrolyte load to the TALH in response to U37883A. As previously shown, increasing late proximal perfusion rate downstream from an obstructing wax block from $15-30$ nl min^{-1} causes V_{ED} to increase from $10-17$ nl min^{-1} and $[Na^+]_{ED}$ from $31-63$ mM reflecting the

increased load to the water-impermeable TALH (Huang *et al*., 1998). Thus, the increase in fluid and electrolyte load to the TALH, as proposed from the present study in response to *systemic* application of U37883A, could fully account for the observed modest rise in $[Na^+]_{ED}$. The latter indicates that U37883A may not have significantly inhibited Na$^+$ reabsorption in TALH, although a minor contribution can not be fully excluded. Further supporting this notion and confirming previous reports (Romano *et al*., 1995), we found that systemic application of furosemide, the action of which is restricted to inhibiting Na$^+$-2Cl$^-$-K$^+$-cotransport in the water-impermeable TALH, elicited a substantial rise in $[Na^+]_{ED}$ ($39-117$ mM) without altering V_{ED} and therefore the fluid load to TALH. Together, these data proposed that, at least in superficial nephrons, effects on water-permeable segments predominantly contributed to the diuretic and natriuretic effect upstream to the early distal tubule in response to *systemic* application of U37883A.

During *systemic* application of U37883A and free-flow collection from early distal tubular site it was further observed that a dose of 5 mg kg^{-1} elicited a similar response up to early distal tubule as 15 mg kg^{-1} as indicated by almost similar rises in V_{ED} and $[Na^+]_{ED}$. However, the observed diuresis and natriuresis were significantly reduced at 5 mg kg^{-1} of U37883A and a kaliuresis was evident, a response which was not observed with 15 mg kg^{-1}. These findings may indicate that U37883A at 5 mg kg^{-1} was not as efficient as 15 mg kg^{-1} to inhibit K$^+$ secretion in the distal nephron. Because a dose of 5 mg kg^{-1} caused an almost similar increase in fluid and Na$^+$ load to the distal nephron, less efficient inhibition of K$^+$ secretion and thus of Na$^+$ reabsorption in this nephron segment is expected to lower natriuresis and diuresis and elicit kaliuresis.

There is evidence that in the proximal convoluted tubule K$_{ATP}$ channels in the basolateral membrane, which are sensitive to glibenclamide, contribute to fluid and Na$^+$ reabsorption (Tsuchiya *et al*., 1992; Beck *et al*., 1993; Welling, 1995). Whereas the latter evidence was derived from studies in rabbits, in the present study, the observed significant fall in fractional fluid reabsorption upstream to the early distal tubule in response to *systemic* application of U37883A could reflect an inhibition of K$_{ATP}$ channels in the proximal convoluted tubule in rat kidney. Further supporting such a notion, subsequent experiments with *systemic* (5 or 15 mg kg^{-1}) or *intratubular* (50 or 100 μM) application of U37883A revealed a significant inhibition of fluid reabsorption in the proximal convoluted tubule. Because proximal tubule fluid reabsorption takes place by virtually isosmotic Na$^+$ reabsorption, these findings indicated that U37883A inhibited Na$^+$ reabsorption

Table 2 Effect of *intratubular* application of U37883A, glibenclamide, or two metabolites of glibenclamide on early distal flow rate, and sodium and potassium ion concentrations (V_{ED}, $[Na^+]_{ED}$, $[K^+]_{ED}$) during stopped-flow perfusion of Henle's loop in MWF rats

Group	n	V_{ED} (nl min^{-1})		$[Na^+]_{ED}$ (mM)		$[K^+]_{ED}$ (mM)	
		Basal	*EXP*	*Basal*	*EXP*	*Basal*	*EXP*
U37883A 10 μM	6	12.3 ± 0.5	12.5 ± 0.8	37.9 ± 0.9	44.5 ± 2.7	1.9 ± 0.2	1.9 ± 0.3
U37883A 50 μM	5	14.1 ± 1.2	13.2 ± 1.2	40.0 ± 2.5	41.3 ± 2.8	1.6 ± 0.2	1.6 ± 0.2
Vehicle (for groups below)	6	16.6 ± 0.6	16.3 ± 0.5	39.1 ± 3.4	41.4 ± 1.6	1.7 ± 0.1	1.6 ± 0.1
Glibenclamide 250 μM	6	16.5 ± 0.5	15.0 ± 0.6	41.2 ± 4.0	37.8 ± 3.3	1.8 ± 0.2	1.7 ± 0.1
WO39580A 100 μM	5	15.0 ± 0.6	15.1 ± 0.5	39.5 ± 1.2	43.0 ± 2.8	1.6 ± 0.1	1.5 ± 0.1
WO39109B 100 μM	6	15.3 ± 0.4	14.8 ± 0.9	37.3 ± 2.0	44.0 ± 4.8	1.8 ± 0.1	1.9 ± 0.2

Data are mean$\pm$s.e.mean. *n*: numbers of nephrons; Basal, basal measurements; EXP, experimental period (after intratubular application of drug or Vehicle).

in the proximal convoluted tubule. The finding that the inhibition of fluid reabsorption in proximal tubule by U37883A appeared not quite as high with stopped-flow perfusion downstream from an obstructing wax block as compared to free-flow collection is not unexpected, because in the experiments with stopped-flow perfusion the part of the proximal convoluted tubule studied did not include the segment between Bowman space and the first superficial proximal tubular loop, a segment which is known to have a high reabsorptive capacity. Furthermore, the observation that both *systemic* as well as *intratubular* application of U37883A inhibited proximal tubular reabsorption during stopped-flow perfusion indicated that application of the drug to either tubular site, i.e., the luminal or basolateral membrane, can be effective. The latter findings, however, do not necessarily implicate that U37883A exerted actions on both the luminal and basolateral membrane, because for example U37883A after being taken up by the cell across the luminal membrane could affect K$_{ATP}$ channels in the basolateral membrane.

It was previously reported that *intratubular* application of U37883A (50 μM) or glibenclamide (250 μM) inhibited the reabsorption of fluid, Na$^+$, and K$^+$ in the loop of Henle in SD rats (Wang *et al.*, 1995a,b). Although the observed significant inhibitory effect of U37883A or glibenclamide on fluid reabsorption in those studies indicated at least an additional action in water-permeable segments, the authors proposed a primary inhibitory effect of U37883A or glibenclamide on reabsorption in the water-impermeable TALH (Wang *et al.*, 1995a,b). Because the present experiments with *systemic* application of U37883A proposed a predominant effect on the proximal convoluted tubule rather than the TALH, additional studies were performed with *intratubular* application of U37883A. Basically further supporting a predominant effect on the proximal convoluted tubule during *systemic* application, it was observed that *intratubular* application of U37883A (10–100 μM) did not significantly affect the reabsorption of fluid, Na$^+$, or K$^+$ in the loop of Henle.

Similar negative findings were observed for glibenclamide (250 μM) as well as for two of its metabolites which in contrast to glibenclamide can be found in the urine (Heptner *et al.*, 1969). In comparison, as shown previously under similar experimental conditions, *intratubular* application of furosemide substantially reduced Na$^+$ and K$^+$ reabsorption without changing fluid reabsorption in the loop of Henle (Huang *et al.*, 1998), which is in accordance with the effect observed in response to *systemic* application of furosemide in the present study. The different response in loop of Henle reabsorption in the present and the two previous studies appears not to be related to the rat strain used, since we could confirm our negative results obtained in MWF rats on the effect of U37883A on loop of Henle reabsorption in SD rats. Thus, independent of the interpretation of the results of the two previous studies, the reason(s) for the different response in loop of Henle reabsorption to *intratubular* application of U37883A or glibenclamide in the present and the two previous studies remains unclear. Two types of K$_{ATP}$ channels were

identified in the luminal membrane of rat TALH using patch clamp technique (Bleich *et al.*, 1990; Wang, 1994). One of these two channels revealed an intermediate K$^+$ conductance (about 70 pS) and the other a low K$^+$ conductance (about 30 pS). Although both types of K$_{ATP}$ channels are present in the apical membrane of TALH, the intermediate conductance K$^+$ channel appears to functionally dominate and represent about 80% of the apical K$^+$ conductance (Wang & Lu, 1995). Interestingly, neither U37883A nor glibenclamide have been reported to inhibit the intermediate conductance K$_{ATP}$ channel in TALH. Whereas U37883A and glibenclamide were shown to inhibit the low conductance K$_{ATP}$ channel (Wang *et al.*, 1995a,b), the intermediate conductance K$_{ATP}$ channel in TALH proved insensitive to glibenclamide (Wang, 1994). Whether the functional contribution of small and intermediate conductance K$_{ATP}$ channels can be altered in the loop of Henle or TALH under certain conditions, which could account for the different findings with *intratubular* application of U37883A or glibenclamide in the present and previous studies (Wang *et al.*, 1995a,b), remains to be determined.

It was further observed that *systemic* application of U37883A increased SNGFR during fluid collection from the late proximal but not the early distal tubular site. These findings may indicate that U37883A elicited an upward pressure on SNGFR which might be independent from the tubular effect (e.g., renal vasodilation in response to U37883A could be the result of direct inhibition of renin release (Vallon *et al.*, 1998a,b)). The upward pressure on SNGFR of U37883A was balanced out during fluid collection from the early distal tubular site implicating a role of tubuloglomerular feedback which may have been activated as a result of inhibition of proximal tubular reabsorption. The upward pressure became evident and the SNGFR increased, however, when the tubuloglomerular feedback signal was prevented from changing as during fluid collection from the late proximal tubular site.

In summary, the present findings suggest that the diuretic and natriuretic response to *systemic* application of the K$_{ATP}$ channel blocker U37883A involves, in addition to effects on the distal nephron, an inhibition of reabsorption in water-permeable segments upstream to the distal tubule such as the proximal convoluted tubule, where a role in tubular reabsorption for K$_{ATP}$ channels has been implicated in the basolateral membrane. Furthermore, evidence is provided that *systemic* application of U37883A exerted an upward pressure on GFR by an yet unknown mechanism, the influence of which, however, appeared to be balanced by the tubular effects of U37883A, such that GFR remained unchanged.

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