Vasodilatation of afferent arterioles and paradoxical increase of renal vascular resistance by furosemide in mice

Mona Oppermann,1 Pernille B. Hansen,2 Hayo Castrop,3 and Jurgen Schnermann1

1National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland; 2Department of Physiology and Pharmacology, University of Southern Denmark, Odense, Denmark; and 3Institute of Physiology, University of Regensburg, Regensburg, Germany

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Oppermann M, Hansen PB, Castrop H, Schnermann J. Vasodilatation of afferent arterioles and paradoxical increase of renal vascular resistance by furosemide in mice. Am J Physiol Renal Physiol 293: F279–F287, 2007. First published May 9, 2007; doi:10.1152/ajprenal.00073.2007.—Loop diuretics like furosemide have been shown to cause renal vasodilatation in dogs and humans, an effect thought to result from both a direct vascular dilator effect and from inhibition of tubuloglomerular feedback. In isolated perfused afferent arterioles preconstricted with angiotensin II or $N^\omega$-nitro-$L$-arginine methyl ester, furosemide caused a dose-dependent increase of vascular diameter, but it was without effect in vessels from NKCC1−/− mice suggesting that inhibition of NKCC1 mediates dilatation in afferent arterioles. In the intact kidney, however, furosemide (2 mg/kg iv) caused a 50 ± 3% reduction of total renal blood flow (RBF) and a 27% reduction of superficial blood flow (SBF) accompanied by a marked and immediate increase of tubular pressure and volume. At 10 mg/kg, furosemide reduced RBF by 60.4 ± 2%. Similarly, NKCC1−/− mice responded to furosemide with a 45.4% decrease of RBF and a 29% decrease of SBF. Decreases in RBF and SBF and increases of tubular pressure by furosemide were ameliorated by renal decapsulation. In addition, pretreatment with candesartan (2 mg/kg) or indomethacin (5 mg/kg) attenuated the reduction of RBF and peak urine flows caused by furosemide. Our data indicate that furosemide, despite its direct vasodilator potential in isolated afferent arterioles, causes a marked increase in flow resistance of the vascular bed of the intact mouse kidney. We suggest that generation of angiotensin II and/or a vasoconstrictor prostaglandin combined with compression of peritubular capillaries by the expanding tubular compartment are responsible for the reduction of RBF in vivo.

renal blood flow; superficial blood flow; candesartan; decapsulation; tubular pressure; NKCC1 knockout

LOOP DIURETICS LIKE FUROSEMIDE or bumetanide are widely used antihypertensive and diuretic drugs. Diuresis and loss of extracellular fluid result from an inhibitory interaction of the diuretics with the Na-K-2Cl cotransporter NKCC2, the major NaCl uptake pathway across the apical membrane of the thick ascending limb (TAL). In addition, loop diuretics also inhibit NKCC1, the second isoform of the Na-K-2Cl cotransporter that is more widely expressed than NKCC2 (11). In polarized cells, NKCC1 is typically located in the basolateral membrane, and NKCC1-mediated NaCl uptake therefore is an early step in transepithelial NaCl secretion. In nonpolarized cells, NKCC1 is believed to play a role in cell volume regulation and maintenance of membrane potential. NKCC1 is widely expressed in the vasculature, and inhibition of this transporter may account for direct vascular effects of loop diuretics that appear to be vasodilatory in most vascular beds (8). The interaction between the diuretics and the two different transporter isoforms occurs with similar affinity (12). The apparently preferential renal effect is presumably due to the fact that the diuretics are secreted into the tubular lumen and concentrated so that higher concentrations are reached in the tubular lumen than in the plasma.

The well-described actions of NKCC cotransporters would suggest that the hemodynamic effect of loop diuretics in the kidney should consist of vasodilatation. In addition to a possible direct renal vascular dilator effect of NKCC1 inhibition, a reduction of renal vascular resistance should result from the removal of the tonic constrictor effect exerted by the tubuloglomerular feedback (TGF) mechanism (40). Nevertheless, the experimental data on the renal vascular effects of loop diuretics are conflicting and difficult to reconcile, particularly when comparing results from different species. The expected increase of renal blood flow (RBF) has in fact been observed in several studies in humans (34) and dogs (10, 26, 30). In contrast, in the majority of studies in the rat, furosemide has been found to cause an ∼10–20% reduction of RBF (6, 15, 31, 32, 35, 44), although a hemodynamically neutral outcome has also been reported (17, 41). When they occur, the renal hemodynamic effects of NKCC inhibition in the rat appear to be unrelated to the primary actions of the diuretic on tubular and vascular functions. Rather, they may be dominated by secondary effects on RBF that are activated by the diuretic and may be related to the size of the kidneys. In addition, loop diuretics are known to rapidly stimulate renin secretion (3), and to stimulate prostaglandin formation so that differences in the response of these regulatory systems to the diuretic could cause species-specific responses (38).

The present studies were performed in mice to assess the net effect of furosemide on total renal and superficial renal vascular resistance in a species that is closer in size to rats than it is to dogs or humans, and to compare the global effect of furosemide with that observed in isolated renal resistance vessels. Our studies show that furosemide causes a robust, highly reproducible, and dose-dependent increase of renal vascular resistance in wild-type mice that is qualitatively similar to, but quantitatively more pronounced, than that previously observed in rats (6, 15, 31). Elevation of renal vascular resistance occurred despite the fact that the loop diuretic elicits direct vasodilatation of afferent arterioles through inhibition of

Address for reprint requests and other correspondence: J. Schnermann, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bldg. 10, Rm. 4D51, 10 Center Drive-MSC 1370, Bethesda, MD 20892 (e-mail: jurgens@intra.niddk.nih.gov).

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NKCC1. Our data indicate that the mechanisms responsible for the resistance increase in vivo are probably multifactorial. Since angiotensin II receptor blockade and cyclooxygenase inhibition diminished the blood flow reduction caused by furosemide, direct or indirect effects of angiotensin and of a vasoconstrictor prostaglandin may play a role. In addition, removal of the kidney capsule attenuated the furosemide effect indicating that compression of peritubular capillaries by the expanding tubular compartment may contribute to the reduction in blood flow, an effect that may be more pronounced in the smaller kidney of the mouse.

METHODS

Animals. Male wild-type mice used in these studies had a mixed 129J/C57BL6 genetic background. Male NKCC1+/+ and NKCC1−/− mice from a subcolony of the original strain generated by Flagella et al. (9) were generously supplied by Dr. S. Wall from Emory University. All mice were kept on standard rodent chow and tap water. Animal care and experimentation were approved and carried out in accordance with National Institutes of Health principles as outlined in their Guide for the Care and Use of Laboratory Animals.

Animal preparation. Mice were anesthetized with 100 mg/kg thiobutabarbital (inactin) intraperitoneally and 100 mg/kg ketamine subcutaneously. Body temperature was maintained at 38.0°C by placing the animals on an operating table with a servo-controlled heating plate. The trachea was cannulated, and a stream of 100% oxygen was blown toward the tracheal tube throughout the experiment. The left femoral artery was catheterized with hand-drawn polyethylene tubing for continuous measurement of arterial blood pressure. A catheter was also inserted into the right jugular vein for an intravenous maintenance infusion of saline at a rate of 12 μl·g body wt⁻¹·h⁻¹. The bladder was catheterized for urine collections.

Measurements of total RBF and superficial renal blood flow. For measurements of total RBF, the right renal artery was approached from a flank incision and carefully dissected free to permit placement of an ultrasonic flow probe (0.5PSB nanoprobe) connected to a T402 perivascular flowmeter module (Transonic Systems, Ithaca, NY). Superficial renal blood flow (SBF) was measured in the same mice simultaneously using a real-time laser Doppler perfusion probe (418-2 Master Probe) with a straight microtip (B500). The probe was placed on the renal surface and connected to a PF 5010 LDPM flowmeter Unit/Periflux System 5000 (Perimed AB, Jarfalla, Sweden). Measurements of SBF are expressed as arbitrary perfusion units (PU). Both probes were held in place with micromanipulators. RBF and SBF signals were digitized and analyzed using PowerLab software (ADInstruments, Colorado Springs, CO). Mean arterial blood pressure was determined after 1, 3, 5, and 10 min. In another set of experiments, the involvement of nitric oxide (NO) in the furosemide-mediated vasodilation was investigated. The arterioles were exposed to the NO synthase (NOS) antagonist Nω-nitro-L-arginine methyl ester (L-NAME; 50 μM; Sigma) for 20 min before furosemide (1.5 mmol/l) was added to the bath in the continued presence of l-NAME. The ability of 100 mM K⁺ to elicit constriction was tested after all experiments.

Statistical analysis. Unpaired t-test was used to compare two values between different groups. Integration as the area under the curve over the 30-min time interval was conducted using GraphPad Prism. Multiple groups were analyzed with ANOVA followed by Bonferroni posttest. A P value <0.05 was considered significant.

RESULTS

Effect of furosemide on total and superficial blood flow. As shown in an exemplary recording in Fig. 1, an intravenous injection of furosemide (2 mg/kg body wt) caused an acute reduction of total (RBF) and superficial RBF in anesthetized mice without a concomitant reduction of blood pressure. On average, RBF decreased from a baseline of 1.67 ± 0.10 to 1.25 ± 0.11 ml/min within 1 min and reaching a minimum of 0.83 ± 0.07 ml/min after 10 min (Fig. 2), a decrease of 50.5 ± 2.8% from the baseline value (n = 8). Similarly, SBF decreased from a baseline of 340 ± 15 PU to a minimum of 250 ± 18 PU after 15 min, a decrease of 27.0 ± 2.8% (n = 6). Arterial blood pressure tended to increase slightly after furosemide injection, but at no time point was there a significant elevation vs. baseline when tested by repeated-measures ANOVA. At times above 10 min, arterial pressure fell in all study groups. On average, there was a maximal increase in RVR of 123.3 ± 11.6% and a maximal increase of SVR of 52.4 ± 6.2% (Table 1). Furosemide increased urinary flow from 1.41 ± 0.14 μl/min to a maximum of 25.6 ± 2.3 μl/min after 20 min. Blood pressure started to decrease after 20 min coincident with the peak in urine flow (Fig. 2).

In five mice we tested the effect of 10 mg/kg furosemide (Fig. 2). RBF fell from 1.67 ± 0.25 to 0.69 ± 0.14 ml/min at 5 min, a fall of 60.4 ± 2% (P < 0.05 vs. 2 mg/kg furosemide). Arterial blood pressure before and 5 min after furosemide was 93.5 ± 3 and 92.2 ± 4.3 mmHg. Furosemide-induced urine flow was 645 ± 23 μl/30 min, significantly higher than the mean value of 398 ± 40 μl/30 min caused by 2 mg/kg (P < 0.01).

Candesartan. To assess a possible role of angiotensin II in the furosemide-induced reduction of RBF, mice received an intravenous injection of the AT1 receptor antagonist candesartan (2 mg/kg). After a stabilization period of 20 min, the effect
of furosemide on RBF was determined. Candesartan increased RBF from 1.38 ± 0.05 to 1.88 ± 0.05 ml/min (+37.4 ± 4.8%), and SBF from 364 ± 21 to 408 ± 23 PU (+12.6 ± 2.9%), while arterial pressure fell by 22.8 ± 2.4% from 93.0 ± 4.0 to 71.9 ± 4.1 mmHg (n = 9, P < 0.001 each). Subsequent furosemide administration decreased both superficial and total renal blood flow, but the relative reductions were significantly diminished in the presence of candesartan compared with control (Fig. 3). The change of RVR caused by furosemide, expressed as percent change integrated over time (area under the curve, %change × time), averaged 2.563 ± 336 while the effect was reduced to 1.041 ± 186 by candesartan treatment (P < 0.001; Fig. 4). While the difference in total urine volume excreted during the course of the experiment (30 min) did not reach significance between vehicle- and candesartan-treated mice (398 ± 40 vs. 286 ± 29 μl; n = 8 vs. n = 5; P = 0.073), peak urine flow was significantly reduced after candesartan probably as a result of the blood pressure reduction (15.8 ± 1.9 vs. 25.6 ± 2.3 μl/min; P < 0.01). The efficiency of AT1 receptor blockade was verified at the end of each experiment by determining the blood pressure response to a bolus injection of angiotensin II (data not shown).

Indomethacin. Since furosemide is known to stimulate prostaglandin production, we tested the effect of furosemide following the administration of the nonspecific cyclooxygenase (COX) inhibitor indomethacin (5 mg/kg). Furosemide reduced RBF in indomethacin-treated mice from 1.2 ± 0.17 ml/min to a nadir of 0.78 ± 0.11 ml/min (P < 0.01; Fig. 3), a maximum decrease by 35.2 ± 0.3%. Arterial blood pressure of 92 ± 9 mmHg was not significantly altered by furosemide. The time-
integrated relative reductions of RBF and the increase in RVR were significantly less than in control mice (605 ± 82 vs. 2.563 ± 336; P < 0.001; Fig. 4). Peak urine flow achieved by furosemide in indomethacin-treated mice was significantly reduced compared with control animals (15.7 ± 4.3 vs. 25.6 ± 2.3 μl/min; P < 0.05).

Decapsulation. To determine whether kidney hypoperfusion after furosemide may be related to changes in tissue hydrostatic pressure resulting from increased urine flow, RBF in response to furosemide was determined after removal of the kidney capsule (decapsulation) with and without concomitant AT1 receptor blockade. Removal of the kidney capsule did not change baseline RBF (1.54 ± 0.07 vs. 1.61 ± 0.09 ml/min, P = 0.11) or arterial blood pressure (83 ± 2.7 vs. 82 ± 2.9 mmHg, P = 0.32). However, SBF increased slightly (393 ± 31 vs. 458 ± 29 PU, P = 0.011, n = 6 each). After decapsulation furosemide reduced RBF and SBF by 36.9 ± 3.3 and 26.3 ± 5.1% after 4 min (n = 5), respectively. Application of candesartan following decapsulation further attenuated the effect of furosemide on RBF (RBF decrease by 21.9 ± 4.0% and SBF decrease by 15.9 ± 5.0% after 10 min, n = 4) with the furosemide-dependent decrease in RBF being significantly less compared with decapsulation alone. The time-integrated increase of RVR was reduced to 923 ± 173 by decapsulation and to 358 ± 42 by decapsulation and candesartan (Fig. 4). Differences in total urine excretion over 30 min did not reach significance levels of 5% (decapsulation: 368 ± 42 μl, P = 0.63 vs. WT; decapsulation + candesartan: 279 ± 53 μl, P = 0.11 vs. WT, P = 0.22 vs. decapsulation).

Free-flow proximal tubular pressure. Following furosemide free-flow proximal tubular pressure (P_{\text{FF}}) increased from 16.3 ± 0.8 mmHg (n = 15) to 40.0 ± 4.3 mmHg (n = 8; Fig. 5) within 1–2 min, a total increase of 146% (P < 0.001) accompanied by a macroscopic swelling of the kidney. Removal of the kidney capsule resulted in a significant decrease in P_{\text{FF}} to 11.7 ± 0.8 mmHg (n = 14, P < 0.001 vs. control), and a reduced P_{\text{FF}} increase following furosemide injection to 29.0 ± 2.8 mmHg (+149%, n = 10, P < 0.001). During the

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<td>Control (n = 6–8)</td>
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<td>Candesartan (n = 5)</td>
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<td>Indomethacin (n = 4)</td>
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<td>Decapsulation (n = 5)</td>
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*P < 0.05, †P < 0.01 vs. control.

Maximum (max.) percent changes (means ± SE) at corresponding time points (means ± SE) in total renal vascular resistance (RVR) and superficial vascular resistance (SVR) after 2 mg/kg iv furosemide (control), after pretreatment with the AT1-receptor antagonist candesartan (2 mg/kg iv), after removal of the kidney capsule (decapsulation), or after 5 mg/kg indomethacin are shown. Significant differences are indicated by asterisks (*P < 0.05, †P < 0.01 vs. control). The time-integrated increases of RVR and SVR were: Control: 1.4 ± 0.4% (n = 5), Candesartan: 2.0 ± 0.6* (n = 5), Decapsulation: 2.7 ± 0.4 (n = 5), Candesartan + decapsulation: 0.7 ± 0.4 (n = 5), Indomethacin: 0.7 ± 0.4 (n = 5).

Fig. 3. Time course of total RBF (A), SBF (B), MAP (C), and urine flow (D) after intravenous administration of 2 mg/kg furosemide in wild-type mice (control, n = 6–8), following intravenous pretreatment with candesartan (2 mg/kg, n = 5) or indomethacin (5 mg/kg, n = 4), pretreatment by removal of the kidney capsule (decapsulation, n = 5) or both (decapsulation + candesartan, n = 4). Initial RBF or SBF values at time 0 were not significantly different from control in any of the experimental groups when tested by 1-way ANOVA and Bonferroni post hoc test.
course of these experiments, arterial blood pressure dropped significantly in both groups (control: 91.4 ± 1.9 to 76.5 ± 5.6 mmHg after 21–25 min, P < 0.01; decapsulation: 86.3 ± 1.9 to 78.8 ± 1.0 mmHg, after 16–20 min, P < 0.01). Free-flow proximal tubular pressures in both groups returned to baseline after 26–30 min (Fig. 5).

NKCC1-deficient mice. To explore whether the effect of furosemide on RBF in vivo is modified by inhibition of NKCC1, we examined the hemodynamic effect of furosemide in NKCC1-deficient mice (n = 5). Like in wild-type controls, RBF and SBF decreased rapidly in NKCC1−/− mice with maximum decreases after 5 min averaging 45.4 ± 4.6% (from 1.37 ± 0.12 to 0.76 ± 0.09 ml/min) for RBF (P = 0.32) and 28.8 ± 3.7% (from 347 ± 11 to 246 ± 14 PU) for SBF (P = 0.51, n = 6) as shown in Fig. 6. No differences between genotypes were found in arterial blood pressure, total urine excretion after 30 min (NKCC1+/+: 389 ± 50 μl, NKCC1−/−: 370 ± 42 μl, P = 0.78), or the relative changes and duration of the observed blood flow decrease after furosemide injection.

Isolated perfused afferent arterioles. To assess the direct vascular effects of furosemide, we determined the response of isolated perfused afferent arterioles to bath application of the drug. Experiments were performed in arterioles preconstricted with 0.5 nM angiotensin II in the bath solution causing a vessel diameter reduction from 8.8 ± 0.6, 5.9 ± 0.5, and 7.8 ± 0.6 for 30 μmol/l, 300 μmol/l, and 1.5 mmol/l of furosemide, respectively, (n = 11). Figure 7A shows representative photographs of the furosemide-mediated dilatation in a mouse-perfused arteriole. Vasodilatation was not the result of loss of angiotensin-mediated constriction since time control experiments showed that vessel constriction caused by angiotensin without furosemide was maintained throughout a 10-min application period with no significant changes in diameter (5.8 ± 0.7, 5.5 ± 0.5, and 5.5 ± 0.6 μm, 3, 5, and 10 min, respectively). To verify the role of NKCC1 in afferent arteriolar vasodilatation, experiments were repeated in vessels dissected from kidneys of NKCC1-deficient mice. Like in vessels from wild-type mice, angiotensin II (0.5 nM) reduced the diameter of afferent arterioles (from 9.4 ± 0.5 to 5.1 ± 0.4 μm), but in contrast to wild-type arterioles, addition of furosemide to the bath at increasing concentrations (30 μM, 300 μM, 1.5 mM) did not alter afferent arteriole diameter of afferent arterioles of NKCC1−/− mice (5.1 ± 0.5, 4.9 ± 0.5, and 5.9 ± 0.4 μm at 30 μmol/l, 300 μmol/l, and 1.5 mmol/l, respectively, n = 7; Fig. 7A).

To address the question whether the vasodilatory effect of furosemide is dependent on the mode of preconstriction, we...
tested in an additional set of experiments the effect of furosemide after inhibition of NOS activity by L-NAME. L-NAME application to afferent arterioles of wild-type mice decreased the diameter of the specimen \((n = 4)\) from \(8.5 \pm 0.6\) to \(5.5 \pm 0.6\) \(\mu m\) \((P < 0.05)\). Subsequent administration of furosemide induced a significant increase in the luminal diameter of the vessel averaging \(8.6 \pm 1.0\) \(\mu m\) \((+58.7 \pm 20.4\%)\).

Fig. 6. Time course of total RBF \((A)\), SBF \((B)\), MAP \((C)\), and urine flow \((D)\) after intravenous administration of 2 mg/kg furosemide in NKCC1+/+ \((n = 5)\) and NKCC1−/− \((n = 6)\) mice.

Fig. 7. \(A\): effect of furosemide on inner luminal diameter of isolated perfused afferent arterioles from NKCC1+/+ \((n = 11)\) and NKCC1−/− \((n = 7)\) mice. Arterioles were preconstricted with angiotensin II. *\(P < 0.05\) vs. basal. \#\(P < 0.05\) vs. angiotensin II. \(B\): images of a perfused afferent arteriole from a NKCC1 wild-type mouse under resting conditions (Basal) and during bath addition of angiotensin II \((0.5\) nM\) and furosemide \((30\) \(\mu M\), \(300\) \(\mu M\)). \(C\): effect of 1.5 mM furosemide on inner vascular diameter of perfused afferent arterioles from NKCC1+/+ mice \((n = 4)\) after preconstriction with 50 \(\mu M\) \(N^3\)-nitro-L-arginine methyl ester (L-NAME). *\(P < 0.05\) vs. basal.
DISCUSSION

Despite numerous previous investigations, the effects of loop diuretics on RVR have remained an issue of contention. In view of the clinical importance of this class of drugs and their use as an experimental tool, it would be desirable to arrive at a better understanding of the principles that may underlie the response of the renal vasculature to loop diuretics and to an increase in urine flow in general. The present study was therefore performed in mice to reinvestigate the effect of the loop diuretic furosemide on RVR. Our data show that furosemide caused a vasodilatation of isolated perfused afferent arterioles resulting from inhibition of NKCC1. Despite the vasodilator potential of furosemide in vitro, we found a robust and dose-dependent reduction in total and superficial renal blood flow in the in situ kidney that was fast in onset and occurred well before any decrements in mean arterial blood pressure were observed. The in vivo studies are in qualitative agreement with several previous results in rats that have shown a consistent increase in RVR in response to furosemide (6, 15, 31). There is evidence that furosemide causes a preferential reduction of medullary blood flow by \( \sim 30\% \) while cortical blood flow fell by only \( \sim 10\% \) (6, 7). The present data in the mouse are in agreement with the possibility of a more pronounced reduction of medullary blood flow since the relative change of total RBF was markedly greater than that of the superficial cortex. Nevertheless, the magnitude of the reduction of total RBF indicates that furosemide elevates vascular resistance throughout the entire vascular bed of the mouse kidney. We acknowledge that for unknown reasons, the observation of an RBF reducing effect of furosemide has not been made in all rat studies (17, 41). Overall, our and previous observations suggest a species-specific response of RBF to furosemide, with renal resistance increases being greater in mice than in rats and absent in humans and dogs.

The markedly different hemodynamic responses to loop diuretics in humans and dogs on the one hand and rats and mice on the other are unlikely to be related to differences in the inhibitory interactions of the diuretic with NKCC cotransporters. There are no discernible differences in the efficacy of loop diuretics to inhibit NKCC2 as judged from comparable diuretic potencies of these agents across species. Furthermore, loop diuretics have been shown to cause inhibition of TGF in both dogs and rats (25, 40). Thus it is highly unlikely that the different hemodynamic responses to furosemide in these species reflect a difference in diuretic-induced TGF inhibition and vasodilatation. Finally, direct vasodilator actions of loop diuretics have previously been observed in various blood vessels of a number of species including humans, dogs, rabbits, and rats (8). The current studies confirm that furosemide can cause relaxation of isolated perfused afferent arterioles of the mouse preconstricted with angiotensin II. Like in other studies of the in vitro effect of furosemide on vascular tone, relatively high concentrations of furosemide were required to observe dilator effects (for reference, see Ref. 8). The causes for the relative insensitivity of excised vessels to furosemide are unclear but may reflect changes in basal variables such as the membrane potential or absence of vasoactive cofactors. Since a dilator effect was not seen in arterioles dissected from NKCC1-deficient mice, relaxation appears to be due to inhibition of the widely expressed NKCC1 isofrom of the cotransporter. This direct demonstration of a role for NKCC1 in vascular effects confirms earlier notions based on isoform expression patterns that the vascular actions of loop diuretics are caused by NKCC1 inhibition (1). Nevertheless, a direct NKCC1-dependent vasodilatation is unlikely to modify the in vivo response of the renal vasculature to furosemide to a significant extent since the increase in RVR was not augmented in NKCC1-deficient compared with wild-type mice.

The mechanism by which NKCC1 inhibition leads to a vasodilatation of afferent arterioles was not addressed in the present study. However, recent patch-clamp studies in juxtaglomerular cells of the afferent arteriole have shown that furosemide induced an increase in an outward current that was carried by potassium and caused marked cell hyperpolarization (3). Alternatively or additionally, a decreased intracellular Cl\(^-\) concentration after inhibition of NKCC1-dependent chloride transport by furosemide might either inhibit voltage-dependent calcium channels directly (27) or indirectly by driving HCO\(_3^-\) out of the cell via the Cl\(^-\)/HCO\(_3^-\) exchanger (42). In either case, the result would be a reduction in cytosolic Ca\(^{2+}\) in smooth muscle cells of the afferent arteriole leading to a decrease in vascular tone. Vascular dilatation by furosemide was also suggested to be in part mediated by endothelium-dependent generation of NO (33). For the afferent arteriole of the mouse, however, this mechanism does not seem to be of major relevance, since vessels preconstricted by application of the NOS inhibitor \( L\)-NAME showed an unaltered dilatory response upon addition of furosemide. This is in agreement with the observation that maximal inhibition of the myogenic response by the diuretic bumetanide was independent of NOS blockade by \( L\)-NAME, although the time course of the vasodilatation was changed (37).

Administration of loop diuretics leads to a rapid increase in renin secretion, an effect that is at least in part mediated by inhibition of NKCC2 in the macula densa segment of the TAL (3). Our experiments using the AT1 receptor antagonist candesartan suggest that angiotensin-dependent vasoconstriction may contribute to the fall in RBF observed after furosemide administration since a dilator effect of the AT1 receptor antagonist candesartan suggests that angiotensin-dependent vasoconstriction may contribute to the fall in RBF observed after furosemide administration.

![Figure 8](http://ajprenal.physiology.org/)

**Fig. 8.** Relationship between furosemide-induced urine flow (\( \mu \)L/30 min) and the relative time-integrated change of renal blood flow (% change \( \times \) 30 min) for different experimental conditions studied. The line indicates the linear regression function.
infusion, a finding which is in agreement with previous studies in the rat (15). This mechanism would be consistent with the dilator action of furosemide in vitro in that angiotensin-mediated resistance changes may not be detectable in the in vitro-perfused arteriole since the generation of angiotensin II and the exposure of the vessel to sufficiently high angiotensin concentrations are unlikely to occur in this setting. Since infusion of angiotensin II in control experiments suggested a rather complete blockade of angiotensin receptors by candesartan, additional mechanisms must contribute to the decrease of RBF after furosemide infusion.

Numerous studies have confirmed the original observation that stimulation of prostaglandin production is another consequence of furosemide administration (38). This effect is at least in part the direct result of inhibition of NKCC2 in TAL cells and subsequent stimulation of COX-2 activity (4, 23). Our observations indicate that the increase in RVR caused by furosemide is diminished following indomethacin. This finding suggests that furosemide may lead to the release of a vasoconstrictor prostaglandin, perhaps as a consequence of the increased pelvic pressure associated with the increased urine production (21, 24). It is possible that the magnified decrease of RBF in the mouse is caused by the direct effect of a more vasoconstrictor PG spectrum, although the effect of furosemide on urinary prostaglandin excretion appears to be a generalized activation of prostaglandin synthesis across different species (5, 18).

In view of absence of discernible species-specific differences in the response of the renin and prostaglandin systems to furosemide, differential vascular responses to these agents may not be an entirely satisfying explanation for the divergent hemodynamic effects in different species. An analysis of our data shows that there is a good correlation between furosemide-induced urine flow and the reduction of RBF (Fig. 8). Although we are aware of the limitations of such correlations, one may interpret this finding as indicating an influence of urine flow per se on RBF. The underlying mechanism could be a compression of peritubular capillaries by the abrupt rise in tubular pressure. Several arguments could be made in favor of such a scenario. First, this mechanism would be effective independent of any direct or indirect vasomotor responses of vascular smooth muscle cells and would therefore be compatible with loop diuretics being vasodilator in nature. Second, the blood flow reduction caused by furosemide was significantly less after decapsulation, and this was associated with a lesser increase of proximal tubular pressure. Third, the precipitous early fall of RBF coincides with the prompt rise in tubular pressure suggesting that the abrupt addition of nonabsorbed tubular fluid cannot be accommodated by the major urinary flow resistance in the collecting ducts in the early phase consistent with the observation that the rise of tubule pressure precedes the onset of a marked diuresis by several minutes. Fourth, compression of peritubular capillaries following furosemide has recently been directly observed by two-photon fluorescence microscopy of the kidney surface of rats (19). Fifth, the effect of increased tubular pressure to compress peritubular capillaries may well be a function of the size of the kidney and may therefore represent the factor that differs between mice and larger species. It is noteworthy that the number of nephrons per unit kidney weight is four to five times higher in mice than it is in humans. It would therefore seem possible that an expansion of swollen tubules into the interstitium without encroaching on capillary space may occur in larger kidneys, but may not be possible in the more tightly packed rat and mouse kidneys. In fact, furosemide has been found to increase proximal tubule pressure in dogs, but this was not accompanied by an increase in interstitial pressure, and therefore probably not with capillary compression (20). It is also possible that the urinary flow resistance is lower in humans and dogs compared with rats or mice and that this would permit an increase in urine flow without increasing proximal pressure and volumes to a similar extent. This is somewhat supported by the greater increase in proximal tubule pressure in our study compared with an earlier observation in dogs (20). Sixth, a remarkable vulnerability of the renal papilla has been observed in numerous mouse models in which urine flow is chronically elevated regardless of the cause of the diuresis (22, 29, 36, 43). This papillary and medullary atrophy appears to be a distinct feature of the mouse since Brattleboro rats do not show a comparable phenotype (2), and it may be the end result of a diuresis-induced impairment of the renal circulation that is specific for the mouse kidney.

In summary, we found marked reductions of total and superficial renal blood flow after furosemide administration in anesthetized mice despite the fact that furosemide caused vasodilatation of microperfused preconstricted arterioles through inhibition of NKCC1. The reduced RBF-lowering effect of furosemide by candesartan or indomethacin treatment indicates that the generation of angiotensin II and of a vasoconstrictor prostaglandin may contribute to the increased RVR caused by the diuretic. In addition, compression of peritubular capillaries by the expanding tubular compartment may contribute to the impairment of blood flow by furosemide.

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