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Everything we always wanted to know about furosemide but were afraid to ask

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Huang X, Dorhout Mees E, Vos P, Hamza S, Braam B. Everything we always wanted to know about furosemide but were afraid to ask. Am J Physiol Renal Physiol 310: F958–F971, 2016. First published February 24, 2016; doi:10.1152/ajprenal.00476.2015.—Furosemide is a widely used, potent natriuretic drug, which inhibits the Na⁺-K⁺-2Cl⁻ cotransporter (NKCC)-2 in the ascending limb of the loop of Henle applied to reduce extracellular fluid volume expansion in heart and kidney disease. Undesirable consequences of furosemide, such as worsening of kidney function and unpredictable effects on sodium balance, led to this critical evaluation of how inhibition of NKCC affects renal and cardiovascular physiology. This evaluation reveals important knowledge gaps, involving furosemide as a drug, the function of NKCC2 (and NKCC1), and renal and systemic indirect effects of NKCC inhibition. Regarding renal effects, renal blood flow and glomerular filtration rate could become compromised by activation of tubuloglomerular feedback or by renin release, particularly if renal function is already compromised. Modulation of the intrarenal renin angiotensin system, however, is ill-defined. Regarding systemic effects, vasodilation followed by nonspecific NKCC inhibition and changes in venous compliance are not well understood. Repetitive administration of furosemide induces short-term (braking phenomenon, acute diuretic resistance) and long-term (chronic diuretic resistance) adaptations, of which the mechanisms are not well known. Modulation of NKCC2 expression and activity in kidney and heart failure is ill-defined. Lastly, furosemide’s effects on cutaneous sodium stores and on uric acid levels could be beneficial or detrimental. Concluding, a considerable knowledge gap is identified regarding a potent drug with a relatively specific renal target, NKCC2, and their actions and improve rational use of furosemide in pathophysiology of fluid volume expansion.

extracellular fluid volume; natriuresis; renal function; chronic kidney disease; heart failure

DIURETICS BLOCKING THE Na⁺-K⁺-2Cl⁻ cotransporter (NKCC) have an important place in the treatment of fluid overload, specifically in the context of kidney disease and heart failure (64). Of the drugs that inhibit the NKCC2 in the loop of Henle (furosemide, bumetanide, torsemide, ethacrynic acid), furosemide is most commonly applied (66) and >40 million prescriptions are dispensed every year in the USA (66a). However, many uncertainties remain about intrarenal and systemic actions of furosemide, including its two main targets NKCC1 and NKCC2.

Clinically, there are a number of undesirable consequences of the use of furosemide, of which the pathophysiological mechanisms have not been sufficiently investigated. Furosemide has been associated with worsening of kidney function in patients treated for volume overload admitted for acute heart failure (104) and even glomerular filtration rate (GFR) responses to furosemide in healthy subjects are variable (5, 13, 14, 31, 42, 51, 71, 91, 100, 102, 115, 120, 121, 133, 147, 158, 169). The very strong and short actions of furosemide have been associated with rebound sodium retention, and it has been brought to question whether furosemide would allow the reaching of a new steady state. Furthermore, knowledge about interactions between factors affecting loop of Henle transport and macula densa sensing of chloride is incomplete. This is important in relation to the function of the tubuloglomerular feedback (TGF) mechanism (72, 167) and to regula-
tion of renin release (81). Altogether, this makes the impact of furosemide on natriuresis, on volume status, and on renal hemodynamic function unpredictable. Scientifically, these and other observations teach us that NKCC inhibition has substantially more consequences than merely inhibiting loop of Henle sodium and potassium reabsorption.

What makes furosemide an interesting scientific tool is that it has a specific target and impacts multiple aspects of integrative physiology. This paper reviews the actions of furosemide, focuses on unanswered questions, and provides areas where more scientific information could lead to a better understanding of targeting NKCC with furosemide. This eventually could lead to a better knowledge of NKCC transporters and of extracellular volume control. Understanding the entire profile of actions of the inhibition of NKCC better would eventually make furosemide a more effective clinical tool.

**Pharmacology, Pharmacodynamics, Pharmacokinetics of Furosemide**

Furosemide, 4-chloro-N-(2-furyl-methyl)-5-sulfamoylanthranilic acid (94), is a 330.7 mol wt member of the sulfonamides, which inhibits the NKCC in the thick ascending limb of the loop of Henle (114). Although insoluble in water, it remains stable in gastric and duodenal juice, bile, and urine (11). Furosemide strongly binds to plasma proteins (91–99%) (34), particularly to anionic sites on albumin (20). Most intestinal furosemide absorption occurs in the stomach and small intestine (30, 58, 160). Although the mean availability of oral furosemide is ~60%, absorption ranges from 10–100%. This is due to differences in product formulation, gastric pH and emptying, to timing of dosing in relation to food ingestion, and to disease conditions, like congestive heart failure (59). Renal actions peak within 1 h after oral and within 5 min after intravenous administration. There is substantial interindividual variability in bioavailability. The half-time (T½) of furosemide ranges from 0.5–2 h (160), but can be prolonged in renal failure. The duration of natriuretic effect is supposedly ~6 h after oral administration (lasts Six) and ~2 h after single-dose intravenous administration (165), yet can vary substantially. Of note, the tubular concentration of furosemide determines its natriuretic effect, and the urine concentration of furosemide has been used as a surrogate for the tubular concentration (165). Furosemide mainly enters the proximal tubular lumen via secretion by the organic anion transporter-1 (OAT1), while glomerular filtration is limited due to the high protein binding (164). Approximately 65% of furosemide is excreted unchanged in urine (11). Furosemide is also metabolized by renal and hepatic glucuronidation and subsequent secretion in urine and in bile (82, 126). The renal glucuronidation by renal UDP-glucuronosyltransferases UGT1A9 and UGT1A1 and clearance has been suggested to be most important (82). Furosemide directly increases urinary Na⁺, K⁺, and Cl⁻ excretion. Furosemide increases kaliuresis indirectly by promoting K⁺ secretion by increased distal tubular fluid flow (99). Distal Ca²⁺ reabsorption is facilitated by the reduced luminal charge created by diminished sodium reabsorption and potassium recycling, effectively transforming NKCC into an electronegic NaCl transporter (92). Furosemide can inhibit proximal tubular carbonic anhydrase (CA), leading to increased urinary excretion of HCO₃⁻ and phosphate (26).

**Furosemide Targets the NKCC Transporters, CA, and GABA Receptors**

The gene encoding for the NKCC has two isoforms, NKCC1 and NKCC2. The NKCC1 isoform is present in a wide variety of tissues, including the basolateral membrane of secretory epithelia, smooth muscle cells, fibroblasts, and red blood cells (55). Interestingly, in vascular endothelial cells, an increase in extracellular tonicity as small as 10 mOsm can cause significant stimulation of NKCC activity, which in turn increases cell volume (113). In contrast, the NKCC2 isoform is exclusively localized in the kidney, where it resides in the luminal membrane of the tubular epithelial cells of the thick ascending limb of the loop of Henle and of the macula densa (56). At least three different full-length isoforms of NKCC2 are derived from differential splicing: NKCC2A, NKCC2B, and NKCC2F. Each isoform is expressed differently along the thick ascending limb of Henle. Although the functional significance of these three isoforms is uncertain (117, 136), and factors affecting the level of expression are not fully understood, differential splicing of NKCC2 seems to be modulated by dietary salt intake. Dietary sodium restriction enhanced the expression of the high-affinity NKCC2B isoform and reduced the low-affinity NKCC2A isoform (136). Reports regarding selectivity between NKCC1/2 of furosemide are limited, yet indicate that furosemide has no selectivity for the two isoforms when NKCC1 is measured in the active state (60).

Both in vitro and in vivo studies show that furosemide inhibits CA by SO₂⁻NH₂ moieties acting as an effective zinc-binding property of CA (130). Inhibition of different isoforms of CA I, II, and XI by furosemide varies (26, 130, 148, 150). The inhibition of CA I has been reported to result in vasodilatation and a reduction in arterial blood pressure (BP) (129). Furosemide is also an antagonist at GABA_A receptors (67), perhaps by allosteric modulation (88). GABA_A receptors have been implicated in certain signal transduction cascades, such as mitogen-activated protein (MAP) kinase cascade (119). Reports of all of these actions of furosemide are limited, and the relevance of these effects is not well defined.

**Questions Pertaining to the Natriuretic Response to Furosemide**

Are renal or systemic hemodynamic effects of furosemide important for its natriuresis? Furosemide could limit or enhance its own diuretic actions in several ways. Since this subject has not been extensively investigated, scientific underpinning is relatively poor. A bolus injection of furosemide results in a strong natriuresis with fractional sodium excretion in healthy individuals exceeding 25% (13) and with peak Na⁺ excretion of ~5 mmol/min. Obviously, this would decrease plasma volume (PV), if refill rates from the extracellular fluid volume (ECFV) are not sufficient to keep up with the rate of sodium and volume loss, resulting in 1) a decrease in BP and pressure natriuresis; 2) activation of the renin-angiotensin system (RAS) (on top of what already happens if the NKCC2 transporter in the macula densa is blocked); and 3) activation of the sympathetic nervous system. Indeed Tucker and Blantz (159) reported a decrease in mean arterial pressure in animals.
treated with furosemide, but without volume repletion (159); this was accompanied by a decrease in single nephron glomerular filtration rate (SNGFR), surprisingly mediated by a decrease in glomerular permeability coefficient LpA, but not by a decrease in net ultrafiltration pressure. Surprisingly, Costa et al. reported similar decreases in MAP after furosemide administration in rats in normovolemic vs. volume-expanded animals (33). To complicate this further, furosemide could elicit a natriuresis in an individual with decompensated heart failure, leading to a more favorable end-diastolic pressure and increased cardiac output (98). Clearly, a decrease in BP and renal perfusion pressure will limit the diuretic actions of the drug, yet whether this occurs under normovolemic and hypervolemic states is not well characterized.

Activation of the RAS is similarly complex. While there is ample data to support that furosemide leads to increases in circulating levels of renin, data documenting how furosemide (and other diuretics) affects components of the intrarenal RAS is absent. This is particularly relevant with respect to intratubular and intrarenal levels of ANG II, which shows very substantial compartmentalization and have been reported to be up to 1,000 and 100 times plasma levels of ANG II, respectively (19, 143). Conversely, data about the natriuresis in response to furosemide in the absence and presence of inhibitors of the RAS is limited and not consistent. Two studies indicated that the fractional sodium excretion was diminished when the angiotensin-converting enzyme (ACEi) captopril was acutely administered before furosemide in humans with heart failure; however, the captopril dose led to substantial hypotension (48, 101). Motwani et al. (107) observed that an ultralow dose of captopril (1 mg), which did not decrease MAP in heart failure patients, enhanced the natriuretic actions of furosemide, whereas a higher dose (25 mg) decreased MAP and attenuated the natriuresis. In a chronic setting, ACEi seems to enhance the natriuretic response to furosemide in heart failure (53). It would be an interesting option to specifically target the renal RAS [e.g., by using lysozyme-modified captopril (61)] to prevent the acute BP effects of ACEi. Altogether, there is an uncertainty about the regulation of systemic and renal RAS in response to furosemide and, conversely, about the renal response to furosemide in the presence and absence of an intact RAS.

Data about the renal sympathetic nervous system are more complex. Several studies have demonstrated that furosemide can directly activate the renal sympathetic nervous system (124), independent from the RAS (123). This could well be elicited by a change in the local micro-environment of the afferent nerve ending in the renal interstitium (52). Nevertheless, renal denervation did not affect the natriuresis evoked by furosemide acutely (146) in lambs or chronically (122) in rats under physiological conditions. Other studies revealed an enhanced natriuretic response to furosemide in healthy animals with acute unilateral denervation (125). This, however, might be difficult to interpret, since unilateral denervation will lead to a reno-renal reflex. Data on renal nerves, furosemide, and natriuresis in (experimental) chronic kidney disease (CKD) or heart failure is absent. Altogether, it remains unclear under which conditions direct activation of the afferent renal nerve endings by furosemide inhibits the natriuretic response to furosemide.

The two remaining issues in this section concern the two mechanisms that form the basis for autoregulation of renal blood flow (RBF) and GFR, myogenic response (MR) and TGF. The group of Louzzenhiser has shown that furosemide affects MR, so that autoregulatory behavior is impaired (163). Although others reported less attenuation of autoregulation (75), any attenuation of MR would render sodium excretion upon furosemide administration more dependent on renal perfusion pressure. This is particularly relevant in conditions with fluid congestion and hypotension, such as advanced heart failure, in which furosemide would be applied. Altogether, furosemide can impair renal autoregulation; whether this is relevant for sodium excretion is unknown. As mentioned, furosemide can block the TGF system by inhibiting the sensing of the Cl\(^-\) concentration in the macula densa. This would de-activate the TGF system and increase single nephron GFR and thereby filtered load. Conversely, furosemide increases the macula densa NaCl delivery and could activate the TGF system, depress GFR, and diminish filtered load. It is not clear whether tubular concentrations achieved by pharmacological use of furosemide tip the balance between these opposing forces toward an attenuated or activated TGF response. To illustrate this further, Fig. 1 shows the response to low-dose administration of furosemide and of the CA inhibitor acetazolam ide in healthy subjects. Acetazolamide led to a consistent increase in lithium clearance (which can be used as estimate of distal delivery) and a consistent decrease in GFR. Furosemide led to a highly variable response in GFR, although never clearly increased GFR. How GFR would respond to furosemide in patients on multiple medications and with multiple co-morbidities is entirely unclear.

What determines the braking phenomenon and is there a fundamental difference with short-term furosemide resistance? The braking phenomenon is the decrease in the response to furosemide after the first dose (165) and is considered a physiological response to avoid ECFV contraction. Some data suggest that it can be prevented by restoring the diuretic-induced loss of ECFV (21). Confusingly, other data suggest that the braking phenomenon induced by the loop diuretic, bumetanide, is volume independent (4). In short-term therapy, more pronounced volume depletion will trigger more compensatory mechanisms to preserve the ECFV, which has also been denoted as acute diuretic resistance (57). Both the braking phenomenon and short-term resistance could be caused by postdiuretic Na\(^+\) retention (7). Dietary Na\(^+\) is a critical factor (43): during high Na\(^+\) intake, the compensatory increase in Na\(^+\) reabsorption between doses can lead to neutral Na\(^+\) balance; to achieve a negative Na\(^+\) balance, a dietary Na\(^+\) restriction is required (7, 165).

Although a potential mechanism explaining rebound sodium retention is that furosemide could induce hyperaldosteronism, increases in aldosterone similar after 1 and 3 days of furosemide administration and 4 wk administration furosemide with or without spironolactone had similar effects on sodium balance (96). Moreover, neither ACEi nor ARB treatment could prevent acute diuretic resistance, suggesting that activation of RAAS is not responsible (81, 90). Altogether, it seems more plausible that acute volume depletion is to a certain extent related to acute resistance (57), yet the available data do not firmly rule out other mechanisms (90).
Incomplete understanding of braking and acute resistance is illustrated by the absence of good strategies to prevent these phenomena. A strategy could be to administer furosemide continuously and intravenously, allowing stable tubular drug delivery. Indeed, some studies report continuous furosemide infusion to be more effective to reduce ECFV in heart failure and severe fluid overload (118, 128). A large study in heart failure patients, however, was unable to demonstrate that continuous intravenous furosemide was more effective in decreasing volume overload than bolus injections (46). Altogether, this leaves the nature of the mechanisms of braking and resistance unanswered as well as how this could be prevented.

Could long term use of furosemide lead to enhanced reabsorption in other nephron segments? Several mechanisms could lead to enhanced reabsorption in other segments than the loop of Henle during long-term use of furosemide. Empirically this has led to the notion of diuretic synergism: thiazides are used to block the adapted sodium reabsorption in the distal nephron to enhance the diuretic effect of chronic furosemide therapy (7, 43).

First of all, furosemide could increase the expression of Na+/H+ transporters in other segments. Indeed, long-term use of furosemide can increase the abundance of NKCC2 in the thick ascending limb and OAT1 in the proximal tubule (84). Furthermore, furosemide infusion increases the abundance of all three subunits of the epithelial Na+ channel in connecting tubules, cortical collecting ducts, outer medullary collecting ducts, and inner medullary collecting ducts (108). Chronic furosemide administration has also been associated with an increased Na+/H+-K+-ATPase expression in the distal convoluted tubule and in cortical collecting duct (135), suggesting the possibility of enhanced capacity in sodium reabsorption in these segments. However, using micropuncture furosemide has been shown to enhance reabsorptive capacity of distal segment only in rats during sodium depletion (144). The question remains whether this adaptation is significant for the effect of chronic furosemide?

Second, furosemide could lead to hypertrophy of tubular segments. Furosemide administration for 6–7 days causes hypertrophy of the distal tubule (44, 77), connecting tubule and principal cells of the collecting ducts (78). This hypertrophy is associated with increases in Na+ transport capacity (43). Continuous infusion of furosemide results in a substantial increase in the size of distal cells (43). The same adaptation of distal convoluted tubule has been demonstrated in humans during long-term use of furosemide (96). As a compensatory process, Na+ that escapes from the loop of Henle could, therefore, be partially reabsorbed at more distal sites, decreasing overall...
diuresis (84). According to Kaisling and Stanton (78), furosemide-induced increases in distal Na\(^+\) concentration is a stimulus for epithelial cell growth in distal tubules in animal models.

Proximal tubular Na\(^+\) reabsorption in response to furosemide treatment could, on the one hand, be diminished by direct furosemide actions, or, on the other hand, be enhanced by indirect actions of furosemide to stimulate renin and ANG II. In dogs, furosemide did not affect proximal tubular sodium reabsorption, yet decreased GFR. Meanwhile, furosemide inhibited the reabsorption of a saline drop in the proximal tubule so that the decrease in GFR might have obscured the effects of furosemide on proximal tubular reabsorption (86). Micropuncture experiments in dogs (39) and monkeys (12) do not show diminished proximal tubular reabsorption during short-term furosemide administration. However, administration of furosemide inhibits proximal tubular reabsorption in the rat, if great care is exercised to prevent retrograde flow of tubule fluid (22).

Another study in rats indicates no inhibition of reabsorption, unless filtration rate was reduced to 50% of normal (37). All of these studies come with some limitations. For example, repetitive sampling of tubular fluid using stop flow techniques to analyze segmental tubular reabsorption have limitations, since they might alter reabsorption in downstream segments (39). Moreover, assessment of segmental tubular sodium handling during prolonged furosemide therapy in the absence, but also the presence of CKD or heart failure, has not been studied. Therefore, results regarding inhibition of proximal tubular transport are conflicting in physiological situations and incomplete in pathophysiology.

This leaves several issues. First, furosemide affects expres- sion of transporters, not only in the loop itself, but also in other segments. This means that, if NKCC2 activity varies, this links to stimuli that modulate the expression of other transporters. What the exact stimuli are, is not resolved. Moreover, at a more physiological level, it seems that there is an intrinsic set point of the kidney for a certain degree of Na\(^+\) reabsorption related to total body sodium (132); while this might affect very local phenomena, such as tubular fluid, tubular osmolality, and interstitial composition, this might also feed back to the kidney by neurohumoral mechanisms.

Why is the effect of furosemide on sodium balance, ECFV, and BP unpredictable? ECFV expansion can increase total peripheral resistance and BP by evoking total body autoregulation in response to overperfusion of tissues. A logical approach to reduce BP is to reduce ECFV using diuretics. Verification of this concept would require reliable and easily applicable ECFV measurements. Radioisotope measures are reliable but not easy to use. Mono- and multifrequency bioimpedance measurements of ECFV are reliable but have hardly been employed to research the subject. Only one study documented a 1.1-liter decrease in ECFV after initiation of furosemide therapy in patients with CKD (compared to subjects treated with nondiuretic antihypertensives) (170). To obtain insight into how furosemide affects ECFV regulation, further studies are needed to better characterize who will benefit from furosemide therapy and which regimen (dose, dose frequency, route of administration) is effective to reduce ECFV to normal.

Several studies in the 1970s indicate that dosing of fu- rosemide once per day induces a natriuresis over \(~6–8\) h followed by sodium retention during the rest of the day (165). The mechanism of this sodium retention has not been entirely resolved; it could be related to 1) activation of the RAS; 2) activation of the SNS; and 3) acute reduction in PV followed by decreased renal perfusion pressure and “pressure natriuresis.” A stronger natriuretic response to furosemide would lead to a more pronounced decrease in PV. A decrease in PV of 16% (580 ml) was observed in hypertensive subjects after 100- to 200-mg intravenous furosemide (36). Of note, the stability of the effective circulating volume also is related to the refill rate from the interstitial space and the dynamics of the venous capacitance (see below). A study in heart failure patients demonstrated a net refill volume (and perhaps also venous-to-arterial fluid redistribution volume) exceeding the diuresis induced by furosemide (140). Refill rate cannot be easily assessed, although a methodology has been developed to measure refill rate in hemodialysis (24). Starling forces primarily determine refill rate and include the permeability of the capillary membrane, which is not fixed but influenced by numerous factors, including ANG II (168), atrial natriuretic peptide (142, 153), inflammatory cytokines (23, 93), and nitric oxide (NO) (28, 149). Characterization of capillary refill in complex states like advanced kidney failure would, therefore, contribute to the understanding of consequences of rapid alterations in volume status with furosemide.

Unpredictable responses to furosemide can also result from differences between the PV/ECFV and the PV/BP relationships of patients. Patients with non-nephrotic CKD have a steeper slope of the PV/ECFV and PV/BP relationship compared with patients with nephrotic syndrome (Fig. 2). Therefore, an acute

![Fig. 2. Effects of furosemide in patients with CKD or nephrotic syndrome (NS). Both groups of patients have expansion of the extracellular fluid volume (ECFV); however, the relationship between ECFV and plasma volume (PV) is different.](http://ajprenal.physiology.org/)

[Adapted from Koomans et al. (89).]
reduction in PV upon diuretic treatment in non-nephrotic patients is more likely to cause an acute reduction in BP compared with nephrotic CKD. In heart failure, the PV/ECFV and PV/BP relationships have hardly been studied. One study reported decreases in blood volume measured by labeled albumin and Cr-51 labeling of red cells in subjects with acute heart failure and pulmonary edema treated with furosemide (47). Unfortunately, methodology is not readily available to easily assess PV/ECFV and PV/BP relationships in humans or animals, and there is little knowledge of these relationships in various disease states, including the response to diuretic therapy.

Closely related to this subject is the distribution of blood volume between the venous and arterial compartments. Schuster et al. (140) compared patients with acute heart failure with and without a diuretic response after furosemide. Remarkably, in both groups, there was a decrease in colloid osmotic pressure and a decrease in central venous pressure. This could be explained if furosemide increased venous capacitance, decreased venous pressure, thereby facilitating fluid reabsorption from the interstitial space and subsequent decreases in plasma oncotic pressure (140). Others have reported such an acute increase in venous capacitance upon furosemide (38). The mechanism, as well as the physiological importance of variations in venous capacitance by furosemide is unknown; it is even not known whether this is mediated via the NKCC2 transporters. In one study, in human umbilical vein endothelial cells, NKCC2 gene expression was induced by inflammatory cytokines, but whether such induction is present in intact veins and is physiologically significant is unknown (157).

Questions Regarding Hemodynamics and Vascular Regulation and the RAS

What determines the effects of furosemide on GFR and RBF? In 18 studies about the actions of furosemide on GFR and RBF in healthy subjects, changes in GFR and/or RBF were reported. In the majority of studies, GFR increased (5, 13, 14, 71, 120, 121, 133, 169) or remained stable (31, 42, 102, 147); in five studies GFR decreased (51, 91, 100, 158). RBF increased in five (71, 102, 115, 121, 169), remained stable in three (42, 51, 120), and decreased in one (51), and was not reported in the other studies (5, 13, 14, 31, 91, 100, 133, 147, 158). This clearly illustrates how complex the actions of furosemide on renal hemodynamics are. Furosemide can affect GFR and RBF by acting on BP, on hydrostatic pressure in Bowman’s space, on afferent and efferent resistance, on the glomerular surface area and permeability, and on plasma colloid osmotic pressure. Obviously, furosemide can decrease BP by causing a brisk natriuresis and, consequently, a decrease in ECFV and PV (36). The impact of furosemide on renal function might be different from other hypotensive agents, since furosemide can affect autoregulation (see below), and subjects treated with furosemide, typically patients with heart and/or renal failure, might have impaired autoregulation. Furthermore, furosemide can cause a direct vasodilation by acting on NKCC1 in the vascular wall. The extent to which this can cause relevant changes in BP in disease models and in humans with heart failure or CKD is not well determined.

Furosemide can increase tubular pressure and thereby increase pressure in Bowman’s space, which will directly decrease net ultrafiltration pressure. Determinants of tubular pressure are tubular flow and resistance. Resistance, in turn, is determined by the tubular diameter, depending on tubular compliance and on renal interstitial pressure. Since the kidneys have a tight capsule, Starling forces governing fluid fluxes between the capillaries and the interstitium determine interstitial fluid volume and thereby interstitial pressure. The net response of all factors on tubular pressure after furosemide is unpredictable. Holstein-Rathlou and Leyssac (63) reported an acute increase in tubular pressure upon intraluminal administration of furosemide of ~5–7 mmHg, which is relevant with respect to an estimated net ultrafiltration pressure of 20–25 mmHg. The increase is likely due to an increase in tubular flow due to the combined effects of inhibition of loop of Henle reabsorption, and an increase in SNGFR due to inhibition of TGF. Since this was a single nephron study, it lacks effects on systemic hemodynamics and on the interstitium. Tucker and Blantz (159) reported the intrarenal responses to systemic administration of furosemide in rats with and without supplement with saline solution to compensate for urinary sodium excretion. Without volume repletion, SNGFR decreased substantially; with volume repletion it remained stable. Interestingly, tubular pressure was slightly decreased after furosemide without volume repletion (from 13 to 11 mmHg), but increased during volume repletion (from 13 to 18 mmHg). Remarkably, the permeability coefficient decreased after furosemide with volume repletion. Finally, Oppermann et al. (116) reported a very strong increase in free flow proximal tubular pressure upon systemic administration of furosemide, which was attenuated by decapsulation. These studies underscore that relevant changes in tubular pressure can occur after (intraluminal) furosemide administration. Nevertheless, the effects of furosemide on tubular pressure (and GFR and RBF) during high dietary sodium, fluid volume expansion, or disease models have hardly been studied.

Regarding afferent resistance, Oppermann et al. (116) reported NKCC1-dependent vasodilation of isolated perfused afferent arterioles preconstricted with ANG II or the NO synthesis inhibitor L-nitro-L-arginine methyl ester. Wang et al. (163) were able to demonstrate NKCC1, but not NKCC2, protein expression in afferent arterioles and showed diminished MR after furosemide and bumetanide. Bumetanide also strongly inhibited the vasoconstrictor response to ANG II; furosemide was not tested. Despite this, Oppermann et al. (116) reported an acute decrease in RBF in response to systemic furosemide, which was explained by compression of the intrarenal vasculature after increased tubular volume. Altogether, these studies leave the possibility that furosemide can decrease GFR through increased interstitial pressure and decrease RBF due to compression of the intravascular vasculature and the inability of the MR to compensate for this. That said, effects of furosemide on MR and vascular contractility have not been studied during relevant physiological perturbations and in disease models of fluid volume expansion (heart failure and CKD).

Generally, in patients with heart failure, plasma oncotic pressure is decreased (6), and PV and ECFV are substantially expanded (166). Hemoconcentration due to diuretic therapy could, therefore, increase plasma oncotic pressure and thereby decrease GFR. Whether increases in plasma oncotic pressure...
as a consequence of decreases in PV upon furosemide treatment can decrease GFR has not been studied.

Are direct vascular actions of furosemide important and what is the mechanism? Multiple factors could contribute to the controversy surrounding direct vasodilator effects of furosemide (41). Inconsistencies about this subject can be traced back to the 1970s. Furosemide has been documented to cause vasodilation in animal experiments (1). In humans, an immediate fall in left ventricular filling pressure was detected preceding the natriuresis after furosemide administration in congestive heart failure after acute myocardial infarction, presumably due to a markedly increased venous capacitance (38). Patients with pulmonary edema reportedly have immediate relief of symptoms by furosemide before any diuretic effect is observed (127). Whether or not furosemide causes increases in venous capacitance are likely also strongly related to the dose being administered. In this context, it is relevant to emphasize that, in healthy subjects, low doses already exert natriuretic effects (e.g., 5 mg iv; see Fig. 1), yet in patients with advanced CKD or heart failure, very substantial dosages are applied (up to 1,000 mg/24 h iv).

Furosemide can induce vasodilation indirectly via the synthesis of prostaglandins (70) since co-administration of indomethacin with furosemide can abolish a change in venous capacitance (127, 131). Conversely, furosemide induced a decrease in medullary hypertonicity, decreased intramедullary prostaglandin activity, and caused a fall in medullary perfusion (40). Confusingly, other studies showed that renal hemodynamic effects of furosemide were not mediated by prostaglandins but were, in fact, direct (29). Furosemide also induced a direct relaxation of the renal, iliac, and carotid vasculature, independent of prostaglandins and persisting after bilateral nephrectomy (9). The direct vasorelaxing effect of furosemide on isolated vessel segments was suggested to be endothelium independent (152). Furosemide was unable to cause vasodilation in afferent arterioles of NKCC1−/− mice (116). Paradoxically, acute administration of furosemide has also been reported to cause vasoconstriction, presumably due to increased angiotensin II (41, 116), norepinephrine, and AVP levels (50).

Altogether, the exact mechanism and vascular site (arterial, arteriolar, venous) of the vascular actions of furosemide remain ambiguous, although it seems to involve a direct action on the NKCC1 transporter.

Does furosemide differentially affect the systemic and intraluminal RAS? Recapitulating, furosemide can increase renin release by activation of the sympathetic nervous system (15, 32), a decrease in afferent arterial perfusion pressure, and by direct inhibition of NKCC2 in the macula densa (27, 151). In addition, macula densa neuronal NO synthase (nNOS)-mediated NO generation can stimulate renin release (18). The increase in renin release on furosemide reportedly is inhibited in the presence of nonspecific or specific nNOS blockers (137), yet furosemide did not affect renal nNOS gene expression in healthy rats (139). Conversely, furosemide can reduce renin release by increased luminal Na+ concentration at the level of the macula densa (105, 138). That leaves uncertainty about the net effect of furosemide on renin release.

To assess the intrarenal RAS, one can measure RAS components in whole kidney (cortex) tissue (17, 49) or in fluids thought to reflect the activity of the intrarenal RAS: lymph (83), interstitial fluid (112), tubular fluid (19, 109, 143), and urine (16, 87). Activity of the systemic RAS does not always parallel activity of the intrarenal RAS (16, 25, 49, 103). We have been unable to find any studies on kidney vs. systemic, or tubular fluid vs. systemic RAS levels during furosemide administration. However, Khuri et al. (83) demonstrated that intravenous furosemide increased renal lymphatic renin more substantially than plasma renin in Mongrel dogs. This could indicate that the macula densa sodium sensing was blocked, and the resulting renin release increased the interstitial and lymph renin levels selectively. Urinary ANG I and ANG II excretion have been suggested to reflect intrarenal RAS activity, although limited evidence is available (162), and there is substantial ANG I and II breakdown. The same group reported increased urinary ANG I and II excretion after furosemide (16). This still does not resolve the question whether there can be divergent responses in systemic and renal RAS. There is currently considerable interest in urinary angiotensinogen as an indicator of the intrarenal RAS, yet we have been unable to find studies reporting the responses to furosemide.

Why would it be important if furosemide causes a disproportionate or diverging increase in renal RAS levels? Here we are left with one of the most intriguing observations in the RAS literature: ANG II infusion increases renal ANG II levels (161). Yet what this means in terms of renal function is obscure. Is the ANG II buffered? Does it reach levels where ANG II might become natriuretic (141)? All in all, the effects of furosemide on the intrarenal RAS are not well documented, and the implications for renal function remain unclear.

Questions Regarding Furosemide in Kidney Disease, Urate, and the Third Compartment

Does kidney disease affect the actions of furosemide? Whether furosemide is equally potent in healthy individuals vs. patients with CKD is related to the pharmacology of furosemide and the tubular mechanisms that lead to Na+ retention in CKD. Regarding the pharmacology, intestinal reabsorption of 40–90% has been reported in subjects with advanced CKD (65), yet altogether reabsorption seems diminished (154). Although a clear relationship between reabsorption and measures of GFR has not been documented in CKD, this could contribute to furosemide resistance. Moreover, furosemide T½ is increased in renal failure (10) and is highly variable, up to >20 h in some individuals. This could contribute to “resistance”, since in some subjects it could accumulate so that additional dosages do not elicit a natriuresis. Conversely, having plasma levels over a more sustained period could prevent resistance. Another pharmacological issue is that, in states with high-grade proteinuria, the glomerular filtrate might contain so much albumin that it diminishes the free furosemide levels to such an extent that this diminishes its actions (145). In that regard, it is noted that Agarwal et al. (2) showed that displacing furosemide from plasma proteins with a sulphonamide (sulfoxazole) did not correct resistance in nephrotic patients. There is still controversy about whether and how albumin infusion could increase the natriuretic actions of furosemide in the nephrotic state (45). Taken together, diminished reabsorption, increased T½, and high-grade proteinuria in patients with CKD could limit the natriuretic response.

A more physiological issue is that, when GFR declines in CKD, fractional excretion of Na+ has to increase to maintain
Na\(^+\) balance. A healthy subject with an estimated GFR of 120 ml\(\cdot\)min\(^{-1}\)\(\cdot\)1.73 m\(^2\), therefore, has a 10\(\times\) lower fractional sodium excretion (0.5–1.0\%) than a subject with advanced CKD and an estimated GFR of 12 ml\(\cdot\)min\(^{-1}\)\(\cdot\)1.73 m\(^2\) (5–10\%), assuming similar intake and assuming sodium balance. This leads to the important question how the remnant nephrons in CKD decrease their Na\(^+\) reabsorption. Since NKCC2-mediated reabsorption is so substantial, one option is that NKCC2 is suppressed. Data about CKD and expression of NKCC2 are limited. One study demonstrated an initial increase in NKCC2 expression after 5/6th nephrectomy in rats, later returning toward normal (85). Two studies in rats after uninephrectomy and salt loading demonstrated decreased expression of the NKCC2 (73, 74). In deoxycorticosterone acetate-salt hypertension (8), NKCC2 seems to decrease, whereas, in ANG II-induced hypertension (110), NKCC2 seems only decreased in the renal medulla. Altogether, data in renal failure about NKCC2 expression and activity are limited.

**ISSUE**

A. FUR can inhibit NHE3; mechanism and relevance unclear
B. FUR increases proximal tubular urate transport; direct or indirect?
C. FUR can inhibit carbonic anhydrase; mechanism and relevance unclear
D. Under which circumstances does tubular pressure increase upon FUR?
E. Can FUR cause an NKCC1-mediated decrease in afferent arteriolar tone?
F. Can FUR differentially inhibit TGF responses and enhance renin release?
G. Does FUR lead to a relevant increase in NCC gene and protein expression?
H. Similarly, can FUR increase in ENaC gene and protein expression?
I. Is FUR induced increased distal tubular fluid load and consequently increased distal tubular reabsorption similar under all circumstances?
J. Does FUR increase DCT and CCD Na\(^+\)-K\(^+\)-ATPase activity?
At a translational level, in relatively severe CKD, the natriuresis to 1.5 mg/kg furosemide in CKD was directly proportional to GFR (inulin clearance), yet the increase in fractional Na\(^+\) and water excretion upon furosemide did not decline with lower GFR (54). Another report indicated an increase in fractional Na\(^+\) excretion to 40% upon high-dose furosemide in patients with very low GFR (4–8 ml·min\(^{-1}\)·1.73 m\(^2\))(3).

Lastly, one report indicated maintained responses in urinary Na\(^+\) excretion after furosemide given orally or intravenously in patients with mild and with advanced (134) CKD, indicating an enhanced response to furosemide is unclear, and whether there is regulation of sodium transporters in CKD with different severity and etiology is unclear.

Are effects of furosemide on urate levels detrimental for BP regulation and kidney integrity? One of the most important side effects of diuretics is that it can provoke a gout attack. Several mechanisms have been suggested. Furosemide inhibits the human sodium phosphate transporter 4 (hNPT4) in proximal tubules, which releases urate into the tubular lumen (76). Moreover, hyperuricemia often coexists with hypertension, CKD, and cardiovascular disease (CVD). Yet it is hard to discriminate whether these issues are induced by elevated uric acid level directly or secondary to the coexistent conditions or drugs (69). Notably, in animal experiments, increased uric acid level resulted in systemic and glomerular hypertension due to elevated renal vascular resistance and reduced RBF (69). Similarly, in the 5/6 remnant kidney model, rats with hyperuricemia displayed more renal hypertrophy, arteriolosclerosis, glomerular injury, and interstitial fibrosis than those with similarly elevated BP but without hyperuricemia (80). Uric acid was also found to induce endothelial dysfunction via impairing NO release, which would impair vasodilation (68).

This poses an intriguing problem: could furosemide aggravate hypertension and CKD and even associated CVD?

Data on the negative effects of uric acid are not consistent. This inconsistency can be due to the duality of uric acid. Uric acid can also act as an antioxidant. Increased serum antioxidant capacity was found in hyperuricemia in individuals with atherosclerosis. This finding could indicate compensation of the oxidative stress caused by CVD and CKD (111). The antioxidant activity of uric acid could be mediated by increasing the activity of SOD1 and SOD3 (62). Others have suggested that uric acid only functions as an antioxidant in the extracellular space, and indeed a reciprocal relationship has been demonstrated between uric acid and NO level in serum (79). So, returning to the effects of furosemide on uric acid levels, what determines whether furosemide could cause aggravation of CKD and CVD?

How does furosemide affect the “third compartment”? In recent years, the notion developed that Na\(^+\) could distribute to cutaneous tissue, where it could bind to glycosaminoglycans in a nonosmotic fashion (156). This nonosmotic sodium possibly

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**Fig. 4. Mechanism of furosemide action in the systemic environment where questions are remaining.** PVR, pulmonary vascular resistance; CO, cardiac output; VC, vasoconstriction; VD, vasodilation.

**Issue**

A A brisk natriuresis after FUR can affect PV and ECFV differently depending on the clinical syndrome

B The mechanism of direct vasodilation in the systemic circulation is unknown

C Whether and how FUR can increase venous compliance is not well known

D Can stimulation of tubular reabsorption by ANG II and Aldosterone counteract the natriuresis after FUR?

E Can renin-induced vasoconstriction supersede the direct FUR-induced vasodilation?

F How does FUR-induced hyperuricemia leads to vasoconstriction?

G Does FUR affect the skin sodium stores and the systemic VEGF-C levels?

H Is a volume shift towards the venous site of the vascular bed after FUR relevant for blood pressure control?
elicits an inflammation response, with release of VEGF-C as a consequence, which would offset hypertensive consequences of high sodium intake (155). Gradually, evidence is accumulating that the subcutaneous sodium storage is expanded in different disease states, such as hypertension (97) and end-stage renal disease (35). A question that is unanswered at this moment is whether any diuretic therapy can mobilize the subcutaneous sodium, and whether this would improve or deteriorate systemic hemodynamic function. A similarly interesting question is how sodium transport takes place between the subcutaneous stores and the lymphatic vessels. It is imaginable that this recruitment of salt from these stores is a regulated process (95). In that case, it may very well involve Na$^+$ transporters that are similar to transporters in the kidney to facilitate movement of sodium from the intercellular space back into the lymphatic vessels. If furosemide would exclusively remove sodium from the extracellular space and not recruit sodium from the subcutaneous stores, would furosemide be beneficial or harmful for the skin-VEGF axis?

Summary

This evaluation has revealed important knowledge gaps, involving furosemide as a drug itself, the function of NKCC2 (and NKCC1), and renal and systemic indirect effects of NKCC inhibition. Regarding the kidney, remaining questions are indicated in Fig. 3, regarding the systemic circulation; remaining issues are indicated in Fig. 4. Major themes are as follows: 1) diuretic resistance and the braking phenomenon; 2) systemic and intrarenal activation of the RAS; and 3) systemic vascular effects, specifically furosemide-induced increases in venous capacitance. An important aspect of the analysis of the actions of furosemide is that the brisk and short diuresis strongly disturbs the steady state with numerous and heterogeneous consequences. Resolving the many remaining questions could help to better understand NKCCs and their actions and improve the application of furosemide in the pathophysiology of fluid volume expansion.

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