Research Article

Renal Nerve Deafferentation Attenuates the Fall in GFR during Intravenous Infusion of Furosemide in Anesthetized Rats

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Keywords

Volume replacement · Loop diuretic · Renal nerves · Renal vascular resistance · Angiotensin receptor blocker

Abstract

Introduction: Furosemide reduces the glomerular filtration rate (GFR) and increases the renal vascular resistance (RVR) despite inhibiting tubuloglomerular feedback but increases proximal tubule pressure, renin release, and renal nerve activity. **Objective:** This study tested the hypothesis that the fall in GFR with furosemide is due to volume depletion or activation of angiotensin type 1 (AT1) receptors or renal nerves. **Methods:** Furosemide was infused for 60 min at $1.0 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ in groups of 5–8 anesthetized rats. Additional groups received intravenous volume replacement to prevent fluid and Na^+ losses or volume replacement plus losartan or plus sham denervation or plus renal denervation or renal nerve deafferentation. **Results:** At 60 min of infusion, furosemide alone reduced the GFR ($-37 \pm 4\%$; $p < 0.01$). This fall was not prevented by volume replacement or pretreatment with losartan, although losartan moderated the increase in RVR with furosemide ($+44 \pm 3$ vs. $+82 \pm 7\%$; $p < 0.01$). Whereas the GFR fell after furosemide in rats after sham procedure ($-31 \pm 2\%$), it was not changed significantly after prior renal deafferentation. Proximal tubule pressure increased significantly but returned towards baseline over 60 min of furosemide, while urine output remained elevated, and GFR and renal blood flow depressed. **Conclusions:** The fall in GFR over 60 min of furosemide infusion is independent of volume depletion or activation of AT1 receptors but is largely dependent on renal afferent nerves.

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Introduction

Furosemide is prescribed widely for patients with edema or hypertension. It inhibits the Na^+ - K^+ - 2Cl^- cotransporter (NKCC2) in the thick ascending limb of the loop of Henle to cause a potent natriuresis and diuresis. However, furosemide reduces the glomerular filtration rate (GFR) and renal blood flow (RBF) and increases the renal vascular resistance (RVR) of anesthetized rats, despite inhibition of tubuloglomerular feedback (TGF) that should cause renal vasodilatation [1]. The fall in GFR with furosemide in human subjects [2–5] is poorly understood [6] but is a major concern in the treatment of edema and congestion [7]. Studies in mice have suggested that the fall in GFR with loop diuretics is related to blood volume depletion, activation of the renin-angiotensin system (RAS), or increases in the intratubular or interstitial pressures, but their relative importance is not clear [8].

Both the afferent and efferent renal nerves can regulate renal hemodynamics in animals and in humans [9–11]. Renal efferent sympathetic nerve activity contributes to hypertension [12] and can increase sodium reabsorption and renal angiotensin II [13] and reduce the GFR and RBF depending on the intensity of renal nerve activation [14]. Renal afferent nerve activity regulates blood pressure [15], renal efferent nerve activity, and renal function via a renorenal reflex [16]. The renal afferent nerves contribute to progressive renal damage in a rat model of chronic kidney disease (CKD) and high salt intake [16] and to hypertension and vasoconstriction in patients with CKD [17]. Reflex activation of renal efferent nerves accompanies a fall in central venous pressure as can occur with loop diuretics [18]. Afferent renal nerves are activated by renal mechanoreceptors in response to a rise in hydrostatic pressure and renal chemoreceptors in response to a rise in salt intake [16, 19].

A single dose of furosemide given to normal human subjects decreases their GFR by ~23% [5], increases the plasma angiotensin II by >150%, the plasma norepinephrine by 40–60% [3, 4], and the heart rate by 15% and decreases the plasma volume by ~10% [3, 4, 20]. Thus, although furosemide blocks the TGF that should increase the GFR, it activates the RAS and the sympathetic nervous system and reduces the blood volume that should reduce the GFR [1]. The mechanism responsible for the hemodynamic effects of furosemide remains largely unclear [6]. Accordingly, we tested the hypothesis that the change in GFR during a 1-h infusion of furosemide was due to volume depletion or to activation of angiotensin type 1 (AT1) receptors or renal nerve activity. Inhibition of tubular fluid reabsorption will raise intratubular pressure that might physically curtail the GFR. This was assessed from free flow micropuncture measurements of the hydrostatic pressure in the proximal tubule (P_{PT}).

Materials and Methods

Male Sprague-Dawley rats weighing 250–300 g were maintained on a standard rat chow (0.3 g·100 g⁻¹ Na⁺ content) with free access to food and water until the day of the study. The use of animals was approved by the Georgetown University Animal Care and Use Committee and performed according to the National Institutes of Health guidelines for the conduct of experiments in animals. On the day of the study, rats were anesthetized with Inactin (50 mg/kg body weight (BW)⁻¹, i.p.; Research Biochemicals). Other rats were prepared for renal clearance and micropuncture, as previously described [1, 21]. The left kidney was placed in a Lucite cup. Rats received an i.v. infusion of 1% bovine serum albumin (Sigma) at 0.3 mL·100 g BW⁻¹·h⁻¹ to which was added [³H] inulin and [¹⁴C] para-aminohippurate (PAH) to estimate the GFR and effective renal plasma flow. After stabilization, urine was collected for 20 min during infusion of vehicle (saline) and for three 20-min periods during constant infusions of furosemide at 1.0 mg·kg BW⁻¹·h⁻¹. Plasma samples and hematocrit were obtained from the

femoral artery at the midpoint of each urine collection. Hydrostatic pressure in the proximal tubule (P_{PT}) was measured by micropuncture using free-flow conditions during 3 min before and during the infusion of furosemide, as previously described [1]. An average of three nephrons per period was recorded and the values averaged.

Study Design

Series 1: Effects of Volume Depletion and AT1 Receptor Activation

Three groups of rats were studied: group 1 – furosemide ($1.0 \text{ mg} \cdot \text{kg BW}^{-1} \cdot \text{h}^{-1} \times 1 \text{ h}$; [$n = 8$]); group 2 – furosemide + volume replacement: furosemide ($1.0 \text{ mg} \cdot \text{kg BW} \cdot \text{h}^{-1} \times 1 \text{ h}$) while continuously replacing urine losses with a variable infusion of isotonic Ringer solution (NaCl 154 mM, KCl 5.6 mM, CaCl_2 2.2 mM, NaHCO_3 25 mM, pH 7.4) at rates to match urine output ($n = 7$); group 3 – furosemide + volume replacement + losartan: furosemide ($1.0 \text{ mg/kg BW}^{-1} \cdot \text{h}^{-1} \times 1 \text{ h}$) while replacing urine losses after i.v. administration of losartan (10 mg/kg BW^{-1}) immediately before furosemide ($n = 7$).

Because GFR and RBF were slightly elevated in the first 20 min of clearance experiments, a study of RBF was undertaken in 6 rats with a transit time blood flow probe around the left renal artery [22]. Mean arterial pressure (MAP) was measured continuously through a femoral catheter connected to an electrically damped output of a pressure transducer (Powerlab system, AD Instruments). These rats were given 30 min to recover from surgery and infused for 30 min with furosemide i.v. to study the initial changes in RBF and RVR.

Series 2: Effects of Renal Denervation and Renal Nerve Deafferentation

Three groups of rats underwent survival surgeries 10 days before study. Rats were anesthetized with 2% isoflurane. The abdomen was shaved and disinfected with a povidone iodine prep pad. A midline abdominal incision was made, and the fat surrounding the renal artery and vein was dissected gently away from the vessels to expose the renal nerves. After each procedure, the incision was closed; the rats received a subcutaneous injection of buprenorphine ($0.05\text{--}0.1 \text{ mg/kg}$) and were observed for 1 h before returning to the animal facility. Group 4 – sham-operated rats: these rats underwent a bilateral approach to the kidneys but without renal nerve transection ($n = 6$); group 5 – renal denervation: all visible renal nerves were stripped from both renal arteries, and both renal arteries were painted with 10% phenol in ethanol ($n = 6$); group 6 – renal nerve deafferentation: after the dissection of the renal vein and artery, a small piece of gauze soaked in capsaicin solution (capsaicin, 33 mM in 5% ethanol, 5% Tween 80, and 90% normal saline) was wrapped around each renal artery for 15 min for selective ablation of renal afferent nerves ($n = 8$). Renal nerve deafferentation has been shown to drastically decrease renal pelvic content of the afferent nerve marker CGRP, while renal denervation decreased the efferent nerve marker norepinephrine [23, 24]. On the day of the experiment, each rat received furosemide ($1.0 \text{ mg} \cdot \text{kg BW}^{-1} \cdot \text{h}^{-1} \times 1 \text{ h}$) while replacing urine volume losses as in groups 2 and 3.

Statistics

Comparison of results after furosemide among different groups used one-way ANOVA and Turkey multiple comparison tests. Repeated measures one-way ANOVA and Dunnett's multiple comparison tests were used to compare the time course of effects of furosemide with baseline (vehicle) in each group. Results are presented as mean $\pm$ SE. A p value ≤ 0.05 was considered statistically significant.

Fig. 1. Mean $\pm$ SE values for mean arterial pressure, renal blood flow measured by a flowmeter, and renal vascular resistance before (–10 to 0 min) and during 20 min of furosemide infusion. Compared to before: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.005$ ($n = 6$ rats).

Results

Short-Term Effects of Furosemide on RBF and RVR Studied by Transit Time Flowmetry

Within 5 min of starting the furosemide infusion, the RBF was reduced and the RVR increased. These changes increased over the 20 min of furosemide infusion (Fig. 1).

Effects of Furosemide Alone or during Volume Replacement or AT1 Receptor Blockade

After 60 min of furosemide infusion, there were similar fractional reductions in GFR and RBF of circa 40% after furosemide alone or with volume replacement or losartan (Fig. 2A, B). RVR increased by $82 \pm 7\%$ with furosemide and by $92 \pm 10\%$ with furosemide plus volume replacement (NS) but only by $44 \pm 3\%$ with furosemide after the losartan ($p < 0.01$; Fig. 2C) because of a similar fall in RBF with losartan despite a fall in MAP ($p < 0.01$; Fig. 2D). The urine output (UV) after 60 min of i.v. infusion of furosemide was similar in the three groups with volume replacement (Fig. 2E), whereas the hydrostatic pressure in the proximal tubule (P_{PT}) after 60 min was significantly increased only in rats that received losartan ($p < 0.01$; Fig. 2F). There were no changes in heart rate or hematocrit, but the filtration fraction was reduced after losartan (Table 1). The time course of the absolute values for renal function and hemodynamics before and during furosemide in groups 1–3 are shown in Figure 3. The GFR and RBF fell significantly and the RVR increased progressively in all groups after 40 and 60 min following

Fig. 2. Mean $\pm$ SE values for fractional changes in renal hemodynamics, mean arterial pressure, urine output, and proximal tubule pressure after 60 min of a constant systemic infusion of furosemide (open bars, $n = 8$), furosemide with volume replacement (solid bars, $n = 7$), and furosemide with volume replacement and losartan (grey bars, $n = 7$). Compared to furosemide alone: * $p < 0.05$, ** $p < 0.01$.

furosemide infusion (** $p < 0.01$ vs. vehicle; Fig. 3A–C). The MAP was quite well maintained except after losartan, where it fell progressively (* $p < 0.05$ vs. vehicle; Fig. 3D). The UV increased more in the two groups with volume replacement than after furosemide alone (** $p < 0.01$ vs. vehicle; Fig. 3E). The hydrostatic pressure in the proximal tubule was elevated after 20 min in all groups (** $p < 0.01$ vs. vehicle) but fell progressively after 40 min, except in the losartan group (** $p < 0.01$ vs. vehicle; Fig. 3F).

Effects of Furosemide and Volume Replacement after Sham Procedures, Renal Denervation, and Renal Nerve Deafferentation

After 60 min of furosemide infusion with volume replacement, the GFR of rats with sham procedures or renal denervation was reduced by 31 ± 3 and $27 \pm 6\%$, respectively, but in rats with renal nerve deafferentation the GFR was reduced by only $13 \pm 8\%$ ($p < 0.05$ vs. other groups; Fig. 4A). There were no significant differences in the fall in RBF with furosemide in these groups (Fig. 4B) but the RVR after furosemide increased less in the group with renal deafferentation ($p < 0.05$; Fig. 4C). The UV and the P_{PT} increased similarly after furosemide in all three groups (Fig. 4E, F). There were no changes in heart rate or hematocrit but the filtration fraction increased in each group (Table 2).

Table 1. Heart rate, hematocrit, and filtration fraction of rats during infusion of Veh and after 20-min periods of intravenous infusion of F, F/VR, or F/VR/Los

	F	F/VR	F/VR/Los
<i>Heart rate, bpm</i>			
Veh	359±14	350±37	350±17
20 min	359±15	368±40	355±16
40 min	350±15	379±41	342±14
60 min	340±17	371±37	335±14
<i>Hematocrit, %</i>			
Veh	46±1	44±2	48±2
60 min	46±1	44±2	46±2
<i>Filtration fraction</i>			
Veh	0.49±0.03	0.42±0.02	0.51±0.03
20 min	0.51±0.03	0.40±0.02	0.47±0.02
40 min	0.55±0.04	0.41±0.03	0.45±0.03
60 min	0.55±0.04	0.43±0.03	0.44±0.03*

Values are mean ± SE ($n = 5$ –8 per group). Veh, vehicle (before); F, furosemide; F/VR, furosemide plus volume replacement; F/VR/Los, furosemide plus volume replacement plus losartan; 20, 40, 60 min, time into furosemide infusion. * $p < 0.05$ vs. baseline (Veh).

The time course of changes in the absolute values for renal function and hemodynamics during furosemide and volume replacement are shown in Figure 5. The GFR and RBF rose and the RVR fell in all groups after 20 min of furosemide (Fig. 5A–C). While the RBF decreased significantly in all groups after 60 min, a significant decrease in GFR was observed only in the sham group at 60 min, compared to its baseline (vehicle) (Fig. 5A, B). The RVR increased similarly in all three groups after 40–60 min (Fig. 5C). The UV increased significantly over this time in all groups, especially in the group with deafferentation (Fig. 5E). The P_{PT} increased progressively in all groups until 40 min of furosemide infusion and remained elevated at 60 min (Fig. 5F).

Discussion/Conclusion

We confirm that an intravenous infusion of furosemide into rats reduces the GFR by ~35% over 60 min [1]. The main new findings are that the progressive fall in GFR after furosemide is accompanied by a parallel reduction in RBF. There is a rise in urine output and a rise in P_{PT} that peaks after 20–30 min. These renal hemodynamic changes are unaffected by volume repletion or by AT-1 receptor blockade, although losartan moderated the rise in RVR over this time because of a fall in MAP. The fall in GFR and RBF in this study with furosemide is similar to a ROMK inhibitor that also blocks NaCl reabsorption in the loop of Henle [1]. Thus, the renal hemodynamic response to furosemide can be ascribed to its renal tubular actions. The fall in GFR with furosemide infusion was prevented by bilateral renal deafferentation.

The changes in GFR during furosemide have been shown by micropuncture methods to represent complex interactions between mechanisms of vasoconstriction/vasorelaxation and physical forces caused by inhibition of tubular fluid reabsorption [25, 26] that cannot be deciphered unequivocally by clearance methods [6]. There was a tendency for the GFR and RBF measured by 3H inulin and ^{14}C PAH clearance to increase initially, but the RBF measured constantly during 20 min by a transit time flowmeter was reduced progressively within 5 min of starting the furosemide infusion. The abrupt increase in urine flow with furosemide likely

Fig. 3. Mean $\pm$ SE absolute values for renal hemodynamics, mean arterial pressure, urine output, and proximal tubule pressure before (vehicle) and during 60 min of infusion of furosemide alone (solid circles and continuous lines, $n = 8$), furosemide and volume replacement (open circles and dashed lines, $n = 7$), and furosemide with volume replacement and losartan (open squares and dotted lines, $n = 7$). Compared to each respective vehicle: * $p < 0.05$; ** $p < 0.01$.

washed out filtered inulin and PAH that had been concentrated in tubular fluid that led to an artifactual early increase in the estimated GFR and RBF by clearance methods. A new steady state was achieved by 40–60 min. Therefore, the artefact should not influence the conclusions of this study.

The GFR of severely fluid-depleted rats infused with furosemide is maintained by a high efferent arteriolar resistance that is due to angiotensin since the glomerular capillary pressure (P_{GC}) and the GFR collapse after angiotensin-converting enzyme inhibition [27].

However, careful restoration of fluid and Na^+ losses alone, or in combination with losartan, did not modify the fall in GFR with furosemide. Surprisingly, furosemide given without volume replacement did not increase the hematocrit, despite considerable diuresis. This may relate to its venodilating effect that can redistribute fluid into the blood stream [28]. There was indirect evidence that losartan reduced the efferent arteriolar resistance from a lesser increase in RVR and a fall in filtration fraction. Thus, activation of AT1Rs modulated the renal hemodynamic response to the loop diuretic but was not an essential component of the reduction in the GFR.

Fig. 4. Mean $\pm$ SE values for fractional changes in renal hemodynamics, mean arterial pressure, urine output, and proximal tubule pressure before (vehicle) and after 60 min of a constant systemic infusion of furosemide with volume replacement 10 days after sham surgery (diagonal hatched bars, $n = 6$), bilateral renal denervation (double crosshatched bars, $n = 5$), or bilateral renal nerve deafferentation (horizontal lined bars, $n = 8$). Compared to sham surgery: * $p < 0.05$.

Prior studies with loop diuretics have disclosed some underlying mechanisms that affect renal hemodynamics. TGF vasoconstricts the renal afferent arterioles during increases in reabsorption of Na^+ , K^+ , and Cl^- by NKCC2 in the macula densa cells. Furosemide blocks NKCC2 reabsorption and thereby blocks TGF. This occurs rapidly after furosemide is infused directly into the tubular lumen of the loop of Henle or infused intravenously into rats [1]. Therefore, inhibition of TGF by furosemide should dilate the renal afferent arterioles and increase the GFR and RBF. However, renal vasodilation was not detected by the constant, direct measurement of RBF during the first 20 min of furosemide (Fig. 1). Rather, other factors causing a progressive reduction in GFR and RBF and increase in RVR presumably overcame any effect of furosemide to block TGF.

The net glomerular ultrafiltration pressure in the rat outer cortical glomeruli averages only about 16 mm Hg, despite a glomerular capillary hydrostatic pressure of about 49 mm Hg, because of the offsetting effects of the plasma protein oncotic pressure of about 15 mm Hg and the intratubular hydrostatic pressure of about 17 mm Hg [29]. Accordingly, the

Table 2. Heart rate, hematocrit, and filtration fraction of rats during infusion of vehicle (Veh) and during 20-min periods of intravenous infusion of F/VR 10 days after sham surgery (Sham), bilateral renal denervation, or deafferentation

	Sham	Denervation	Deafferentation
<i>Heart rate, bpm</i>			
Veh	319±14	303±32	332±14
20 min	319±13	295±32	332±14
40 min	319±13	289±31	330±13
60 min	321±13	286±30	326±14
<i>Hematocrit, %</i>			
Veh	46±2	45±1	44±1
60 min	45±2	45±1	45±1
<i>Filtration fraction</i>			
Veh	0.48±0.03	0.52±0.03	0.44±0.02
20 min	0.53±0.05	0.56±0.01	0.48±0.02
40 min	0.58±0.02	0.62±0.04	0.54±0.02*
60 min	0.62±0.03**	0.68±0.03**	0.58±0.03*

Values are mean ± SE ($n = 5$ – 8 per group). Veh, vehicle (before); F/VR, furosemide plus volume replacement; 20, 40, 60 min, time into furosemide infusion. * $p < 0.05$; ** $p < 0.01$ vs. baseline (Veh).

increase in P_{PT} of ~10 mm Hg in the first 20 min of furosemide might have been expected to reduce the GFR profoundly. However, the rise in P_{PT} after furosemide is matched by a similar rise in the intrarenal tissue pressure [30] that itself is matched by a rise in the glomerular capillary hydrostatic pressure (P_{GC}) [25, 26]. A rise in intrarenal tissue pressure with furosemide [30] could collapse the peritubular capillaries and add to the flow resistance of the arterioles that would cause a passive rise in RVR. However, some of the increased RVR was apparently active vasoconstriction from angiotensin II since losartan moderated the progressive rise in RVR and reduced the FF, as previously reported [27].

Furosemide causes a sharp rise in the volume of the distal tubule of the rat, but the volume of the proximal tubule is maintained [31]. This may be explained because the proximal tubule is separated from the site of furosemide action in the loop of Henle by the thin loops of Henle that are the site of the maximum flow resistance in the nephron [32]. In contrast, the distal tubules have a low flow resistance, but likely develop a steep rise in pressure during increases in tubular fluid flow produced by furosemide because of a high flow resistance downstream in the collecting ducts [32]. An increase in resistance to flow through the nephron caused by increased fluid presentation to segments with high flow resistance presumably itself causes some reduction in clearance of glomerular filtrate. Thus, the increase in P_{PT} during furosemide likely represents an increase in distal tubule volume and pressure, leading to a rise in renal interstitial pressure [30]. The return of P_{PT} towards baseline, despite ongoing tubular effects of furosemide, may represent offsetting effects of reduced tubular filling from a sharply falling GFR [33, 34] and a reduction in the resistance of the renal capsule to stretch that moderates over time [35].

Furosemide activates renal efferent nerves in the rat [36–38] that would be anticipated to reduce the GFR, yet renal denervation does not prevent a reduction in GFR after furosemide in conscious lambs [39]. Furosemide also activates renal afferent nerves in anesthetized cats [40]. The sharp rise in P_{PT} likely reflects an increase in intrapelvic pressure that activates renal afferent baroreceptors that also are limited to renal afferent nerves [41]. Indeed, increases in urine flow rate that raise the pelvic pressure by as little as 3 mm Hg increase the rate of firing

Fig. 5. Mean ± SE absolute values for renal hemodynamics, mean arterial pressure, urine output, and proximal tubule pressure before (vehicle) and during 60 min of infusion of furosemide with volume replacement 10 days after sham surgery (solid triangles and continuous lines, $n = 6$), renal denervation (open triangles and dashed lines, $n = 5$), or renal nerve deafferentation (solid squares and dotted lines, $n = 8$). Compared to each respective vehicle: * $p < 0.05$; ** $p < 0.01$.

of the renal afferent nerve fibers [42]. Moreover, an increase in the urinary sodium concentration should stimulate the renal pelvic chemoreceptors. Thus, furosemide likely activated renal afferent nerves via chemoreceptors and/or baroreceptors in the kidneys [43, 44]. Furosemide increases the renal efferent sympathetic nerve discharge and reduces the GFR by 20%. The finding that renal deafferentation blunted the fall in GFR with furosemide implicates the renal afferent nerves, but these studies leave unresolved what mediates this effect.

Stimuli that activate renal afferent nerves either acutely [11, 41–46] or over a prolonged period [12, 13, 16, 17], generally reflexly activate the renal efferent nerves, and this causes renal vasoconstriction and a fall in GFR. Accordingly, renal nerve deafferentation and renal nerve denervation were undertaken to assess the roles of renal innervation. Both maneuvers prevented a significant reduction in GFR with furosemide (Fig. 5A), but a puzzling finding was that only renal deafferentation prevented a reduction in the fractional fall in GFR (Fig. 4). Renal denervation depletes renin [47, 48], blunts responses to nitric oxide [49] and adenosine [50, 51], and reduces the BP [12, 52], all of which may modulate the hemodynamic response. Renal denervation lowered the basal MAP, RBF, and GFR (Fig. 5). Loop diuretics block the TGF mechanism [1] and thereby impair renal autoregulation especially when the perfusion

pressure is initially low and the range of myogenic relaxation is almost exhausted [53]. Thus, the fall in BP after renal denervation in these studies may have caused a fall in RBF and GFR with furosemide and thereby concealed an effect of the denervation procedure to maintain the GFR with furosemide. Therefore, the role of the renal efferent nerves in mediating the response to afferent nerve activation with furosemide in these studies remains unclear.

In conclusion, furosemide triggers an afferent renal nerve signal that causes renal vasoconstriction and a reduction in GFR by an unclear mechanism. A second, perhaps related, component of the fall in GFR is an increase in intrarenal pressure. However, the fall in GFR is not itself a consequence of a reduction in extracellular fluid volume or activation of AT1Rs.

Perspective

The present study suggests that selective pharmacological prevention of renal afferent nerve activation [45, 46] may be beneficial in preserving renal hemodynamics during furosemide treatment. The studies of Ulla Kopp and colleagues have shown the rich intrarenal regulation of renal afferent nerve activation [43] that entails endothelin-1 [45] and endothelin type A and B receptors [44, 46], angiotensin AT1 receptors [46], prostaglandins, adrenergic alpha-1 and alpha-2 receptors [42], and substance P [41] that could be targets to relieve diuretic-induced azotemia or unwanted fluid retention. Our results also caution against routinely attributing rises in serum creatinine to furosemide-induced hypovolemia or activation of the RAS since furosemide caused major reductions in GFR despite volume replacement or losartan in this study.

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Statement of Ethics

This study was conducted ethically in accordance with the World Medical Association Declaration of Helsinki. There were no human subjects involved in the research.

The procedures used to gather the data for the manuscript were approved and monitored by the Georgetown University Institutional Animal Care and Use Committee.

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Author Contributions

M.A., C.S.W., W.J.W. designed the study. M.A. performed all GFR/micropuncture experiments and the denervation/deafferentation surgeries. G.S. performed the short-term RBF experiments. M.A. analyzed the data and prepared figures and tables. M.A., W.J.W., and C.S.W. contributed to the interpretation of the results. M.A. wrote the first draft of the manuscript. C.S.W. wrote the protocol and the final drafts of the manuscript.

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