Studies on the Tubulo-Glomerular Feedback System in the Rat

THE MECHANISM OF REDUCTION IN FILTRATION RATE WITH BENZOLAMIDE

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ABSTRACT The specific mechanisms whereby superficial nephron glomerular filtration rate (sngfr) is reduced after the administration of benzolamide, a carbonic anhydrase inhibitor with a primary inhibitory effect in the proximal tubule, have been examined by measuring pertinent pressures, flows, and glomerular permeabilities in the hydropenic Munich-Wistar rat, a strain with surface glomeruli. Because benzolamide decreases absolute proximal reabsorptive rate, the rate of delivery of tubular fluid to the distal nephron should be at least transiently increased and may reduce sngfr by activating the tubulo-glomerular feedback system. Sngfr fell from 29.2±2.0 to 21.1±3.1 nl/min ($P < 0.01$) after benzolamide (group 1), a percentage reduction equal to kidney glomerular filtration rate and similar to sngfr obtained in collections from distal tubules.

Separate studies (group 2) revealed that if transient increases in distal nephron delivery were prevented by insertion of a long oil block in proximal tubules before control, the decrease in sngfr was prevented (30.3±1.0 vs. 30.3±1.8 nl/min, $P > 0.9$). In paired "unblocked" nephrons in the same rats, sngfr fell in group 2 (33.0±1.0 vs. 25.2±2.3 nl/min, $P < 0.01$). In "blocked" nephrons in which sngfr reduction was prevented, the rate of fluid leaving the proximal tubule increased from 16.9±0.9 to 23.1±1.0 nl/min ($P < 0.01$). In group 1 studies in which sngfr fell and transient increases in flow out of the last segment of the proximal tubule (distal delivery) (≅8 nl/min) were not prevented, steady-state distal delivery was unchanged by benzolamide (13.9±1.1 vs. 14.2±2.2 nl/min). Also, sngfr returned toward control, pre-benzolamide values, when a proximal oil block was placed for 15 min and the rate of distal delivery reduced after benzolamide administration, which suggests that this activation was reversible. These data suggest that activation of tubulo-glomerular feedback by transient increases in distal delivery was responsible for decreases in sngfr.

Analysis of all determinants of glomerular ultrafiltration revealed that the efferent mechanism leading to reduced sngfr after benzolamide was decreased nephron plasma flow (101±13 vs. 66±13 nl/min, $P < 0.01$). Hydrostatic pressures and the glomerular permeability coefficient did not contribute to reductions in sngfr with benzolamide. Because the rate of distal delivery remained constant in spite of large changes in both sngfr and absolute proximal reabsorptive rate, it is suggested that the rate of distal delivery may be the physiologic entity that is regulated by the tubulo-glomerular feedback system via alterations in sngfr.

INTRODUCTION

The renal contribution to volume homeostasis requires a delicate balance between the rates of glomerular filtration and tubular reabsorption (1). The fact that the rate of tubular reabsorption responds appropriately to spontaneous variations in the filtered load has been recognized for some years (1–3). It is now also recognized that either the rate of tubular reabsorption within

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the distal nephron or the rate of delivery of fluid out of the proximal tubule modifies the rate of filtration in single nephrons (4–8). This juxtaposed control system has been termed tubulo-glomerular feedback (5). Impressive evidence for the operation of the tubulo-glomerular feedback system has been provided primarily from microperfusion studies in single nephrons (5, 9–13).

Studies which have demonstrated tubulo-glomerular feedback activity have been performed primarily at the single nephron level, although most investigators propose that the system must assume an important role in the control of whole kidney function in all nephrons. The vast majority of investigative effort in this area has been directed towards the definition of the afferent signal of the feedback system (4, 12, 14–16). The most recent candidate for the entity which constitutes the afferent limb of the system is the rate of chloride reabsorption in the distal nephron, possibly at the macula densa cell (11, 14, 17, 18). The efferent mechanisms that produce changes in nephron filtration rate have not been fully defined. Certain investigators have noted reductions in stop-flow pressure and directly measured glomerular capillary hydrostatic pressure (P_G) when distal delivery was increased by microperfusion, which suggest that changes in glomerular capillary pressure may have some role in the efferent limb of the tubulo-glomerular feedback system (13, 15, 17). We have examined the effects of benzolamide, a carbonic anhydrase inhibitor with few nonrenal effects, on single nephron and kidney glomerular filtration rate (GFR). This diuretic inhibits reabsorption primarily in the proximal tubule and has little or no effect on reabsorptive rate in the distal nephron (19–21). This agent should lead to acute increases in delivery of fluid out of the proximal tubule and may thereby activate the tubulo-glomerular feedback system and result in reduced superficial nephron filtration rate (sngfr). If this were the case, then the mechanisms that produce reduced sngfr could be specifically defined. The goals of the present study are as follows: (*a*) to determine if benzolamide administration leads to reductions in filtration rate, (*b*) to determine if this reduction in sngfr is caused by activation of the tubulo-glomerular feedback system, and (*c*) to define the determinants of sngfr that participate in the efferent limb of the tubulo-glomerular feedback system activated by benzolamide administration.

GLOSSARY OF SYMBOLS

APR	Absolute proximal reabsorptive rate.
AR	Afferent arteriolar resistance.
C	Protein concentration.
C_A	Systemic protein concentration.
EFP	Effective filtration pressure.
ER	Efferent arteriolar resistance.
FR	Fractional reabsorption.
GFR	Glomerular filtration rate.
L_pA	Glomerular permeability coefficient.
p	Plasma count rate.
P_G	Glomerular capillary hydrostatic pressure.
ΔP	Hydrostatic pressure gradient across glomerular capillary.
π	Oncotic pressure.
π_A	Systemic oncotic pressure.
π_E	Efferent oncotic pressure.
rbf	Nephron blood flow.
rpf	Nephron plasma flow.
snff	Superficial nephron filtration fraction.
sngfr	Superficial nephron glomerular filtration rate.
TF	Tubular fluid concentration.
V_f	Volume of tubular fluid collected per minute.
UV	Total count rate of tubular fluid collected per minute.
x^*	Length of glomerular capillary.
—	Bar (superscript) designates mean value.

METHODS

Studies were performed in Munich-Wistar rats (190–260 g body wt), bred and maintained in a colony housed at the Animal Research Facility, Veterans Administration Hospital, San Diego, Calif.

Micropuncture studies on the effects of benzolamide administration: group 1 studies. Surgical preparation of rats for micropuncture was as previously described in recent studies from this laboratory (22–24). All micropuncture studies were paired, with the control period in the hydropenic condition. After preparation for micropuncture, a continuous infusion of [^{14}C]inulin in isotonic NaCl-$NaHCO_3$ (0.5% body wt per h) was begun at ≅40 μCi/h. At the end of a 60-min equilibration period, all measurements were performed. The last segment of surface proximal tubules was localized by injection of small volumes of lightly stained (FD and C green, Allied Chemical Corp., Specialty Chemicals Div., Morristown, N. J.) isotonic NaCl-$NaHCO_3$ (3–5-μm tip pipettes) into random proximal segments. Complete collections of late proximal tubular fluid were obtained over 2–4 min into 8–11-μm tip-sharpened pipettes. Collections of tubular fluid were then tipped with oil to prevent evaporation before volume measurements. Sngfr and absolute and fractional reabsorption (FR) were determined from these samples and concurrent plasma samples for [^{14}C]inulin concentration. Kidney GFR was also measured concurrently with individual nephron observations.

Control period. Five tubular collections were obtained in the control period. At least three samples of efferent peritubular capillary blood were also obtained from "star" vessels on the kidney surface (13–16-μm tip pipettes). Samples of femoral artery blood were also obtained concurrently for microprotein determinations. Details of the micro-adaptation of the Lowry protein method (25) have been described in previous publications from this laboratory (22, 26). Glomerular capillary and Bowman's space hydrostatic pressure were then measured in all available surface glomeruli with a servo-nulling device with 1–3-μM tip pipettes. Methods of pressure measurement and the mechanics of the pressure monitoring device have been described in detail in previous publications from this laboratory (22, 24, 26, 27).

Experimental period. After completion of all control period measurements, benzolamide was administered as a bolus of 2 mg/kg body wt in 250 μl over 5 min and then infused at a rate of 2 mg/kg body wt per h. The agent was suspended in 300 mM $NaHCO_3$ solution and administered at a volume

rate of ≅0.5% body wt per h (19). We have found that the diuresis is modest after benzolamide, and the replacement rate and $NaHCO_3$ concentration were both sufficient to maintain volume constant and serum bicarbonate concentrations equal to the control condition. Urine flow rate was stabilized at 20 min after the onset of benzolamide infusion, and at this time all measurements of pressures, sngfr, tubular reabsorptive rate, and efferent peritubular capillary proteins were repeated. At the completion of each study a sample of renal venous blood was obtained to calculate total kidney filtration fraction and renal plasma flow from the extraction rate for inulin.

In separate studies, sngfr, before and after benzolamide, were evaluated from distal tubule collections. Distal tubules were similarly localized by injections of FD- and C green-stained fluid into random proximal tubules. Collections differed from the proximal tubule only in that 7–8-μm tip glass pipettes were used because of the narrower distal tubular lumens in the Munich-Wistar rat.

Studies on the role of activation of tubulo-glomerular feedback in the reduction in nephron filtration rate: group 2 studies. If a reduction in sngfr occurs after benzolamide administration, it could be the result of activation of the tubulo-glomerular feedback system secondary to early, transient increases in delivery out of the proximal tubule caused by the reduction in proximal tubular reabsorption. If this were the case, then any maneuver that prevents this increase in distal delivery should also prevent the reduction in sngfr. In separate studies on five rats, a long castor oil block was placed in four tubules ≅20–30 min before control measurement of sngfr and vented proximal to the block to allow filtrate to escape onto the surface. Sngfr was then measured proximal to the oil block after a short mineral oil block was placed. After the measurement of sngfr in control blocked tubules, the tubule was again vented proximal to the collection site. Sngfr was also measured in four other tubules that were not blocked in the control period, alternating in sequence with blocked tubules.

Benzolamide was then administered in the same doses as described in the previous studies. In spite of the decrease in absolute tubular reabsorption with benzolamide (19), oil blocks remained stable. It is reasonable to assume that tubular fluid escaped onto the surface in blocked tubules and distal delivery remained at zero in these nephrons. After benzolamide, sngfr was measured in the four blocked tubules by recollection and in four new unobstructed tubules in which distal delivery had been elevated in the nonsteady state. Measurements of sngfr alternated between "blocked" and "unblocked" tubules in control and experimental periods. The change in sngfr between control and post-benzolamide periods were compared between blocked and unblocked tubules. Control studies without benzolamide were also performed to insure that the "blocking" maneuver did not increase sngfr and controlled for the time element during the course of the study. In separate studies the actual increase in distal delivery (or volume of tubular fluid collected per minute, V_f) was evaluated in blocked nephrons by placing the castor oil block at the last segment of the proximal tubule. This site was vented and observations of sngfr and volumes collected were obtained before and after benzolamide to exactly quantitate changes in the rate of fluid leaving the late proximal tubule, but in tubules in which delivery to the distal nephron had been prevented.

Studies on reversibility of activation of feedback mechanisms with benzolamide: group 3 studies. In an additional group of six rats, the reversibility of the effects of benzolamide was examined. Sngfr was measured in five tubules in control conditions and was again measured in five new nephrons after a waiting period of ≅30–40 min after benzolamide administration. Immediately after finishing the collection of filtrate from the nephron, a long castor oil block was inserted distal to the collection site and the tubule vented proximal to the castor oil block, ceasing all flow to the distal portion of the nephron. After a 15-min waiting period, recollection of filtrate was made in the blocked nephron, obtaining paired values for sngfr in the five tubules before and after tubular blockade. Extreme care was taken to assure that the oil block did not flow retrograde and cover the vent holes allowing inulin to concentrate in the proximal tubule such that an artificially high value for sngfr would result from the recollection. The sngfr was remeasured in these tubules by recollection to determine if this period of reduced distal delivery would lead to restoration and increases in sngfr towards the control values.

Analytic methods. Urine and plasma sodium and potassium concentrations were determined on an Instrumentation Laboratories flame photometer (Instrumentation Laboratory Inc., Lexington, Mass.). Serum total CO_2 was determined on a Harleco Micro CO_2 apparatus (no. 64987) (Hartman-Leddon Co., Gibbstown, N. J.). Total filtration rates, renal plasma flow, and renal blood flow were calculated as previously described (23, 27, 29, 30). ^{14}C counts in plasma, urine, and tubular fluid were monitored on a model 2425 Packard liquid scintillation counter (Packard Instrument Co., Inc., Downers Grove, Ill.).

For the microprotein measurements, human albumin (Cutter Laboratories Inc., Berkeley, Calif.) was used for standards. We have previously demonstrated that in a range of 2–10 μg/100 ml protein, human albumin provides identical values in the microprotein assay as does the same range of dilute to concentrated rat plasma from Munich-Wistar littermates (50% albumin:50% globulin) (23). Colloid osmotic pressure on rat plasma standards also correlated with the predictions of Landis and Pappenheimer (30).

Nephron filtration rate (sngfr = UV/p) was calculated from the total count rate of tubular fluid collected per minute (UV) divided by the plasma count rate (p) (corrected for plasma water). Volume of tubular fluid was determined by transferring the sample to a calibrated, constant bore glass capillary tube and by measuring the length of the aqueous column between oil blocks.

Fractional reabsorption (FR) at the late proximal tubule is defined as follows:

$$FR = (1\text{-}p/TF) = (sngfr\text{-}V_f/sngfr),$$

where TF = tubular fluid concentration of [^{14}C]inulin at the late proximal tubule and V_f = the volume of tubular fluid collected per minute (equal to the rate of distal delivery). Absolute proximal reabsorption (APR) is defined as follows:

$$APR = sngfr\ (FR) = sngfr\ (1\text{-}p/TF).$$

Calculations. The calculation of superficial nephron filtration fraction (snff), nephron plasma flow (rpf), nephron blood flow (rbf), afferent arteriolar resistance (AR), and efferent arteriolar resistance (ER) was as previously described (29). Oncotic pressure (π) was determined from the protein concentration by the method of Landis and Pappenheimer (30). The specific modifications to the equations describing these relationships have been shown in previous publications from this laboratory (22, 26).

The sngfr is determined by four factors: (*a*) the hydrostatic pressure gradient acting across the glomerular capillary (ΔP), (*b*) the systemic oncotic pressure (π_A), (*c*) the glomerular permeability coefficient (L_pA), and (*d*) the rpf (31).

The sngfr is a product of the mean effective filtration pressure ($\overline{EFP}$) and the L_pA (sngfr = $\overline{EFP} \cdot L_pA$). The EFP can be

defined as follows:

$$EFP = \Delta P - \pi.$$

π rises along the length of the glomerular capillary (x^*) (normalized to a unit length) as a result of the formation of glomerular ultrafiltrate and the resultant increase in protein concentration (C). The $\overline{EFP}$ is defined as follows:

$$\overline{EFP} = \int_0^1 (\Delta P - \pi) dx^*.$$

Changes in rpf modify the EFP profile by affecting the rate of concentration of protein and π along x^*. By using mathematical models of glomerular filtration as previously described (22, 28) and applying data to the computer, a profile for EFP and the integrated value for $\overline{EFP}$ can be generated. A value for the L_pA is also generated by this method. At filtration pressure equilibrium ($\pi_E \approx \Delta P$), as has been demonstrated for the hydropenic Munich-Wistar rat (22, 32), a specific numerical value for L_pA and $\overline{EFP}$ cannot be defined. At filtration pressure equilibrium only a minimal value for L_pA and a maximal value for $\overline{EFP}$ can be defined (22, 31).

Statistical analysis. Significance of data between control and experimental conditions was determined by analysis of variance and Student's t test where appropriate (33).

RESULTS

Group 1 studies. After the administration of benzolamide, urine flow increased from 1.5±0.1 μl/min in

TABLE I

Rates of Filtration and Tubule Reabsorption before and after Benzolamide Administration in Group I Rats

Rat no.	Period	GFR	sngfr	Absolute proximal reabsorption	Distal delivery	Fractional reabsorption
		ml/min	*nl/min*	*nl/min*	*nl/min*	%
7	Control	0.89	24.1 ±1.9	11.1 ±0.6	13.0 ±1.4	0.49 ±0.05
	Experimental	0.74	19.2 ±1.6	5.7 ±0.7	13.4 ±1.2	0.30 ±0.02
8	Control	1.12	30.3 ±4.2	15.8 ±0.4	15.7 ±3.3	0.51 ±0.05
	Experimental	0.78	16.7 ±3.9	4.5 ±0.9	8.8 ±2.4	0.34 ±0.02
9	Control	1.01 ±0.09*	29.9 ±2.2	15.0 ±1.2	14.8 ±2.5	0.51 ±0.05
	Experimental	0.74	22.6 ±1.4	7.5 ±1.8	15.0 ±1.5	0.33 ±0.08
10	Control	0.58	29.3 ±2.1	17.2 ±1.3	12.1 ±1.3	0.59 ±0.03
	Experimental	0.57	21.6 ±1.4	8.9 ±0.4	12.7 ±1.0	0.49 ±0.01
12	Control	0.77 ±0.14	24.5 ±3.0	14.2 ±1.7	10.2 ±1.4	0.58 ±0.01
	Experimental	0.74	15.8 ±1.4	4.6 ±1.0	10.7 ±1.1	0.29 ±0.06
15	Control	1.09	37.5 ±1.9	19.7 ±1.9	17.8 ±1.1	0.52 ±0.03
	Experimental	0.86	36.8 ±3.7	12.4 ±1.2	24.4 ±2.6	0.34 ±0.01
Overall mean	Control	0.91 ±0.08	29.1 ±2.0	15.5 ±1.2	13.9 ±1.1	0.53 ±0.02
	Experimental	0.74 ±0.04	21.1 ±3.1	7.3 ±1.2	14.2 ±2.2	0.34 ±0.02
P value		<0.01	<0.01	<0.01	NS	<0.01

* ±SEM.

control to 6.2±1.0 μl/min ($P < 0.01$) in the left kidney. Hematocrit remained constant at 56±1%, which suggests that no major alterations in plasma volume occurred during the time course of the study. Mean arterial pressure, however did decrease slightly from 112±2 to 102±3 mm Hg ($P < 0.05$). Plasma total CO_2 before and after benzolamide administration was 21.2±0.9 and 19.5±1.0 mM/liter ($P > 0.2$), respectively, which demonstrates that with the bicarbonate-rich replacement infusion, metabolic acidosis did not develop as a result of benzolamide administration (19).

The kidney GFR decreased from 1.14±0.1 to 0.93±0.04 ml/min/g kidney wt (0.91±0.08 to 0.74±0.04 in absolute values) ($P < 0.01$) which was a similar (25%) reduction to that observed for the sngfr at 29.2±2.0 in control and 21.1±3.1 nl/min after benzolamide (the mean of animal mean sngfr) ($P < 0.01$). The fact that sngfr and kidney GFR fell proportionately (Table I) suggests that changes in sngfr measured in proximal tubules over 2–3 min with replacement of an oil block was an accurate reflection of the degree of change in other nephrons in the kidney.

The sngfr in distal collections in a separate group of rats decreased similarly with benzolamide, from 40.0±2.1 to 33.8±1.7 nl/min ($P < 0.01$). This finding demonstrates that proximal collections yielded valid measurements of sngfr and that 2–3 min interposition of an oil block did not reverse the benzolamide induced reduction in sngfr. Kidney GFR decreased from 1.05±0.05 to 0.90±0.04 ml/min in the same rats ($P < 0.05$) (five rats).

APR in group 1 rats decreased from 15.5±1.2 to 7.3±1.2 nl/min after administration of the drug or less than 50% of control ($P < 0.01$) (Table I). This direct effect of benzolamide on APR should have resulted in an acute, transient increase in distal delivery of ≅8 nl/min (see group 2 studies). However, after benzolamide, FR decreased from 0.53±0.02 to only 0.34±0.02 ($P < 0.001$). This decrease in FR would have been much larger had not the sngfr also decreased. In the steady state, distal delivery remained remarkably similar to the control condition at 13.9±1.1 in control and 14.2±2.2 nl/min ($P > 0.5$) after benzolamide (Table I). One can observe that this tendency for APR and sngfr to decrease by similar absolute quantities also held when each animal was analyzed (Table I).

Examination of the role of tubulo-glomerular feedback systems in the reduction in filtration rate after benzolamide: group 2 studies. The sngfr in blocked and unblocked tubules was evaluated before and after benzolamide administration to determine if preventing completely the increase in the rate of delivery of fluid out of the proximal tubule also interfered with the reduction in sngfr.

In unblocked tubules control sngfr was 33.0±1.0 nl/min and fell to 25.2±2.3 nl/min during benzolamide ($P < 0.01$) (Fig. 1). The kidney GFR fell by 19% in these same animals, which suggests that changes in sngfr in

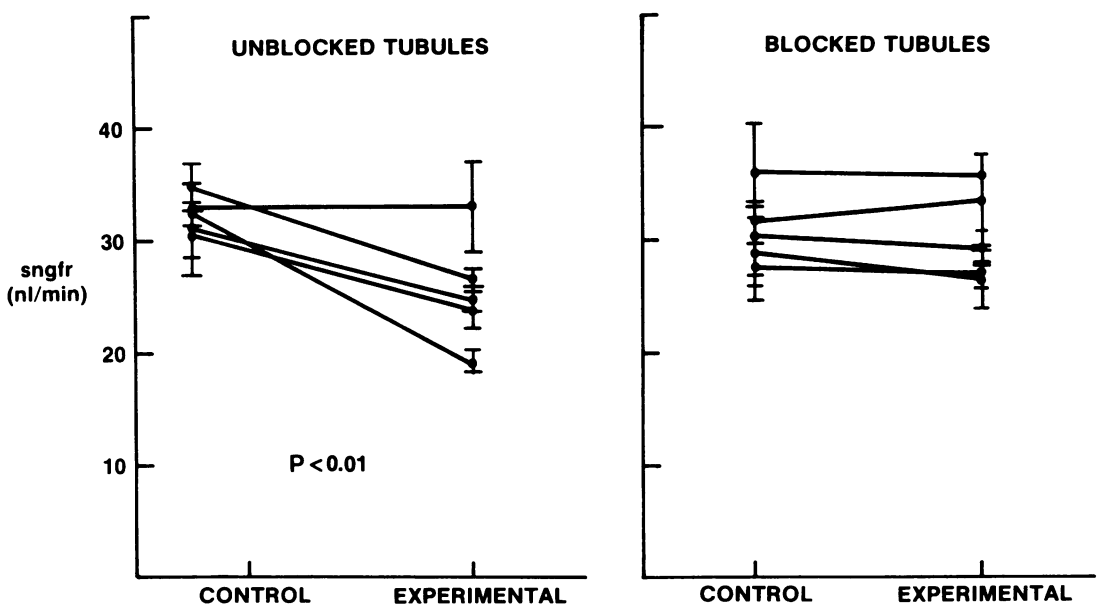

FIGURE 1 A comparison of the effects of benzolamide upon sngfr in group 2 rats in blocked (right) and unblocked tubules (left). Castor oil blocks were inserted into proximal tubules in each rat in control conditions (right) to prevent an increase in distal delivery. This maneuver prevented a reduction in sngfr observed in unblocked tubules in which early increases in distal delivery must have occurred with benzolamide.

unblocked tubules paralleled those in kidney GFR. However the sngfr did not change in tubules that were blocked in the control period to prevent the initial increases in the rate of distal delivery (30.3±1.0 in control and 30.3±1.8 nl/min during benzolamide in blocked tubules) ($P > 0.9$) (Fig. 1). These results indictate that the reduction in sngfr with benzolamide is the consequence of the nonsteady-state increases in distal delivery and most logically a consequence of activation of the tubulo-glomerular feedback system.

The specific effects of prolonged obstruction of tubules upon sngfr was examined in two rats. Blocks were inserted into a total of eight tubules before control measurements and sngfr was measured. Sngfr were also determined in eight control unblocked tubules in these same rats. No benzolamide was administered but blocked tubules were recollected after 60–70 min and sngfr was evaluated in eight unblocked tubules. With time, there was no change in sngfr in either unblocked (32.4±1.3 to 32.2±3.2 nl/min) or blocked (34.0±3.1 to 34.2±3.9 nl/min) tubules. Hematocrits were 54±2 in control and 55±1% after the 60–70-min period. These studies suggest that prolonged tubular blockade alone has no effect upon sngfr.

The actual increase in the V_t was evaluated in a separate group of rats in blocked nephrons. In these studies sngfr was 33.5±1.9 in control and 32.2±1.9 nl/min after benzolamide (NS), similar to other studies described above. The V_t or distal delivery increased from 16.9±0.9 to 23.1±1.0 nl/min ($P < 0.01$) after benzolamide, as a result of the reduction in APR. This increase of 6.2 nl/min in distal delivery (actually onto the surface) was very similar to the 8 nl/min predicted from group 1 studies if sngfr was not decreased by the administration of benzolamide.

Studies on the reversibility of activation of feedback mechanisms with benzolamide: group 3 studies. Sngfr was measured in hydropenia and after administration of benzolamide in six animals in a separate study. Recollections of tubular fluid were made after an initial collection to measure the changes in sngfr on a paired basis during benzolamide infusion after 15 min of cessation of distal delivery. Both the initial collection and the recollection were compared to control values and analyzed on a paired t test to each other. These results are shown on Table II. There was a significant reduction in sngfr after administration of benzolamide ($P < 0.01$), (Table II). After 15 min of distal blockade there was a significant rise in sngfr from the initial collection ($P < 0.01$, Table II) which tended to reverse the decrease in sngfr and return the filtration rate in the blocked tubules toward control values. However, this reversal was not complete and there remained a significant difference between the control hydropenic sngfr and the recollection values ($P < 0.05$). Although the

TABLE II

Effect of Complete Blockade of Distal Delivery (15 min) upon Sngfr after Administration of Benzolamide

No.	Control	After benzolamide administration: Initial collection	After benzolamide administration: Recollection after 15 min blockade
1		26.4	29.6*
		26.5	27.8
		22.0	25.9
		23.5	29.0
	Mean±SE		
	27.2±4.0	24.6±1.1	28.1±0.8
2		26.9	30.9
		29.0	39.5
		32.4	34.8
		22.3	28.1
		38.2	40.1
	Mean±SE		
	39.2±1.3	29.8±2.7	34.7±2.3
3		25.9	30.5
		15.0	28.2
		33.5	36.4
		29.9	42.2
		38.7	39.6
	Mean±SE		
	37.9±3.2	28.6±4.0	35.4±2.6
4		34.5	32.2
		31.4	28.6
		29.1	20.1
		24.2	26.7
		44.4	37.2
	Mean±SE		
	38.7±1.8	32.7±3.4	29.0±2.8
5		29.0	35.1
		21.8	24.3
		25.4	30.0
		24.1	37.1
		23.9	22.0
	Mean±SE		
	30.3±1.2	23.8±1.4	29.7±2.9
6		19.1	28.6
		29.9	24.0
		15.0	23.2
		31.1	33.0
		38.2	34.3
	Mean±SE		
	32.6±1.3	26.7±4.2	28.6±2.3
Group mean±SE	34.3±2.0	27.7±1.4‡	30.9±1.3§

* Paired collections.

‡ $P < 0.01$ compared to control sngfr.

§ $P < 0.01$ compared to initial collection after benzolamide.

reversal of sngfr was not complete, these results indicate that cessation of delivery of fluid out of the proximal tubule to distal portions of the nephron causes sngfr to rise which was not the case in hydropenia (group 2 studies).

Analysis of efferent mechanism of the tubuloglomerular feedback system leading to reduced nephron filtration rate: group 1 rats. All of the determinants of glomerular ultrafiltration were also measured in the same rats in which sngfr fell from 29.2±2.0 to 21.1±3.1 nl/min after benzolamide (Table III). The Bowman's space pressure remained unchanged after benzolamide (13.7±1.1 vs. 13.4±1.0 mm Hg) ($P > 0.3$). P_G was 47.9±1.7 in the control period and 45.9±1.2 mm Hg after benzolamide ($P > 0.2$) (Fig. 2). Therefore ΔP was also unchanged (34.2±1.0 vs. 33.1±0.6 mm Hg) ($P > 0.2$) (Fig. 2). Changes in hydrostatic forces then did not contribute to the alterations in sngfr after benzolamide.

The systemic protein concentration (C_A) fell from 6.0 ±0.1 to 5.2±0.2 g/100 ml ($P < 0.02$) after benzolamide which was a beneficial effect acting to maintain sngfr. π_A fell in proportion to C_A. The oncotic pressure at the efferent end of the glomerular capillary (π_E) fell from 35.4±1.5 to 31.7±1.2 mm Hg ($P < 0.05$) (Fig. 2). In the control period, the effective filtration pressure was not different from zero at the end of the glomerular capillary at −1.4±1.8 mm Hg and remained at filtration equilibrium after benzolamide at +1.4±1.2 mm Hg. Because filtration pressure equilibrium persisted

TABLE III

Pressures, Flows, Vascular Resistances, and Glomerular Permeability before and after Benzolamide Administration on Group I Rats

Rat no.	Period	MAP	GFR	Hct	P_G	P_{BS}	ΔP	sngfr	snff	rpf	rbf	AR	ER	HP_E	π_A	π_E	EFP_E
		mm Hg	*ml/ min*	*%*	*mm Hg*	*mm Hg*	*mm Hg*	*nl/ min*	*%*	*nl/ min*	*nl/ min*	*× 10⁹ dyn-s/ cm⁻⁵*	*× 10⁹ dyn-s/ cm⁻⁵*	*mm Hg*	*mm Hg*	*mm Hg*	*mm Hg*
7	Control	111	0.89	58	47.5	14.3	33.2	24.1 ±1.9	0.27	89 ±4	213 ±8	23.5	10.8	21.8 ±1.4	19.5	31.2	2.0
	Experimental	100	0.74	56	50.2	16.1	34.1	19.2 ±1.6	0.32	60 ±5	136 ±12	28.8	21.3	18.6 ±0.9	17.6	31.8	2.3
8	Control	119	1.12	58	54.4	15.4	39.0	30.3 ±4.2	0.34	89 ±12	209 ±29	24.3	15.7	18.7 ±0.8	19.5	37.0	2.0
	Experimental	106	0.78	57	44.2	10.5	33.7	16.7 ±3.9	0.42	40 ±9	91 ±20	53.8	27.8	18.1 ±0.2	15.7	35.7	−2.0
9	Control	113	1.01	57	48.1 ±0.1	16.1 ±0.3	32.0 ±0.4	29.9 ±2.2	0.22	136 ±10	316 ±23	16.2	7.7	20.1 ±1.5	22.6	33.1	−1.1
	Experimental	97	0.74	56	46.2 ±2.2	14.8 ±1.1	31.4 ±1.1	22.6 ±1.4	0.35	64 ±4	146 ±9	27.4	16.9	19.6 ±1.0	14.4	27.0	4.4
10	Control	106	0.58	54	41.2 ±0.1	8.4 ±0.1	32.7 ±0.8	29.3 ±2.1	0.34	86 ±6	187 ±13	27.2	9.5	22.2 ±1.1	19.5	37.0	−5.7
	Experimental	102	0.57	55	41.8 ±2.4	10.0 ±0.9	31.8 ±1.5	21.6 ±1.4	0.39	55 ±4	123 ±8	38.1	17.8	18.9 ±1.2	14.8	31.2	0.6
12	Control	104	0.77	60	47.0	13.6	33.4	24.5 ±3.0	0.39	63 ±8	105 ±13	41.2	29.0	17.4 ±1.2	19.5	41.1	−7.7
	Experimental	96	0.74	60	46.0	13.9	32.1	15.8 ±1.4	0.34	47 ±4	78 ±7	46.6	33.3	19.9 ±2.0	18.0	33.7	−1.6
15	Control	117	1.09	52	49.2 ±0.3	14.2 ±0.5	35.1 ±0.2	37.5 ±1.9	0.26	144 ±7	300 ±15	17.8	8.3	21.4 ±0.9	21.0	33.1	2.0
	Experimental	114	0.86	54	47.0 ±2.5	15.0 ±1.0	35.3 ±2.4	36.8 ±3.7	0.28	131 ±13	292 ±29	18.1	8.6	19.1 ±0.4	18.5	30.6	4.7
Overall means	Control	112 ±2	0.91 ±0.08	56 ±1	47.9 ±1.7	13.7 ±1.1	34.2 ±1.0	29.2 ±2.0	0.30 ±0.03	101 ±13	222 ±32	25.0 ±3.7	13.5 ±3.3	20.3 ±0.8	20.3 ±0.5	35.4 ±1.5	−1.4 ±1.8
	Experimental	102 ±3	0.74 ±0.04	56 ±1	45.9 ±1.2	13.4 ±1.0	33.1 ±0.6	21.1 ±3.1	0.35 ±0.02	66 ±13	144 ±32	35.5 ±5.4	21.0 ±3.6	19.0 ±0.3	16.5 ±0.7	31.7 ±1.2	1.4 ±1.2
P value		<0.01	<0.05	NS	NS	NS	NS	<0.01	NS	<0.01	<0.01	<0.05	<0.01	NS	<0.02	<0.05	NS

MAP, mean arterial pressure; Hct, hematocrit; HP_E, efferent arteriole hydrostatic pressure; P_{BS}, Bowman's space hydrostatic pressure.
* ±SEM.

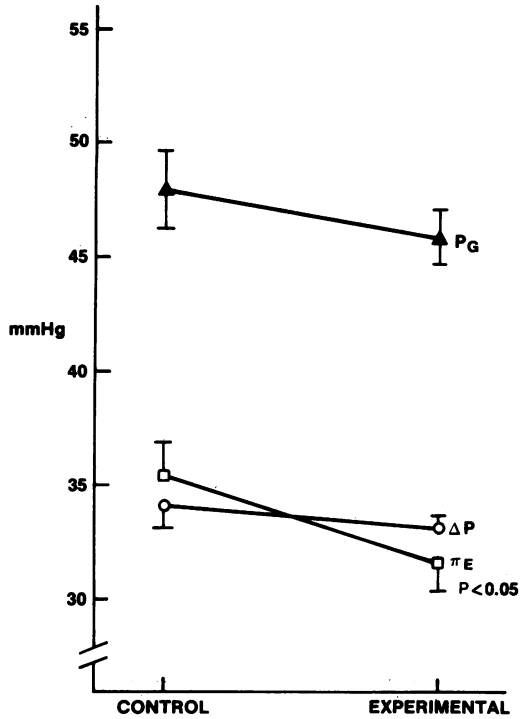

FIGURE 2 The effect of benzolamide upon P_G, ΔP, and π_E in group 1 rats. P_G and ΔP did not change significantly with benzolamide and filtration pressure equilibrium ($\Delta P \approx \pi_E$) persisted before and after benzolamide.

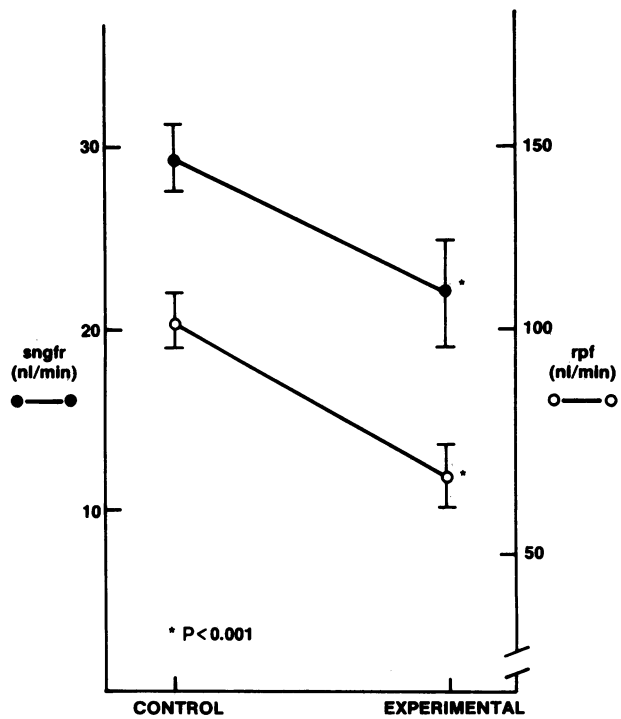

FIGURE 3 The effect of benzolamide on sngfr (closed circles) and rpf (open circles) in group 1 rats. The decrease in rpf was the only determinant that contributed to reduced sngfr after benzolamide.

before and after benzolamide administration, specific values for L_pA could not be determined (rather only minimum value estimates). Therefore, it cannot be determined if L_pA actually decreased with benzolamide. It can be concluded with certainty that changes in L_pA did not contribute to the reduction in sngfr because filtration pressure equilibrium persisted in both control and experimental periods.

Because changes in ΔP, π_A, and L_pA did not contribute to the reduction in sngfr after benzolamide, the reduction must have been solely the result of decreases in rpf. The rpf decreased from 101±13 to 66±13 nl/min ($P < 0.01$) (Fig. 3) because of increases in both afferent arteriolar resistance (AR) (25.0±3.7 to 35.5±5.4 × 10^9 dyn-s-cm^{-5}) ($P < 0.05$) and efferent arteriolar resistance (ER) (13.5±3.3 to 21.0±3.6 × 10^9 dyn-s-cm^{-5}) ($P < 0.01$). The rbf fell from 222±32 to 144±32 nl/min ($P < 0.001$). The snff was not statistically different (0.30±0.03 vs. 0.35±0.02) ($P > 0.1$). The proportional increases in AR and ER permitted P_G to remain constant in spite of the changes in rpf and rbf.

Therefore rpf was the only determinant of glomerular ultrafiltration that constituted the efferent mechanism of the tubulo-glomerular feedback system operating after benzolamide to decrease sngfr. Balanced increases in AR and ER decreased rpf in the absence of any changes in hydrostatic pressures.

DISCUSSION

The existence of a tubulo-glomerular feedback system has been well established in multiple studies over the past several years (2, 9, 11–14, 17, 34). These studies have demonstrated the inverse feedback relationship between the rate of distal delivery and nephron filtration rate, in each case on the single nephron level by micropuncture and particularly microperfusion methods. Much of the experimental activity directed toward further definition of the specific operation of the tubulo-glomerular feedback system has focused upon the afferent or sensor limb of the system. Some controversy remains regarding the character of the specific afferent mechanism (11, 14, 17, 18). Although it seems logical that such a feedback system should contribute in some way to the renal maintenance of volume homeostasis, the specific entity (sngfr, distal delivery, etc.) that is regulated by the feedback system has not been determined.

Considerably fewer investigative studies have addressed the complete definition of efferent mecha-

nisms of the feedback system that affect change in sngfr. As stated previously, the sngfr could change as a result of alterations in one or more of three determinants of glomerular ultrafiltration: (*a*) the rpf, (*b*) ΔP, and (*c*) the L_pA. The studies in the literature that use stop-flow pressure measurements suggest that stop-flow pressure decreases in the same nephron when distal perfusion rate (or distal delivery) is significantly increased above normal rates (9, 13, 17, 34). These studies indirectly imply that P_G changes with activation of the efferent limb of the feedback system, but (*a*) do not allow quantification of the degree to which ΔP changes and (*b*) do not exclude the participation of the other determinants of glomerular ultrafiltration. However, studies on glomerular ultrafiltration in the rat, which have used direct measurements of the determinants of filtration rate, have suggested that alterations in ΔP are rarely the primary influence leading to physiologic alterations in sngfr.

The major reason that the specific mechanisms mediating the efferent limb of the feedback system have not been fully demonstrated is that it remains technically difficult to monitor changes in ΔP, rpf, and L_pA while altering distal delivery by microperfusion on a single nephron level (the only level at which tubulo-glomerular feedback activity had been demonstrated). With the present direct techniques, activation of the efferent mechanisms in all nephrons (or at least all superficial nephrons) is required for a complete analysis of the determinants operating to change nephron filtration rate, because a complete analysis cannot be accomplished at the level of the single nephron.

The renal effects of the carbonic anhydrase inhibitor, benzolamide, fulfill most of the investigative criteria we have outlined. First, the agent decreased absolute reabsorption in the proximal tubule and in the early, nonsteady state must have increased the rate of delivery of fluid out of the proximal tubule by $\cong$8 nl/min or by about 60%. Actual measurements in blocked nephrons revealed an increase of 6.2 nl/min. Benzolamide has little if any effect on reabsorption within the distal nephron (19) and therefore should not have a major influence upon the sensitivity of the afferent limb of the feedback system. Also, the drug has no known primary effects upon either the glomerulus or renal vascular resistance. The studies of Kunau et al. (19) demonstrate that delivery of chloride and HCO_3 out of the proximal tubule is increased as well as the concentration of HCO_3 in tubular fluid. Our previous study on tubulo-glomerular feedback demonstrated that the efficiency and magnitude of the feedback response were not diminished by increasing the concentration of HCO_3 in perfusion fluid to 30 mM (11). Therefore the secondary effect of benzolamide on the concentration of bicarbonate in fluid leaving the proximal tubule should not influence the efficiency of the feedback response. There is also prior evidence in the literature that administration of benzolamide (19, 20) and other carbonic anhydrase inhibitors (35, 36) result in decreased GFR, independent of the volume depleting effects of these agents.

The decrease in sngfr observed in these studies was about 25%, a value approximately equal to the percentage reduction in kidney GFR, which suggests that changes in proximal tubules of superficial nephrons are a valid reflection of changes occurring in all nephrons. Distal collection values also support this conclusion. In addition, these data suggest that acute replacement of a proximal oil block for a period of 2–3 min during the collection of tubular fluid and the reduction in distal delivery to zero for this period does not reverse the reduction in sngfr and cause values to rise to control sngfr. The studies in which transient increases in fluid delivery to the distal nephron were prevented by placement of a long oil block before the control measurements (blocked nephrons) demonstrate that if these increases (caused by reduced proximal reabsorption) were prevented, then sngfr did not decrease in those nephrons, and the rate at which fluid exitted the proximal tubule and escaped onto the kidney surface (or V_f) did increase by 6.2 nl/min. The final type of studies on this issue demonstrated that if sngfr was measured after benzolamide and then an oil block placed into the proximal tubule for approximately 15 min, the recollected values for sngfr tended to rise towards control, pre-benzolamide values (Table II). The degree to which this interruption of distal delivery during benzolamide caused sngfr to return to control values varied among rats. In four of six rats studied, sngfr clearly reverted to control values in almost every tubule. However, the sngfr remained decreased in spite of placement of the oil block in two of the rats. These data in the aggregate suggest that the reduction in sngfr with benzolamide requires at least a transient increase in distal delivery and that by either preventing or reversing this process, sngfr remains at or returns to control values. The most logical conclusion therefore remains that the reduction in sngfr is related to activation of the tubulo-glomerular feedback mechanism.

An alternative explanation for the results does exist and cannot be disproven on the basis of the present data. Benzolamide is a weak acid and highly lipid insoluble (differing from acetazolamide in this regard) (21). The drug is likely secreted by the weak acid secretory system and probably gains access to cells in the more distal tubule from the tubular fluid rather from the peritubular membrane. The present results could also be explained if benzolamide directly activated the affector or sensor cell (possibly macula densa) independent of effects on the rate of distal delivery,

by gaining access to the cell from tubular fluid. Prevention of the increase in distal delivery and the 15-min interruption of distal delivery during benzolamide might prevent the drug from reaching the "sensor" cell. Such an explanation is highly speculative and less likely than explanations based upon activation of tubulo-glomerular feedback mechanisms.

These results are somewhat at variance with the results presented in preliminary form by Persson and Wright (36). These investigators examined the differences in sngfr obtained in proximal and distal tubules in control conditions and shortly after large doses of acetazolamide. The sngfr measured in distal tubules decreased as did kidney GFR after acetazolamide, but sngfr in proximal tubules was not statistically different. However, the difference in proximal collections for sngfr was numerically large, although not significant. The studies of Persson and Wright (36) and the present data both conclude that the tubulo-glomerular feedback system participated in the reduction in sngfr, and the two studies differ because a 2–3-min reduction in distal delivery did not deactivate the efferent mechanisms and restore sngfr to control values in the present analysis, but rather required a period of 15 min.

Since evidence has been provided that sngfr decreased in all surface nephrons with benzolamide as a result of activation of the tubulo-glomerular feedback system, this finding has provided a unique opportunity to examine specifically the determinants of glomerular ultrafiltration which constitute the efferent limb of the feedback system. In the hydropenic control period, the oncotic pressure rose sufficiently along the length of the glomerular capillary to equal the hydrostatic pressure gradient, defining the condition of filtration pressure equilibrium. The finding of filtration equilibrium during hydropenia in the present study is therefore in agreement with previous studies from this laboratory (22, 23, 31) and the studies of Brenner and co-workers (32, 37). After the administration of benzolamide and the reduction in sngfr the EFP remained indistinguishable from zero at the efferent end of the glomerular capillary. Under conditions of filtration equilibrium, specific values for L_pA cannot be defined. However, when filtration equilibrium persists in control and experimental periods, as observed in this study, changes in L_pA cannot contribute to the decrease in sngfr. Changes in the rate of plasma flow are a logical mechanism in the rat, a species in which sngfr has been shown to be highly plasma flow dependent (31, 37).

Hydrostatic factors, glomerular capillary and Bowman's space hydrostatic pressure, also did not change after benzolamide and therefore made no contribution to the reductions in sngfr. The conclusion that changes in the P_G and ΔP did not result from activation of the tubulo-glomerular feedback system and did contribute to a reduction in sngfr is somewhat at variance with the several studies which have examined this issue with microperfusion studies and estimates of glomerular hydrostatic pressure in single nephrons (9, 13, 15, 17). Microperfusion studies in which distal delivery has been increased to 200–400% of normal values have found reductions in both stop-flow pressure (9, 13, 17) and directly measured P_G (15) in parallel with the decrease in sngfr. The fact that sngfr fell in the present study in the absence of major changes in either P_G or ΔP can probably be explained. The lesser increase in distal delivery (60%) which was predicted and observed directly in the early phase after benzolamide in this study, before a reduction in sngfr may not have been a sufficient stimulus to alter P_G and ΔP and was certainly a lesser stimulus than in previous microperfusion studies.

The reduction in sngfr after benzolamide was totally the result of a decrease in rpf and rbf. The reduction in rpf was the consequence of proportionate increases in AR and ER such that P_G remained relatively constant. The present study has provided no insight into the specific mediators of vasoconstriction. Local release of angiotensin remains a serious candidate as the potential efferent mediator which produces the vasoconstriction. The few other biologically active vasoconstrictors seem much less likely potential candidates. It also remains possible that constriction of afferent and efferent vessels occurs by direct activation via the contiguous elements of the juxtaglomerular apparatus without the extracellular release of any humoral substance.

The present study may have also provided insights into the physiologic entity which is regulated by the tubulo-glomerular feedback system. The present data reveal that although sngfr decreased with benzolamide, the sngfr did not decrease to zero, in spite of the large, acute nonsteady-state increase in distal delivery that results from the reduction in proximal reabsorption. The mean sngfr fell by the same volume flow rate as did absolute reabsorptive rate. The net result was that the rate of distal delivery remained relatively constant. Although the tubulo-glomerular feedback system must participate in some way in the renal control of volume homeostasis, the primary regulated entity is not, of necessity, sngfr. The present study supports the hypothesis that the rate of distal delivery may be the primarily regulated physiologic entity. If this were the case, then the efferent mechanisms would be activated in response to increases in distal delivery and produce sufficient vasoconstriction to decrease the sngfr to a level at which distal delivery returns to the original rate. If distal delivery were not regulated by some mechanism, spontaneous increases should result in obligate volume losses, because distal FR either

remains constant or decreases (11, 38) with increases in load, and reabsorptive capacity in the terminal segments of the tubule could be overloaded.

The reduction in sngfr which occurs after benzolamide resulted from increased AR and ER and decreases in rpf. Evidence was provided that this reduction in filtration rate was the consequence of activation of the tubulo-glomerular feedback system via benzolamide induced reductions in absolute proximal tubular reabsorption and transient increases in distal tubular delivery. In this experimental condition, vasoconstriction and reductions in rpf constitute the efferent limb of the tubulo-glomerular feedback system. Alterations in glomerular hydrostatic pressure and the L_pA were not observed to participate in this feedback-mediated reduction in sngfr.

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