

# Regulation of Renal Blood Flow by Plasma Chloride

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**ABSTRACT** Micropuncture studies have shown that glomerular filtration rate (GFR) falls in response to a rise in  $\text{Na}^+$  or  $\text{Cl}^-$  concentrations in the loop of Henle, whereas studies in isolated kidneys have shown that GFR falls in response to osmotic diuresis. To define the separate effects of an acute increase in plasma sodium ( $P_{\text{Na}}$ ), chloride ( $P_{\text{Cl}}$ ) or osmolality ( $P_{\text{osmol}}$ ), changes in renal blood flow (RBF) and GFR were measured during intrarenal infusions of hypertonic NaCl,  $\text{NaHCO}_3$ , Na acetate, dextrose,  $\text{NH}_4\text{Cl}$  or  $\text{NH}_4\text{acetate}$  to denervated kidneys. The infusions raised  $P_{\text{osmol}}$  at the experimental kidney by 30–45 mosmol. RBF increased abruptly by 10–30% with all hypertonic infusions indicating that an acute increase in plasma tonicity causes renal vasodilatation. Renal vasodilatation persisted or increased further during infusion of dextrose,  $\text{NaHCO}_3$  and Na acetate, but GFR was unchanged. In contrast, during infusion of the two Cl-containing solutions, vasodilatation was reversed after 1–5 min and RBF and GFR decreased ( $P < 0.01$ ) below preinfusion levels. Prior salt depletion doubled the vasoconstriction seen with hypertonic NaCl infusions. Overall, changes in RBF were unrelated to changes in  $P_{\text{Na}}$  or fractional Na or fluid reabsorption but correlated with changes in  $P_{\text{Cl}}$  ( $r = -0.91$ ) and fractional  $\text{Cl}^-$  reabsorption ( $r = 0.94$ ). The intrafemoral arterial infusion of the two Cl-containing solutions did not increase femoral vascular resistance. In conclusion, hyperchloremia produces a progressive renal vasoconstriction and fall in GFR that is independent of the renal nerves, is potentiated by prior salt depletion and is related to tubular  $\text{Cl}^-$  reabsorption. Chloride-induced vasoconstriction appears specific for the renal vessels.

## INTRODUCTION

The maintenance of fluid and electrolyte balance by the kidney is achieved by integrated adjustments in glomerular filtration and tubular reabsorption. When

*Received for publication 21 July 1982 and in revised form 24 November 1982.*

the adequacy of the extracellular volume (ECV)<sup>1</sup> is threatened by salt depletion, impaired proximal tubule reabsorption or a high perfusion pressure, renal vasoconstriction restricts the volume of filtrate delivered to the tubules (1–5). A mechanism that can relate glomerular filtration to distal fluid delivery has been identified in micropuncture experiments in which single nephron glomerular filtration rate (SNGFR) has been found to fall during a selective increase in the  $\text{Na}^+$  or  $\text{Cl}^-$  concentrations or osmolality of early distal tubule fluid (6–8).

In previous experiments, we (9) and others (10, 11) showed that intrarenal infusion of hypertonic NaCl solution causes transient vasodilatation followed by sustained vasoconstriction. Schnermann et al. (8) demonstrated that retrograde injection of Cl-containing solutions into the distal tubule regularly elicited a decrease in SNGFR, whereas similar injections of Na-containing solutions did not. In contrast, in experiments on the isolated kidney, Nizet (12–14) concluded that there was no specific effect of increased  $\text{Na}^+$  or  $\text{Cl}^-$  concentration on renal vascular resistance but that the GFR varied in direct proportion to tubular fluid reabsorption. To settle these differences between the responses of the isolated kidney and the nephron perfused retrogradely, changes in renal vascular resistance of the intact kidney occurring in response to an acute increase in plasma sodium ( $P_{\text{Na}}$ ), chloride ( $P_{\text{Cl}}$ ), or osmolality ( $P_{\text{osmol}}$ ) were investigated.

## METHODS

### *Preparation*

Experiments were performed on 48 male greyhounds weighing  $28 \pm 2$  kg (mean  $\pm$  SEM) and fed a diet of meat and dog biscuits; food and water were withheld for 12 h before experiments.

<sup>1</sup> *Abbreviations used in this paper:* ECV, extracellular volume; FBF, femoral blood flow; FF, filtration fraction; GFR, glomerular filtration rate; Hct, hematocrit; MAP, mean arterial pressure; PS, plasma solid; R/F, fractional reabsorption; RPF, renal plasma flow; RBF, renal blood flow; SNGFR, single nephron glomerular filtration rate.

Dogs were anesthetized with intravenous pentobarbital and were ventilated mechanically (arterial blood  $p\text{CO}_2$ , 32–42 mmHg). Mean arterial blood pressure (MAP) was recorded via a cannula in a femoral artery (as the damped electrical output from a pressure transducer [Statham Instruments, Inc., Oxnard, CA]).

The “experimental” kidney was denervated by autotransplantation to the neck of the same dog by anastomosis to the carotid artery and jugular vein as described previously (15, 16). The artery to the experimental kidney was encircled by an electromagnetic flow probe connected to a pulsed-logic blood flow meter (Biotronex Laboratory, Inc., Kensington, MD). About 1 in. distal to the flow probe, the artery was cannulated with an 18-gauge needle to infuse solution I (see below). A tributary of the jugular vein was cannulated to obtain samples of renal venous blood. The ureter was cannulated and urine flow rate was displayed graphically by a drop counter (Devices Ltd., London, England) and its volume measured gravimetrically. The experimental kidney was placed in a box filled with light liquid paraffin mineral oil and maintained at 37°C by a water jacket. The ureter of the contralateral kidney was cannulated, but this kidney was otherwise undisturbed.

Through a cannula inserted into a brachial vein, a solution of two-thirds 0.154 M NaCl solution and one-third 1.10 M mannitol was infused at a rate of 0.04 ml  $\text{min}^{-1}$  kg body  $\text{wt}^{-1}$  (solution 2). The markers used to estimate GFR and renal plasma flow rate (RPF) were added to this infusion. During surgery, the animals received 500 ml of isoosmotic (6 g 100 ml<sup>-1</sup>), dextran (average molecular weight 110,000) in 0.154 M NaCl solution (Dextraven 110, Fisons, Ltd., London, England). Infusion I (intrarenal) was delivered at 0.1 ml  $\text{min}^{-1}$  body  $\text{wt}^{-1}$ . It was initially 0.154 M NaCl solution. 1 h after completing surgery, urine was collected from each kidney for a 15-min collection period and blood samples (10–15 ml) were drawn from the femoral artery and the renal vein at the midpoint. Dextraven 110 was infused to replace blood losses.

### Experimental protocol

**Effects of hypertonic intrarenal infusions on renal function.** The infusion into the artery of the experimental kidney was changed from 0.154 M NaCl to either NaCl,  $\text{NaHCO}_3$ , Na acetate,  $\text{NH}_4\text{Cl}$  or  $\text{NH}_4\text{acetate}$  or dextrose at 1.232 M for 30 min, but the rate of infusion was not changed. Urine was collected for the last 15 min during which blood samples were drawn. Thereafter, the infusion was returned to 0.154 M NaCl solution for a further 30 min and urine was collected for the last 15 min with a blood sample at the midpoint.

**Effects of prior salt depletion on renal hemodynamics during hypertonic NaCl infusion.** In five experiments, the dogs received furosemide (80 mg  $\text{d}^{-1}$  with the food) for 2 d to cause ECV depletion. The second dose was given 18 h before study and no further food or fluid was given. These dogs received the intrarenal infusions of hypertonic NaCl according to the protocol described above.

**Effects of intraarterial infusions of hypertonic solutions on femoral blood flow.** In four dogs, the hypertonic solutions used in protocol A were infused into a femoral artery at 0.1 ml  $\text{min}^{-1}$  kg body  $\text{wt}^{-1}$ . These infusions were given for ~15 min with 0.154 M NaCl infused for 15 min between each one. Femoral blood flow (FBF) was assessed by a non-cannulating blood flow meter placed around the femoral artery at least 1 in. proximal to the site of the infusion.

### Laboratory methods

Details of the analytical methods have been described previously (16). Measurements of  $\text{Na}^+$  and  $\text{K}^+$  concentrations were made with an EEL flame photometer, Cl with a Corning chloride meter (Corning Medical, Corning Glass Works, Corning, NY), osmol with an Advanced osmometer (Advanced Instruments, Inc., Needham Heights, MA), pH,  $p\text{CO}_2$  and  $p\text{O}_2$  of arterial and renal venous blood with a Radiometer blood gas analyzer (Radiometer Co., Copenhagen, Denmark) and plasma  $\text{HCO}_3^-$  calculated from a standard normogram (Siggaard-Andersen). Hematocrit (Hct) was estimated as the mean of two measurements with a microhematocrit centrifuge. Plasma solid concentration (PS; indicating plasma protein concentration) was estimated by desiccating a weighed quantity of plasma to constant weight and reweighing with correction for the measured content of ions. The radioactivity of arterial and renal venous plasma and urine were counted with a Packard autogamma counter (Packard Instruments, Inc., Downers Grove, IL).

**Calculation of results.** The RPF was calculated as the clearance of Hippuran corrected for renal extraction. The renal blood flow (RBF) was calculated from RPF and Hct. The electromagnetic flow meter was used to record the time course of any changes in RBF. The GFR was calculated as the clearance of EDTA and the filtration fraction (FF) calculated as  $\text{GFR}/\text{RPF}$ . Paired comparisons were used and values of  $2P < 0.05$  were taken as statistically significant.

## RESULTS

**Blood pressure and composition before and during hypertonic infusions.** Table I details the initial values of MAP and blood composition at the experimental kidney and the changes produced by the hypertonic infusions. Since there were no differences in initial values between groups, the mean starting values were pooled. The MAP was not altered during any infusion. The  $P_{\text{osmol}}$  at the experimental kidney was increased ~30–45 mosmol. Infusions containing  $\text{Na}^+$  or  $\text{Cl}^-$  increased  $P_{\text{Na}}$  or  $P_{\text{Cl}}$  ~20–25 mM. Infusion of NaCl, dextrose and especially  $\text{NH}_4\text{Cl}$  produced a systemic metabolic acidosis; infusions of  $\text{NaHCO}_3$ , and Na acetate produced a systemic metabolic alkalosis. However, there were no differences between the pH in the renal vein and the systemic circulation except during intrarenal infusion of  $\text{NaHCO}_3$ . The systemic plasma hydrogen ion concentration ( $P_{\text{H}}$ ) was higher during the infusion of  $\text{NH}_4$ -containing solutions than during the corresponding Na-containing solutions.

**Steady-state changes in RBF, GFR and renal clearance during intrarenal hypertonic infusions.** The renal function of the experimental kidney during the initial infusion of 0.154 M NaCl and the relationship of its function to that of the control kidney were strictly comparable to that reported previously (16). There were no differences between the RBF, RPF, or fractional excretion of sodium ( $\text{FE}_{\text{Na}}$ ) or chloride ( $\text{FE}_{\text{Cl}}$ ) between the experimental and control kidneys,

TABLE I  
Blood Pressure and Composition

	Change during infusion of 1.232 M solution						
	Starting value (n = 48)	NaCl (n = 17)	NH <sub>4</sub> Cl (n = 6)	NaHCO <sub>3</sub> (n = 5)	Na Acetate (n = 6)	Dextrose (n = 9)	NH <sub>4</sub> Acetate (n = 5)
MAP, mmHg	164±22	-3±15	+8±15	-3±7	-8±12	-1±4	-4±7
P <sub>osmol</sub> , mosmol kg <sup>-1</sup>	310.2±11.0	+41.6±13.7°	+45.5±16.9°	+39.0±12.7°	+33.7±7.9°	+30.3±12.2°	+37.6±7.5°
P <sub>Na</sub> , mmol l <sup>-1</sup>	147.4±5.8	+22.4±7.4°	-1.0±0.9	+21.0±4.2°	+18.5±2.3°	-6.2±1.7°	-0.8±1.3
P <sub>K</sub> , mmol l <sup>-1</sup>	3.64±0.54	-0.3±0.3°	+1.0±0.3°	-0.3±0.2‡	-0.9±0.6‡	-0.2±0.2‡	+0.6±0.1°
P <sub>Cl</sub> , mmol l <sup>-1</sup>	110.4±15.5	+22.2±5.4°	+27.8±7.1°	-4.4±1.1°	-4.7±2.7§	-6.0±2.7°	-6.6±3.3‡
P <sub>II</sub> , nmol l <sup>-1</sup>	47.6±1.8	+4.8±4.7°	+17.6±7.0°	-16.0±4.3°	-7.8±6.3‡	+4.3±1.7°	+0.5±4.2
P <sub>CO<sub>2</sub></sub> , mmHg	36.7±8.9	+1.2±4.0	+0.3±8.5	+4.5±2.1	+0.8±6.5	-0.5±4.4	-1.5±6.6
P <sub>O<sub>2</sub></sub> , mmHg	83.1±15.2	+1.5±5.1	+4.3±9.8	+0.5±5.0	+5.0±6.6	-1.3±4.4	+1.0±2.6
P <sub>HCO<sub>3</sub></sub> , mmol l <sup>-1</sup>	18.9±0.8	-2.4±2.3°	-5.2±2.7°	+8.1±0.6°	+3.2±2.8‡	-2.2±1.2°	-1.9±1.1‡
PS, g 100 g <sup>-1</sup>	5.74±0.72	-0.53±0.39°	+0.06±0.08	-0.28±0.20‡	-0.29±0.27‡	-0.08±0.14	+0.21±0.22
Hct, %	48.9±7.7	-4.1±3.1°	+1.8±1.8	-1.6±0.9‡	-0.2±3.7	-3.7±1.5°	+4.2±2.5‡

Column 1 shows the mean (±SD) initial value for the entire group (n = 48) and subsequent columns show the mean (±SD) absolute changes produced in the blood perfusing the experimental kidney 15–30 min after starting intrarenal arterial infusions.

\*P < 0.001. †P < 0.05; §P < 0.01;

but the GFR and FF were slightly lower (average, 7%; P < 0.01) at the experimental kidney.

The changes in renal hemodynamics 15–30 min after starting infusions of the four hypertonic solutions that did not contain Cl<sup>-</sup> are shown in Table II. There were significant increases in RBF and RPF for all save NH<sub>4</sub> acetate. There were no significant changes in GFR and FF decreased with each infusion. All these changes in RBF and RPF were reversible, except that there was a persistent fall in FF after the infusion of the two acetate-containing solutions. Thus, hypertonic infusions that did not contain Cl<sup>-</sup> or NH<sub>4</sub><sup>+</sup> led to reversible renal vasodilatation. The changes in renal hemodynamics that occurred after 15–30 min of infusion of hypertonic solutions containing Cl<sup>-</sup> were quite different (Table II). RBF, RPF, and GFR were all reduced significantly and these changes were greater during the infusion of NH<sub>4</sub>Cl than NaCl (P < 0.05). The FF was maintained during infusion of NaCl, but it fell during NH<sub>4</sub>Cl. These changes (except for the fall in GFR with NH<sub>4</sub>Cl) had reversed within 15–30 min after stopping the infusions. Thus, hypertonic Cl<sup>-</sup> infusions produced renal vasoconstriction and a fall in GFR that was more pronounced when NH<sub>4</sub><sup>+</sup> was the cation.

In Fig. 1 are shown the percentage changes in RBF of the experimental kidney alone and relative to the control kidney during the different infusions. During the infusion of Na acetate, dextrose, and NaHCO<sub>3</sub>, RBF increased, whereas during the infusion of the two Cl-containing solutions, it decreased. These changes in RBF were also seen when the RBF of the experimental kidney was expressed as a fraction of its control.

This indicates that the changes in the composition of blood perfusing the experimental kidney caused the changes in vascular resistance. However, the magnitude of the changes in RBF were generally reduced when expressed as a fraction of the control kidney, indicating that the infusions caused similar, although smaller, changes in RBF of the control kidneys, presumably in response to the small changes in composition of the systemic blood.

There was no significant correlation between the group mean changes in RBF and changes in P<sub>Na</sub> (r = 0.18; n = 6; NS). However, there was a very close correlation between group mean changes in RBF and changes in P<sub>Cl</sub> (r = -0.91; n = 6; P < 0.01).

*Relationships between changes in RBF and fractional reabsorption of fluid, Na<sup>+</sup> or Cl<sup>-</sup>.* To examine the hypothesis that changes in GFR or RBF during osmotic diuresis are mediated by inhibition of tubular fluid reabsorption (12–14), the group mean changes in RBF and GFR produced by the hypertonic infusions were compared to changes in fractional reabsorption of fluid (R/F<sub>H<sub>2</sub>O</sub>). No significant correlations were apparent between group mean changes in R/F<sub>H<sub>2</sub>O</sub> and either RBF (Fig. 2A) or GFR (r = 0.37; NS).

During osmotic diuresis or during administration of NH<sub>4</sub>Cl or hypertonic NaCl administration, there is considerable inhibition of proximal tubular reabsorption of fluid, Na<sup>+</sup> and Cl<sup>-</sup> (17–22). Consequently, the changes in the fractional reabsorption of sodium (R/F<sub>Na</sub>) or chloride (R/F<sub>Cl</sub>) during these infusions indicate the delivery of filtered Na<sup>+</sup> and Cl<sup>-</sup> to the loop of Henle. To examine the hypothesis that changes in GFR or RBF were related to changes in the delivery of

TABLE II  
Changes in Renal Hemodynamics during Hypertonic Intrarenal Infusions

Solution	Before compared with	% Change			
		RBF	RPF	GFR	FF
NaHCO <sub>3</sub> (n = 5)	During	+9.7±8.5 P < 0.05	+11.0±8.8 P < 0.05	-9.7±10.5 NS	-21.4±12.7 P < 0.05
	After	+4.5±11.2 NS	+7.3±11.6 NS	+2.2±14.5 NS	-4.4±9.3 NS
Na Acetate (n = 6)	During	+27.6±17.7 P < 0.02	+27.6±15.3 P < 0.01	-13.7±25.3 NS	-29.0±12.6 P < 0.005
	After	+5.6±18.7 NS	+6.8±17.6 NS	-10.0±11.9 NS	-16.4±12.8 P < 0.025
Dextrose (n = 9)	During	+14.7±13.1 P < 0.01	+20.5±13.9 P < 0.005	-8.4±21.0 NS	-21.3±12.7 P < 0.05
	After	-8.8±14.6 NS	-9.0±14.3 NS	+2.2±14.5 NS	-4.4±9.3 NS
NH <sub>4</sub> Acetate (n = 5)	During	+4.8±26.4 NS	-5.2±20.1 NS	-25.6±28.0 NS	-23.8±7.8 P < 0.01
	After	+26.0±29.9 NS	+40.8±49.4 NS	-9.1±27.9 NS	-33.6±12.7 P < 0.005
NaCl (n = 17)	During	-14.4±9.2 P < 0.001	-7.2±10.3 P < 0.01	-9.4±11.4 P < 0.005	-1.9±7.6 NS
	After	-5.9±13.0 NS	+1.6±16.9 NS	+0.5±15.9 NS	+1.2±17.1 NS
NH <sub>4</sub> Cl (n = 6)	During	-44.4±23.7 P < 0.01	-48.0±21.1 P < 0.005	-62.2±14.6 P < 0.001	-26.4±6.2 P < 0.001
	After	-1.0±25.3 NS	-5.0±25.9 NS	-20.7±18.7 P < 0.05	-6.0±16.4 NS

Mean (±SD) percentage changes in RPF, GFR, FF, and RBF 15–30 min after starting and 15–30 min after completion of the infusion of 1.232 M solutions compared to values before these infusions. The mean±SD initial values for the entire group were as follows: RBF, 9.43±3.12 ml min<sup>-1</sup> kg body wt<sup>-1</sup>; RPF, 4.73±1.51 ml min<sup>-1</sup> kg body wt<sup>-1</sup>; GFR, 1.39±0.40 ml min<sup>-1</sup> kg body wt<sup>-1</sup>; FF, 29.6±5.9%.

filtered Na<sup>+</sup> (6) or Cl<sup>-</sup> (8) to the loop of Henle, the relationships between RBF and either R/F<sub>Na</sub> or R/F<sub>Cl</sub> are presented in Fig. 2B and C. There were no significant correlations between group mean changes in R/F<sub>Na</sub> and RBF (Fig. 2B) or GFR ( $r = 0.45$ ; NS). In contrast, there were very close relationships between group mean changes in R/F<sub>Cl</sub> and RBF (Fig. 2C) or GFR ( $r = 0.81$ ;  $P < 0.05$ ).

Since all infusions increased  $P_{\text{osmol}}$ , yet some led to renal vasodilatation and others to renal vasoconstriction, it is clear that the steady-state changes in renal vascular resistance were independent of  $P_{\text{osmol}}$ .

*Time course of changes in RBF during hypertonic infusions.* Fig. 3 shows the records of RBF, MAP, and urine flow from two experiments during the infusion of hypertonic solutions of Na acetate (A) and NH<sub>4</sub>Cl (B). Within the first 2–3 min of starting both infusions, there was an increase in RBF without a rise in MAP indicating renal vasodilatation. However, during the subsequent 30 min, opposite changes in renal vascular resistance occurred. Thus, with Na acetate, RBF increased progressively from 220 to 380 ml min<sup>-1</sup>. In contrast, with NH<sub>4</sub>Cl, RBF fell sharply from 480 to 190 ml min<sup>-1</sup>. Both changes were reversed by ~15

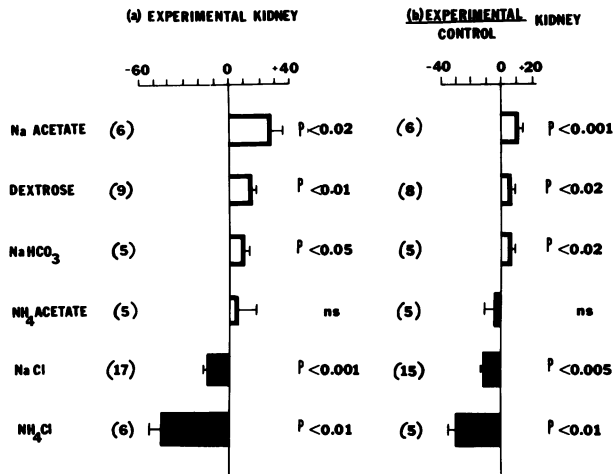


FIGURE 1 Mean ( $\pm$ SEM) values with number of observations in parentheses for (a) percentage change in RBF at the experimental kidney during the period 15–30 min after starting 1.232 M intrarenal infusions of the solutions shown and (b) percentage changes in renal blood flow at the experimental kidney compared to the control kidney during these infusions. The *P* value refers to results of paired *t* tests. The mean ( $\pm$ SD) starting values for the entire group were: RBF,  $9.43 \pm 3.12$  ml  $\text{min}^{-1}$  kg body  $\text{wt}^{-1}$ ; experimental/control RBF,  $97.7 \pm 17.1\%$ .

min after stopping the hypertonic infusions. The infusion of Na acetate reduced urine flow rate, whereas the  $\text{NH}_4\text{Cl}$  increased it initially, although by the end of the infusion when GFR had fallen sharply, urine flow rate also fell.

The time course of changes in RBF were assessed

from the electromagnetic flow meter records. Percentage changes in RBF at 1–2 min and at 30 min after starting the infusions are shown in Table III. Within 1–2 min of starting the infusions of the different hypertonic solutions, RBF increased in 45 of 46 experiments. Compared with the 10.3% mean increase in RBF after 2 min of dextrose infusion, the rise of 35.2% with Na acetate was significantly greater ( $P < 0.02$ ), but the early increase in RBF with the other solutions was not different. Thereafter, RBF increased further during the infusion of dextrose and  $\text{NaHCO}_3$ ; with Na acetate, the RBF increased further in six of seven experiments. During  $\text{NH}_4\text{acetate}$ , there was a large initial rise in RBF that returned towards (but not below) preinfusion values.

The infusion of the two Cl-containing solutions produced an early increase in RBF that was strictly comparable to that seen with the other hypertonic solutions. In contrast, however, in each of the 21 experiments, RBF declined over the period 1–30 min during the infusion of the Cl-containing solutions. By 30 min, RBF was reduced significantly below preinfusion levels with both NaCl and  $\text{NH}_4\text{Cl}$ ; the fall in RBF was greater with  $\text{NH}_4\text{Cl}$  than with NaCl ( $P < 0.01$ ).

*Effects of prior salt depletion on hemodynamic response to hypertonic NaCl infusions.* The effects of prior salt and fluid depletion on the renal hemodynamic response to hypertonic NaCl infusions was assessed by comparing the changes produced by intrarenal infusion of hypertonic NaCl in 17 “euvoletic” dogs with those of five “Na-depleted” dogs. As shown in Table IV, the Na-depleted dogs had a higher FF and a tendency for a lower RPF before the infusion.

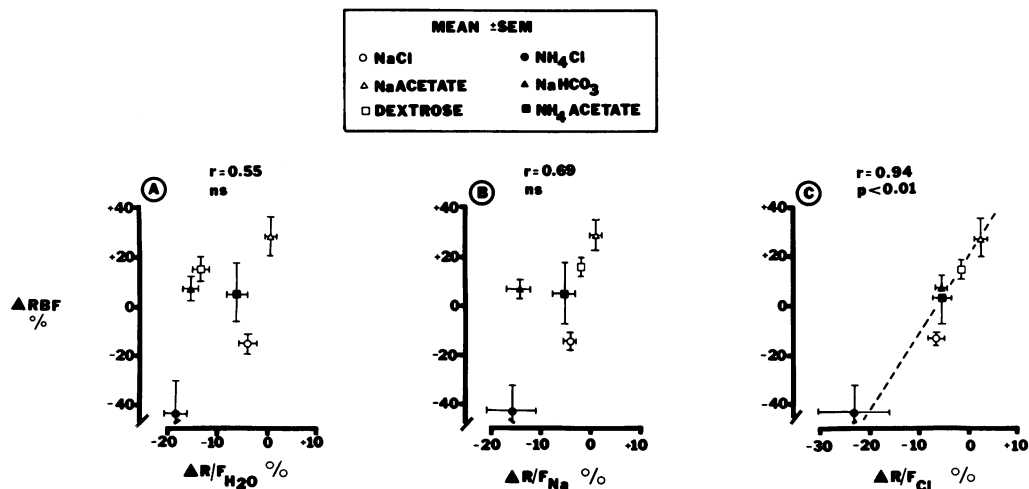


FIGURE 2 Group mean ( $\pm$ SEM) values for percentage changes in renal blood flow are shown as a function of changes in: (A) fractional water reabsorption, (B) fractional sodium reabsorption, and (C) fractional chloride reabsorption.

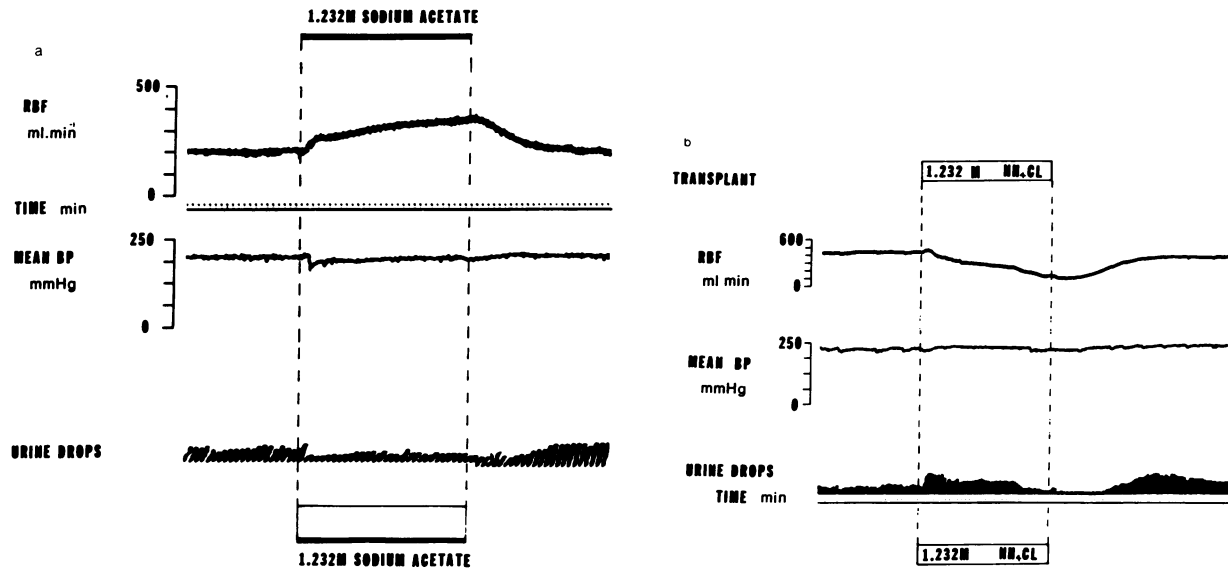


FIGURE 3 Records of two experiments showing the mean RBF recorded with a flow meter, mean BP and urine drop rate. An intrarenal isotonic NaCl infusion was switched to a 1.232-M infusion of (a) Na acetate and (b)  $\text{NH}_4\text{Cl}$  during the periods shown.

During NaCl infusion, the falls in RBF, RPF, and GFR in the salt-depleted dogs were more than twice as great as in the euvoletic dogs, but there were no changes in FF or blood pressure (BP) in either group. Thus, salt depletion augmented the renal vasoconstriction that developed with hypertonic NaCl infusions.

**FBF during intraarterial infusions of hypertonic solutions.** In four experiments, the same six hypertonic solutions were infused into the femoral artery. All six hypertonic solutions led to an abrupt increase in FBF that was maintained for the 15-min infusion periods. Since mean BP did not change, femoral vascular resistance declined during the infusion of each of the solutions.

## DISCUSSION

Infusion of hypertonic solutions into the renal artery caused a biphasic response in renal vascular resistance. The hyperosmolality invariably led to an abrupt renal vasodilatation. Acute vasodilatation, in response to hyperosmolality, is seen at other vessels besides the kidney (9-11, 23, 24), including the aorta (25), intact and isolated muscle arteries (26-30), the pulmonary (31), the mesenteric (10) and the coronary (30), although this response is not always fully maintained (30). Gazitua et al. (32) contrasted the changes in renal vascular resistance produced by hypertonic dextrose and NaCl. As in the present study, they found that both produced an initial fall in resistance but, whereas this vasodilatation was maintained during the

3-min infusion of dextrose, vasoconstriction developed with NaCl. In the present study, only  $\text{NaHCO}_3$  failed to elicit a significant early rise in RBF. This may have been due to alkalosis since this has been shown to potentiate calcium-activated vascular contraction (33, 34).

The addition of hypertonic solutions to blood causes an abrupt shrinkage of erythrocytes that restricts their deformability and increases the blood viscosity (35, 36). In the present experiment, however, an increase in plasma tonicity led to an abrupt reduction in renal vascular resistance and therefore, changes in blood viscosity could only have limited the increase in RBF.

**Late changes in renal vascular resistance.** The uniform early renal vasodilatation during intrarenal infusions of various hypertonic solutions was succeeded by strikingly different patterns of renal vascular resistance that depended on the composition of the infusate. The infusion of dextrose,  $\text{NaHCO}_3$ , and Na acetate led to persistent or progressive renal vasodilatation;  $\text{NH}_4$ acetate did not change renal vascular resistance consistently. Thus, hypertonic solutions can cause a sustained renal vasodilatation regardless of whether they increase  $P_{\text{Na}}$ . In marked contrast, in each of the 21 experiments during which  $P_{\text{Cl}}$  was increased, renal vasoconstriction occurred after the initial vasodilatation.

We showed previously that the degree of renal vasodilatation that occurred during more prolonged infusions of hypertonic NaCl was dependent upon the accompanying metabolic acidosis (9). In the present

TABLE III  
Changes in RBF during Intrarenal Arterial Infusions of Hypertonic Solutions

	n	Percentage changes in RBF during infusion of hypertonic solution	
		After 1 min of infusion	After 30 min of infusion
Dextrose	7	+10.3±5.1	+14.4±6.0
During vs. before		<i>P</i> < 0.005	<i>P</i> < 0.001
30 min vs. 1 min			<i>P</i> < 0.05
Na Acetate	8	+35.2±23.5	+50.7±23.4
During vs. before		<i>P</i> < 0.005	<i>P</i> < 0.001
30 min vs. 1 min			NS
NaHCO <sub>3</sub>	5	+10.8±11.7	+20.8±16.1
During vs. before		NS	<i>P</i> < 0.05
30 min vs. 1 min			<i>P</i> < 0.05
NH <sub>4</sub> Acetate	5	+25.6±20.5	+2.3±29.3
During vs. before		<i>P</i> < 0.05	NS
30 min vs. 1 min			NS
NaCl	14	+11.4±4.9	-12.5±12.6
During vs. before		<i>P</i> < 0.001	<i>P</i> < 0.005
30 min vs. 1 min			<i>P</i> < 0.001
NH <sub>4</sub> Cl	7	+18.6±11.3	-41.3±22.5
During vs. before		<i>P</i> < 0.005	<i>P</i> < 0.005
30 min vs. 1 min			<i>P</i> < 0.001

Data represent changes in RBF (mean±SD) at the experimental kidney as measured with an electromagnetic flow meter. Values refer to percentage changes at 1 and 30 min after starting the infusions of 1.232 M solutions compared to values recorded immediately before the hypertonic infusions. The *P* values refer to results of paired *t* tests; *n* = number of experiments.

TABLE IV  
Effects of Prior Salt Depletion on Renal Vascular Response to Intrarenal Hypertonic NaCl Infusion

	Prior salt depletion	Value before		% Change during hypertonic NaCl infusion	
RBF, <i>ml min<sup>-1</sup> kg<sup>-1</sup></i>	No	9.43±3.12		-14±9	
	Yes	9.31±1.21	NS	-29±13	<0.0125
RPF, <i>ml min<sup>-1</sup> kg<sup>-1</sup></i>	No	4.73±1.51		-7±10	
	Yes	3.88±1.18	NS	-20±11	<0.05
GFR, <i>ml min<sup>-1</sup> kg<sup>-1</sup></i>	No	1.37±0.38		-9±11	
	Yes	1.43±0.52	NS	-25±8	<0.005
FF, %	No	29.6±5.9		-2±8	
	Yes	36.4±3.2	<0.005	-5±9	NS
MAP, <i>mmHg</i>	No	164±23		-2±10	
	Yes	165±15	NS	-1±6	NS

Mean±SD values for renal clearance and mean arterial pressure in dogs without prior salt depletion (*n* = 17) compared to those with salt depletion (*n* = 5). Shown are the values recorded before the hypertonic infusion and the percentage changes occurring 15-30 min during the infusion.

series,  $P_H$  was higher and RBF was lower during the infusions of the two  $NH_4$ -containing solutions than during the infusion of the corresponding Na-containing solutions. This suggests that the vasoconstriction occurring with  $NH_4^+$  infusions could be related to metabolic acidosis (21, 37). The same pattern of changes in renal vascular resistance was apparent when the RBF at the experimental kidney was factored by that at the control kidney. This suggests that the renal vasoconstriction during the infusion of Cl-containing solutions was related to the local increase in  $P_{Cl}$  that was potentiated by an accompanying rise in  $P_H$ .

Prior salt depletion doubled the renal vasoconstriction that occurred during intrarenal hypertonic NaCl infusion. Gerber et al. (10) showed that acute volume expansion with isotonic saline abolished renal vasoconstriction during hypertonic NaCl infusions. An uncontrolled salt intake might explain why renal vasoconstriction was not seen with hypertonic NaCl infusion in one previous study (24). Several other studies have demonstrated vasoconstriction during infusion of hypertonic NaCl solutions in intact and isolated kidneys (9–11, 32).

The intrarenal infusion of all the hypertonic solutions that did not contain  $Cl^-$  (except for  $NH_4$ acetate) produced an increase in RPF but no significant changes in GFR; the FF fell in every case indicating that the renal vasodilatation probably affected afferent and efferent arterioles (38). In contrast, the GFR was reduced during the intrarenal infusion of the two Cl-containing solutions. With NaCl, the fall in GFR was proportional to the fall in RPF and the FF remained unchanged, suggesting that the vasoconstriction affected afferent and efferent arterioles. With  $NH_4Cl$ , however, the FF fell considerably suggesting an additional effect on the glomerular ultrafiltration coefficient (39).

There are several similarities between the Cl-induced fall in RBF and GFR observed in these experiments and the "tubulo-glomerular feedback" mechanism identified at the single nephron level and that is initiated by tubular  $Cl^-$  reabsorption (8, 40–42). Some experiments have indicated that tubular fluid osmolality may also regulate the nephron feedback response although this remains controversial (4, 43). "Feedback inhibition" of SNGFR occurs during infusion of hypertonic NaCl but not hypertonic  $NaHCO_3$  (44), is augmented by prior dietary salt restriction (45), and is independent of the nervous system (46). In one series, feedback inhibition of SNGFR was accompanied by a rise in FF (47) but in a recent study, Ichikawa has shown that although feedback inhibition of SNGFR is accompanied by comparable increases in afferent and efferent arteriolar resistance, the FF falls because

SNGFR is reduced not only by the fall in plasma flow rate, but also by a large fall in the glomerular capillary ultrafiltration coefficient (39). In the present series, sustained renal vasoconstriction was specifically related to hyperchloremia, was related to  $Cl^-$  reabsorption, was initiated by an intrarenal mechanism that was independent of the nervous system, was potentiated by prior salt depletion, was specific for the kidney, and was accompanied by a fall in GFR. We observed no change in FF during Cl-induced renal vasoconstriction with NaCl infusion, but a fall in FF during  $NH_4Cl$ . The  $NH_4Cl$  produced a hyperchloremic acidosis, which has been found to impair proximal tubule  $Na^+K^+$  transport (21, 22), leads to enhanced delivery of filtrate to the distal nephron (48) and increases  $U_{Na}V$  (21, 23, 37). The greater vasoconstriction apparent with  $NH_4Cl$  than with NaCl in the present series and the potentiation of NaCl-induced renal vasoconstriction by metabolic acidosis observed previously (49) may be related to inhibition of proximal fluid reabsorption during metabolic acidosis with a consequent increase in distal  $Cl^-$  delivery and potentiation of the feedback response. Thus, the present studies extend the micropuncture findings to the whole kidney level, demonstrate the specificity of hyperchloremic vasoconstriction for the kidney and indicate a physiological role for the feedback mechanism in regulating renal vascular resistance during hyperchloremia and metabolic acidosis.

The predominant effects of hypertonic infusions on salt and water reabsorption should occur in the proximal tubule. In the isolated tubule, an increase in the NaCl concentration of the perfusate inhibits fluid reabsorption (20). In the intact animal,  $NH_4Cl$  or hypertonic NaCl inhibits proximal reabsorption of fluid,  $Na^+$  and  $Cl^-$  and increases the early distal NaCl concentration (48, 50) whereas hypertonic  $NaHCO_3$  decreases early distal  $Cl^-$  concentration (51). Thus, infusions of  $NH_4Cl$  or NaCl will increase substantially the fraction of filtrate delivered to the loop of Henle (17, 21, 22). Accordingly, changes in  $R/F_{Na}$  or  $R/F_{Cl}$  should indicate changes in distal delivery of filtered  $Na^+$  and  $Cl^-$ . The changes in RBF and GFR were independent of changes in  $R/F_{Na}$  but were very closely correlated with changes in  $R/F_{Cl}$  (Fig. 2B and 2C), suggesting that renal vascular resistance was related to the delivery of  $Cl^-$ , but not  $Na^+$ , to the loop of Henle. Nizet found that the GFR fell during osmotic diuresis or inhibition of proximal-tubular function but that this was related to a fall in  $R/F_{H_2O}$  and was independent of  $Cl^-$  reabsorption (12–14). His studies were conducted during a considerable osmotic diuresis that may have increased the intratubular and intrarenal pressures and could have led to passive falls in GFR and RBF. In our studies, the osmotic diuresis was less severe and



changes in GFR or RBF were independent of  $R/F_{H_2O}$  (Fig. 2A). Whereas Nizet (12) found that  $Na_2SO_4$ , but not NaCl, reduced RBF, Sadowski (11) obtained opposite results using rates of osmolar infusion similar to those in the present experiments. Thus, the mechanisms identified by Nizet to regulate GFR and RBF probably operate at high rates of osmotic diuresis. The present study demonstrates that the regulation of RBF and GFR by  $Cl^-$  can override the effects of hyperosmolality on the renal circulation.

#### ACKNOWLEDGMENTS

The author gratefully acknowledges a Pharmaceutical Manufacturer's Faculty Award and a grant from the National Institutes of Health AM 27952. We thank Miss Paula Dolan for typing the manuscript.

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