Renal Mechanism of Action of Rat Atrial Natriuretic Factor

Chou-Long Huang, John Lewicki, Lorin K. Johnson, and Martin G. Cogan
Cardiovascular Research Institute and Departments of Medicine and Physiology, University of California, San Francisco, California 94143; and California Biotechnology, Inc., Palo Alto, California 94303

Abstract

There has been conflict as to whether crude extracts of atrial natriuretic factor increase renal solute excretion by a hemodynamic mechanism or by direct inhibition of tubular transport. To investigate this issue, seven rats were studied during a euvolemic control period and following continuous administration of pure, synthetic 24 amino acid atrial natriuretic factor. A 10–25-fold increase in urinary sodium and chloride excretion occurred with a brisk kaliuresis but little bicarbonaturia. Atrial natriuretic factor caused whole kidney glomerular filtration rate to increase from 1.17±0.04 to 1.52±0.07 ml/min ($P < 0.005$). A parallel increase in single nephron glomerular filtration rate, from 34±1 to 44±2 nl/min ($P < 0.001$), and from 26±1 to 37±2 nl/min ($P < 0.005$) was measured at the end-proximal and early distal nephron sites, respectively. Appropriate for the higher flows were an increase in absolute proximal and loop reabsorptive rates for bicarbonate, chloride, and water, with a slight decrease in fractional solute and volume reabsorption in proximal and loop segments. To exclude the possibility that atrial natriuretic factor increased filtration rate only in anesthetized animals, eight unanesthetized rats were studied. Glomerular filtration rate increased by 45%, from 2.04±0.17 to 2.97±0.27 ml/min ($P < 0.005$) without significant change in renal plasma flow, as reflected by ^{14}C-para-aminohippurate clearance (5.4±0.5–5.6±0.9 ml/min). The clearance and micropuncture data did not preclude changes in relative blood flow distribution to or in transport by deep nephron segments. In conclusion, atrial natriuretic factor appears to increase renal solute excretion predominantly by a hemodynamic mechanism without directly inhibiting superficial tubular transport.

Introduction

Within the last few years, a vasodilatory, natriuretic compound derived from granules within cardiocytes of the right atrium has been described (1–16). This substance has been very recently characterized as a closely related group of peptides of 21–26 amino acids (2–4) derived from a common precursor polypeptide of 152 amino acids (3, 5). The general term atrial natriuretic factor (ANF)[1] has been used to describe these peptides.

The mechanism of action of this factor is controversial. Studies from one laboratory have suggested that the increase in renal solute excretion is due primarily to changes in hemodynamics, specifically an increase in glomerular filtration rate (GFR) (4, 6–8). Other groups have suggested that the renal effect is not mediated by a change in GFR (1, 9–16) but rather by redistribution of blood flow (10) or by direct inhibition of proximal reabsorption (11), of loop reabsorption (10, 12), or of collecting duct reabsorption (13–16). Difficulties with these studies include: (*a*) use of crude, unpurified extracts of the right atrium (1, 6, 7, 9–16) that may contain contaminants with vasodilatory but not natriuretic properties (17); (*b*) use of volume contracted animals (10, 14, 15); (*c*) use of isolated renal membrane vesicles without correlation to in vivo effects (11); (*d*) use of an in vitro isolated perfused kidney preparation with impaired base-line hemodynamics (4, 6); and (*e*) unstable time controls (14). To date, there have been no micropuncture investigations examining the renal mechanism of action of ANF using a pure peptide in animals in a euvolemic state.

The principal purpose of the present study was to elucidate the mechanism of renal action of ANF using a pure, synthetic peptide in euvolemic animals. Free-flow micropuncture techniques were used to measure single nephron GFR as well as proximal and loop[2] reabsorption of bicarbonate, chloride, and water in response to ANF. Clearance studies were performed in awake animals to examine the hemodynamic response to ANF in the absence of anesthesia or major surgery.

Methods

Animals. Sprague-Dawley rats weighing 233±7 g were used for micropuncture studies ($n = 7$ males) or clearance studies ($n = 8$ females).

Protocol. Animals were prepared for micropuncture (18–20) or for clearance studies (20) as previously described. In the micropuncture experiments, animals were replenished with plasma lost consequent to the preparatory surgery (18, 20). In the clearance experiments, animals were allowed at least 1 h to fully regain consciousness after the short-acting anesthesia (methohexital) used for catheter placement. Infusion of bicarbonate Ringer's solution (0.84 ml/h) with [^{3}H]inulin (100 μCi prime and 150 μCi/h in the micropuncture studies and 15 μCi prime and 15 μCi/h in the clearance studies) and with ^{14}C-para-aminohippurate

Portions of this work have been published in preliminary form in *Kidney Int.*, 1985.

Address correspondence and reprint requests to Dr. Cogan.

Received for publication 9 October 1984 and in revised form 6 November 1984.

J. Clin. Invest.

0021-9738/85/02/0769/05 $1.00
Volume 75, February 1985, 769–773

1. *Abbreviations used in this paper:* ANF, atrial natriuretic factor; GFR, glomerular filtration rate; PAH, para-amino-hippurate; SNGFR, single nephron GFR.

2. The superficial loop is defined for the purposes of this study to include the nephron segment between the last accessible loop of the superficial proximal convoluted tubule and the early distal tubule.

(PAH; 5 μCi prime and 5 μCi/h sustaining in the clearance studies only) was then begun. After an hour equilibration, the first control period commenced, with end-proximal and early distal samples taken in the micropuncture studies, or with four consecutive 15-min urine collections obtained in the clearance studies. All blood withdrawn for inulin, PAH, electrolyte, and blood gas measurements was replaced quantitatively with blood obtained from sibling rats on the day of study.

After the first euvolemic control period, synthetic rat 24 amino acid ANF (California Biotechnology, Inc., Palo Alto, CA) (4) was given intravenously as a prime, 12 μg/kg, and then as a constant infusion, 1 μg/kg·min. ANF was infused in a modified Ringer's solution designed to replace urinary electrolyte losses (in meq/liter: NaCl, 100; KCl, 35; $KHCO_3$, 5; Na_2HPO_4 4; $MgSO_4$, 1; and $CaCl_2$, 1.8) at 30 μl/min in the micropuncture studies and 50 μl/min in the clearance studies, the rate designed to be less than or equal to urinary losses. After 15 min for equilibration, the second micropuncture or clearance period was performed identically to the first.

Measurements and calculations. Methods for measuring radioisotopes and electrolytes in plasma and in urine have been described previously (18–21). Tubular fluid [total CO_2] was measured by microcalorimetry and [chloride] by the microtitrimetric method. Methods for calculating filtration and reabsorption of solutes and water have been previously published (18–21). Statistical significance was assessed using the paired *t* test for results in the same animal.

Results

The results of the micropuncture studies are shown in Table I. Arterial hematocrit, plasma electrolyte composition, and acid-base status were stable after ANF administration and there was only a slight (3 mmHg) decline in blood pressure. Urine sodium and chloride excretion rates increased 10–25-fold and remained stable throughout the study period. The >2.5-fold increase in potassium excretion was normal for the increase in sodium excretion based on previous measurements

Table I. Micropuncture Studies on the Effect of Atrial Natriuretic Factor on Filtration and Proximal Tubule and Loop Reabsorption of Bicarbonate, Chloride, and Water

	Control (n = 7)	ANF	$P<$
Blood			
Pressure (*mmHg*)	113±3	110±3	0.005
Hematocrit (*vol%*)	42.9±0.5	42.6±0.8	NS
[Protein] (*g/dl*)	4.9±0.1	4.7±0.1	0.025
pH	7.41±0.01	7.43±0.01	NS
P_{CO_2} (*mmHg*)	37.5±0.5	36.1±0.7	NS
[Na] (*meq/liter*)	145±1	145±1	NS
[K] (*meq/liter*)	3.6±0.2	3.4±0.1	NS
[Total CO_2] (*mM*)	24.2±0.4	24.5±0.5	NS
[Cl] (*meq/liter*)	103±1	105±1	0.025
Urine (1 kidney)			
GFR (*ml/min*)	1.17±0.04	1.52±0.07	0.005
Excretion rate			
Volume (*μl/min*)	2.6±0.2	14.3±1.9	0.005
Na (*neq/min*)	104±30	2,518±478	0.005
K (*neq/min*)	641±137	1,708±218	0.005
Total CO_2 (*nmol/min*)	19±16	145±57	0.05
Cl (*neq/min*)	324±84	3,601±473	0.001
Fractional excretion rate			
Volume	0.002±0.0002	0.009±0.001	0.001
Na	0.001±0.0002	0.011±0.002	0.005
K	0.146±0.021	0.321±0.023	0.005
Total CO_2	0.001±0.0004	0.003±0.001	0.025
Cl	0.002±0.0006	0.020±0.002	0.001
Tubular fluid			
Proximal			
SNGFR (*nl/min*)	34.1±1.1	44.3±2.2	0.001
Filtered load			
Total CO_2 (*pmol/min*)	907±27	1,193±61	0.001
Cl (*peq/min*)	3,881±108	5,106±247	0.001
End-proximal			
[Total CO_2] (*mM*)	5.1±0.4	8.8±0.7	0.005
[Cl] (*meq/liter*)	135±2	138±3	NS
Delivery out of proximal tubule			
H_2O (*nl/min*)	16.9±0.8	24.1±1.5	0.001
Total CO_2 (*pmol/min*)	86±9	217±28	0.005
Cl (*peq/min*)	2,276±91	3,320±172	0.005
Absolute proximal reabsorption			
H_2O (*nl/min*)	17.3±0.9	20.1±1.3	0.005
Total CO_2 (*pmol/min*)	820±28	976±35	0.005
Cl (*peq/min*)	1,605±103	1,786±182	NS
Fractional proximal reabsorption			
H_2O	0.51±0.02	0.46±0.02	0.005
Total CO_2	0.90±0.01	0.82±0.02	0.005
Cl	0.41±0.02	0.35±0.03	0.025
Distal			
SNGFR (*nl/min*)	26.3±1.4	36.5±2.1	0.005
Collected concentrations			
[Total CO_2] (*mM*)	3.7±0.9	5.2±1.0	0.05
[Cl] (*meq/liter*)	29.4±2.7	47.9±5.0	0.005
Delivery from distal tubule			
H_2O (*nl/min*)	4.1±0.7	8.0±1.1	0.025
Total CO_2 (*pmol/min*)	14±3	37±5	0.005
Cl (*peq/min*)	114±17	377±61	0.005
Absolute reabsorption to distal tubule (proximal + loop)			
H_2O (*nl/min*)	22.2±1.5	28.5±2.1	0.025
Total CO_2 (*pmol/min*)	684±35	947±60	0.01
Cl (*peq/min*)	2,873±35	3,836±234	0.005
Fractional reabsorption to distal tubule (proximal + loop)			
H_2O	0.84±0.03	0.78±0.03	0.05
Total CO_2	0.98±0.01	0.96±0.01	0.025
Cl	0.96±0.01	0.91±0.01	0.005

(n = 186) in euvolemic animals or in animals subjected to volume expansion or carbonic anhydrase inhibition (18–21). Bicarbonaturia was slight.

ANF caused whole kidney GFR to increase from 1.17±0.04 to 1.52±0.07 ml/min ($P < 0.005$) with a parallel increase in single nephron GFR (SNGFR), from 34±1 to 44±2 nl/min ($P < 0.001$) and from 26±1 to 37±2 nl/min ($P < 0.005$) measured at the end-proximal and early distal nephron sites, respectively, as illustrated in Fig. 1. There was an increase in absolute proximal total CO_2, chloride, and water reabsorption, with a decline in fractional reabsorption of each (Table I). These changes in proximal solute and volume transport were appropriate for the increase in SNGFR as seen in Fig. 2. The shaded areas in Fig. 2 represent the normal reabsorptive rates for euvolemic Sprague-Dawley rats (n = 26) with spontaneous variation of SNGFR over the range observed in the present study (20, 22). Similarly, at the early distal micropuncture site, measurements of absolute total CO_2, chloride, and volume reabsorption were increased after ANF administration. Again, the slight decline in fractional solute and volume reabsorptive rates (e.g., the decrease in fractional chloride reabsorption from 0.96±0.01 to 0.91±0.01, $P < 0.005$) was consistent with the normal response to increased flow by loop transport processes (23, 24).

Studies in awake animals were performed to preclude the possibility that ANF increased GFR only in anesthetized animals. As shown in Table II, blood pressure and plasma electrolyte composition were relatively stable. Base-line urinary electrolyte excretion rates were higher than had been obtained in the micropuncture experiments but ANF still caused a stable, 5–7-fold increase in sodium and chloride excretion with a brisk kaliuresis and slight bicarbonaturia. GFR increased by 45%, from 2.04±0.17 to 2.97±0.27 ml/min ($P < 0.005$), as shown in Fig. 1. However, the increase in GFR was not accompanied by a rise in renal plasma flow, as estimated by PAH clearance (5.4±0.5–5.6±0.9 ml/min, NS). PAH clearance is an accurate reflection of renal plasma flow, since extraction of PAH is unaltered by ANF and PAH clearance has correlated well with plasma flow measured by electromagnetic flowmeter during ANF administration (8). Assuming 90% extraction of PAH, the calculated filtration fraction rose from 0.35 to 0.50.

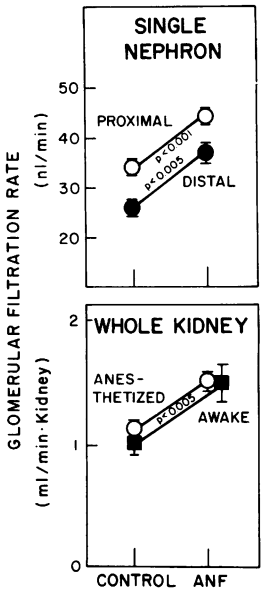

Figure 1. Change induced by ANF in single nephron glomerular filtration rate (*Top*) measured at the end-proximal (open circles) and early distal (closed circles) sites and in whole kidney GFR (*Bottom*) measured in anesthetized (open circles) and awake (closed squares) euvolemic rats.

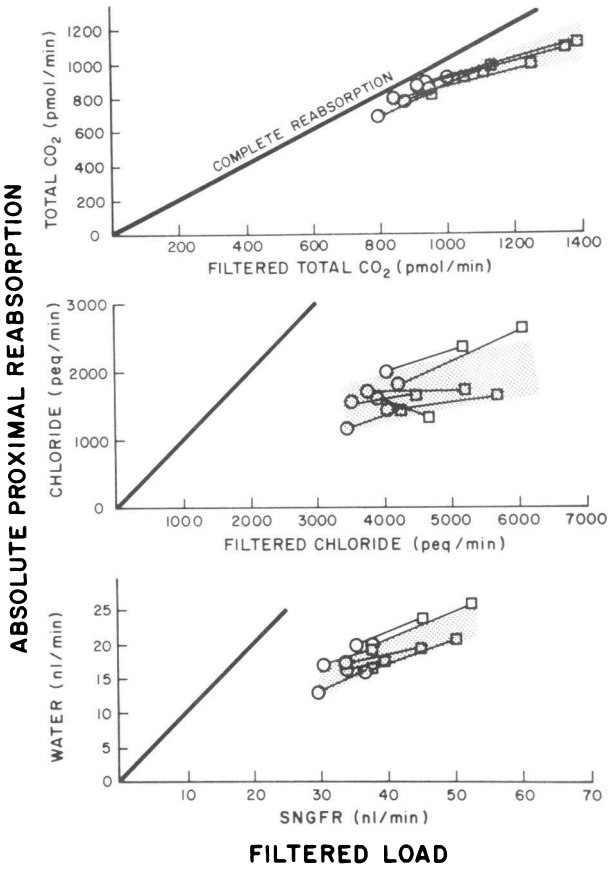

Figure 2. Effect of ANF (open squares) compared with control euvolemic values (open circles) on absolute proximal reabsorption of total CO_2 (*Top*), chloride (*Middle*), and water (*Bottom*) as a function of filtered load. Shaded areas represent normal euvolemic reabsorptive values at comparable filtered loads from previous studies of 26 Sprague-Dawley rats (20, 22).

Discussion

The present study is in agreement with those of Atlas and his colleagues (4, 6–8) in finding that ANF increases renal solute excretion principally by hemodynamic means rather than by direct tubular transport inhibition.

The increment in absolute proximal bicarbonate, chloride, and volume reabsorption in response to the increase in SNGFR induced by ANF appeared to be appropriate based on previous observations (Fig. 2).[3] The lack of substantial bicarbonaturia also makes the proximal tubule an unlikely site of direct generalized inhibition by ANF. Similarly, the superficial loop of Henle appeared to increase solute reabsorption normally in

3. While a depression in filtration fraction and peritubular protein concentration selectively decreases proximal NaCl reabsorption (18–20), it has yet to be demonstrated that a rise in filtration fraction, as observed in these studies, is associated with an increase in proximal NaCl reabsorption under normal free-flow conditions.

Table II. Clearance Studies in Awake Rats on the Effect of Atrial Natriuretic Factor on Whole Kidney Filtration Rate and Urinary Solute Excretion

	Control ($n = 8$)	ANF	$P<$
Blood			
Pressure (*mmHg*)	114±1	112±1	NS
Hematocrit (*vol%*)	42.4±0.9	45.2±0.6	0.005
[Protein] (*g/dl*)	5.4±0.1	5.7±0.1	0.005
[Na] (*meq/liter*)	141±1	139±1	NS
[K] (*meq/liter*)	3.7±0.1	3.5±0.1	NS
[Cl] (*meq/liter*)	96±1	95±1	NS
Urine (2 kidneys)			
GFR (*ml/min*)	2.04±0.17	2.97±0.27	0.005
PAH clearance (*ml/min*)	5.40±0.46	5.58±0.91	NS
Excretion rate			
Volume (*μl/min*)	33±3	118±10	0.001
Na (*neq/min*)	1,819±265	13,353±1,129	0.001
K (*neq/min*)	1,450±133	4,097±216	0.001
Total CO_2 (*nmol/min*)	147±68	1,024±352	0.05
Cl (*neq/min*)	2,637±231	13,864±1,079	0.001
Fractional excretion rate			
Volume	0.017±0.0016	0.042±0.004	0.005
Na	0.007±0.0010	0.034±0.003	0.001
K	0.201±0.021	0.414±0.044	0.001
Total CO_2	0.003±0.0013	0.012±0.005	NS
Cl	0.014±0.0012	0.051±0.004	0.001

response to the increased load (Table I). The small decrease (−5%) in fractional sodium chloride reabsorption observed as flow increased is expected (23, 24), and is more than enough to account for the observed increase in fractional sodium and chloride excretion (1–2%). In addition to the change in delivered load, however, the present data do not preclude a small direct or indirect (i.e., by medullary washout) effect by ANF on loop transport of juxtamedullary nephrons (8, 10, 12). Finally, although a major effect by ANF on cortical collecting tubule function has been proposed (13–16), this appears to be unlikely because the large stimulation of urinary potassium excretion was appropriate for the natriuresis (18–21). This kaliuretic response to ANF stands in contrast to what is expected with diuretics that act directly on the cortical collecting tubule, such as amiloride or triamterene.

The 30–45% acute increase in GFR was associated with a 1–4% increment in fractional sodium and chloride excretion. However, a question arises as to whether this excretory response was appropriate, and not excessive, for the increased load. Put another way, it is not clear a priori that the normal renal response to an acute rise in GFR is for 6–10% of the increment in filtered sodium load to escape tubular reabsorption, as was observed in these studies. There have been only a few previous studies which have examined the load dependence of renal sodium reabsorption when GFR is acutely raised above normal values. In these studies, disruption of renal autoregulation or infusion of substances thought not to have direct tubular effects, such as glucagon or glycine, have acutely increased GFR by 17–50% above normal, and 5–19% of the increment in filtered sodium load has been excreted (25–28). Thus, the present changes in solute excretion evoked by ANF are not inconsistent with those expected in the normal renal response to a large, acute augmentation in GFR.

In addition to the rise in GFR induced by ANF, redistribution of intrarenal blood flow may also contribute to the observed natriuresis and chloriuresis (10). This possibility is not rigorously excluded in the present studies, although the increase in superficial SNGFR was proportional to the increase in whole kidney GFR (Table I and Fig. 1).

The increase in GFR might be mediated by a substantial alteration in the hydraulic permeability-filtration area coefficient (K_f), if filtration disequilibrium is obtained. Alternatively, if there were filtration equilibrium, then a rise in net transmembrane hydraulic filtration pressure occurred, mediated by afferent arteriolar vasodilation and efferent vasoconstriction. The changes in afferent and efferent resistances must have been reciprocal, since systemic blood pressure and renal plasma flow (as reflected by PAH clearance), and hence total renal vascular resistance, were unchanged, as has been found by others (8). The impressive increase in filtration fraction that resulted, from 0.35 to 0.50, is unprecedented when compared with other vasoactive substances (29). Thus, these results suggest either that K_f increased substantially or, more likely (given the relatively high filtration fraction in the control period), that glomerular capillary hydraulic pressure increased to ≥64 mmHg from a control value of 45 mmHg.[4] Direct measurement of intraglomerular pressures will be needed to confirm these predictions.

In conclusion, atrial natriuretic factor markedly increases GFR in euvolemic rats without a demonstrable direct inhibition of proximal or loop transport in superficial nephrons. The supernormal GFR appears to account for the rise in urinary solute excretion, although a change in relative blood flow distribution to or in transport by deep nephron segments cannot be excluded as contributing to the natriuresis and chloriuresis.

Acknowledgments

The authors are grateful to Dr. John Baxter for help in obtaining the rat atrial natriuretic factor. We also wish to thank Dr. Floyd C. Rector, Jr. for his ongoing support and advice.

This work was supported in part by a Clinical Investigator Award to Dr. Cogan (1-KO8-AM-01015) and a grant (AM-27045) from the National Institute of Arthritis, Diabetes, and Digestive Diseases.

References

1. DeBold, A. J., H. B. Borenstein, A. T. Veress, and H. Sonnenberg. 1981. A rapid and potent natriuretic response to intravenous injection of atrial myocardial extract in rats. *Life Sci.* 28:89–94.

2. Currie, M. G., D. M. Geller, B. R. Cole, N. R. Siegel, K. F. Fok, S. P. Adams, S. R. Eubanks, G. R. Galluppi, and P. Needleman.

4. Glomerular capillary hydraulic pressure was assumed to be greater than or equal to the sum of efferent arteriolar oncotic pressure and Bowman's space hydraulic pressure. Efferent arteriolar protein oncotic pressure was calculated by the method of Brenner et al. (30), and it was assumed that: efferent protein concentration equals afferent protein concentration/(one minus filtration fraction); filtration fraction equals GFR/(PAH clearance/0.9); and Bowman's space hydraulic pressure equals 10 mmHg.

1984. Purification and sequence analysis of bioactive atrial peptides (Atriopeptins). *Science (Wash. DC).* 223:67–69.

3. Sagnella, G. A., and G. A. MacGregor. 1984. Cardiac peptides and the control of sodium excretion. *Nature (Lond.).* 309:666–667.

4. Atlas, S. A., H. D. Kleinert, M. J. Camargo, A. Januszewicz, J. E. Sealey, J. H. Laragh, J. W. Schilling, J. A. Lewicki, L. K. Johnson, and T. Maack. 1984. Purification, sequencing and synthesis of natriuretic and vasoactive rat atrial peptide. *Nature (Lond.).* 309:717–719.

5. Yamanaka, M., B. Greenberg, L. Johnson, J. Seilhamer, M. Brewer, T. Friedemann, J. Miller, S. Atlas, J. Laragh, J. Lewicki, and J. Fiddes. 1984. Cloning and sequence analysis of the cDNA for the rat atrial natriuretic factor precursor. *Nature (Lond.).* 309:719–722.

6. Camargo, M. J. F., H. D. Kleinert, S. A. Atlas, J. E. Sealey, J. H. Laragh, and T. Maack. 1984. Ca-dependent hemodynamic and natriuretic effects of atrial extract in isolated rat kidney. *Am. J. Physiol.* 246(*Renal Fluid Electrolyte Physiol.* 15):F447–F456.

7. Vaughan, E. D., Jr., D. N. Marion, J. E. Sealey, M. J. F. Camargo, H. D. Kleinert, T. Maack, and J. H. Laragh. 1983. Atrial natriuretic extract: effects on renal hemodynamics in the rabbit. *Surg. Forum.* 34:690–692.

8. Maack, T., D. N. Marion, M. J. F. Camargo, H. D. Kleinert, J. H. Laragh, E. D. Vaughan, Jr., and S. A. Atlas. 1984. Effects of synthetic atrial natriuretic factor on kidney function and the renin-aldosterone system in the dog. *Am. J. Med.* In press.

9. Keeler, R. 1982. Atrial natriuretic factor has a direct, prostaglandin-independent action on kidneys. *Can. J. Physiol. Pharmacol.* 60: 1078–1082.

10. Borenstein, H. B., W. A. Cupples, H. Sonnenberg, and A. T. Veress. 1983. The effect of a natriuretic atrial extract on renal haemodynamics and urinary excretion in anaesthetized rat. *J. Physiol.* 334:133–140.

11. Dousa, T. P., T. G. Hammond, A. N. K. Yusufi, and F. G. Knox. 1984. Synthetic atrial natriuretic factor inhibits Na-coupled solute reabsorption in proximal tubules. *Kidney Int.* 208A.

12. Sonnenberg, H., C. K. Chong, and A. T. Veress. 1981. Cardiac atrial factor—an endogenous diuretic? *Can. J. Physiol. Pharmacol.* 59: 1278–1279.

13. Keeler, R., and A. M. Azzarolo. 1983. Effects of atrial natriuretic factor on renal handling of water and electrolytes in rats. *Can. J. Physiol. Pharmacol.* 61:996–1002.

14. Sonnenberg, H., and W. A. Cupples. 1982. Intrarenal localization of the natriuretic effect of cardiac atrial extract. *Can. J. Physiol. Pharmacol.* 60:1149–1152.

15. Pollock, D. M., and R. O. Banks. 1983. Effect of atrial extract on renal function in the rat. *Clin. Sci.* 65:47–55.

16. Briggs, J. P., B. Steipe, G. Schubert, and J. Schnermann. 1982. Micropuncture studies on the renal effects of atrial natriuretic substance. *Pfluegers Arch. Eur. J. Physiol.* 395:271–276.

17. Cole, B. R., J. G. Boylan, M. A. Kuhnline, M. G. Currie, D. M. Geller, and P. Needleman. 1984. Atriopeptins: natriuresis is dissociated from spasmolytic properties. *Fed. Proc.* 43:454.

18. Cogan, M. G., D. A. Maddox, M. S. Lucci, and F. C. Rector, Jr. 1979. Control of proximal bicarbonate reabsorption in normal and acidotic rats. *J. Clin. Invest.* 64:1168–1180.

19. Cogan, M. G., and F. C. Rector, Jr. 1982. Determinants of proximal bicarbonate, chloride and water reabsorption during carbonic anhydrase inhibition. *Am. J. Physiol.* 242(*Renal Fluid Electrolyte Physiol.* 11):F274–F284.

20. Cogan, M. G. 1983. Volume expansion predominantly inhibits proximal reabsorption of NaCl rather than $NaHCO_3$. *Am. J. Physiol.* 245(*Renal Fluid Electrolyte Physiol.* 14):F272–F275.

21. Cogan, M. G., and M. R. Mueller. 1983. $NaHCO_3$ therapy corrects the increased urinary Na, K, Cl and H_2O excretion induced by NH_4Cl. *Miner. Electrolyte Metab.* 9:132–136.

22. Cogan, M. G. 1984. Physiologic regulation of proximal $NaHCO_3$ and NaCl reabsorption by renal nerve activity. *Kidney Int.* In press.

23. Schnermann, J. 1968. Microperfusion study of single short loops of Henle in rat kidney. *Pfluegers Arch. Eur. J. Physiol.* 300:255–282.

24. Morgan, T., and R. W. Berliner. 1969. A study by continuous microperfusion of water and electrolyte movements in the loop of Henle and distal tubule of the rat. *Nephron.* 6:388–405.

25. Baer, P. G., L. G. Navar, and A. C. Guyton. 1970. Renal autoregulation, filtration rate, and electrolyte excretion during vasodilatation. *Am. J. Physiol.* 219:619–625.

26. Johannesen, J., M. Lie, and K. Kiil. 1977. Effect of glycine and glucagon on glomerular filtration and glucagon on glomerular filtration and renal metabolic rates. *Am. J. Physiol.* 233(*Renal Fluid Electrolyte Physiol.* 2):F61–F66.

27. Levy, M. 1975. Further observations on the response of the glomerular filtration rate to glucagon: comparison with secretin. *Can. J. Physiol. Pharmacol.* 53:81–85.

28. Levy, M., and N. L. Starr. 1972. The mechanism of glucagon-induced natriuresis in dogs. *Kidney Int.* 2:76–84.

29. Dworkin, L. D., I. Ichikawa, and B. M. Brenner. 1983. Hormonal modulation of glomerular function. *Am. J. Physiol.* 244(*Renal Fluid Electrolyte Physiol.* 13):F95–F104.

30. Brenner, B. M., I. R. Ueki, and T. M. Daugharty. 1972. On estimating colloid osmotic pressure in pre- and postglomerular plasma in the rat. *Kidney Int.* 2:51–53.