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Atrial Natriuretic Factor Ameliorates Chronic Metabolic Alkalosis by Increasing Glomerular Filtration

Abstract. *The kidney maintains the elevated plasma concentration of bicarbonate that occurs in chronic metabolic alkalosis. A reduction in the glomerular filtration rate (GFR) can maintain the filtered bicarbonate load at a normal level so that a normal rate of bicarbonate reabsorption suffices to prevent urinary excretion of this anion. It is also possible that bicarbonate reabsorption might increase so as to maintain the alkalosis if GFR were not reduced. To examine this latter possibility, atrial natriuretic factor was used in alkalotic rats to restore a more normal GFR and to increase the amount of bicarbonate filtered by the glomerulus. Proximal bicarbonate reabsorption remained relatively static. Higher than normal amounts of bicarbonate were then delivered out of the proximal tubule, bicarbonate appeared in the urine, and the plasma concentration of bicarbonate fell. A reduction in GFR is thus necessary for the maintenance of chronic metabolic alkalosis. Normalizing GFR induces bicarbonaturia and initiates repair of the alkalosis.*

MARTIN G. COGAN
Cardiovascular Research Institute and
Department of Medicine,
University of California,
San Francisco 94143

Metabolic alkalosis, one of the four major acid-base disorders, is characterized by an elevated concentration of the principal blood buffer, bicarbonate. The kidney is responsible for sustaining this disorder because it does not allow the excess bicarbonate to be excreted in the urine. Traditionally, the renal mechanism thought to prevent bicarbonate excretion in the setting of a high filtered load of bicarbonate was the stimulation of proximal tubular bicarbonate reabsorption. Such augmentation of proximal bicarbonate transport was ascribed to simultaneous extracellular volume contraction and potassium depletion.

Alternatively, recent evidence suggests that the high plasma concentration of bicarbonate found in chronic metabolic alkalosis is sustained by a reduction in the glomerular filtration rate (GFR) (*1*, *2*). This depression in GFR prevents an increase in the filtered bicarbonate load, such that a normal rate of proximal bicarbonate reabsorption is sufficient to prevent bicarbonaturia. However, it can be argued that a decrease in GFR is coincidental with, but not necessary for, the maintenance of alkalosis; enhanced bicarbonate reabsorption might maintain the alkalosis if GFR did not fall. To address this possibility, extracellular volume expansion has been used to restore more normal levels of GFR. Proximal bicarbonate reabsorption under these conditions did not rise, so that bicarbonaturia developed and the alkalosis began to diminish (*1*). This observation supported the view that a reduction in GFR was critical for the maintenance of the alkalosis. However, the method for normalizing GFR (extracellular volume expansion) might have exerted effects on proximal acidification independent of changes in GFR and filtered bicarbonate load (*3*, *4*).

A means of increasing GFR without altering extracellular volume recently has been afforded by the purification, sequencing, and synthesis of atrial natriuretic factor (ANF), a cardiac-derived vasoactive hormone (*5*). ANF increases single nephron and whole kidney GFR (*6*, *7*). ANF has been shown by micropuncture and in vitro microperfusion techniques to have no inhibitory effect on proximal tubular transport (*7*, *8*). Specifically, it does not have a direct effect on proximal or whole kidney acidification, independent of the changes in tubular flow rate it induces (*7*). The purpose of the present study was to assess whether proximal bicarbonate reabsorption can increase to supernormal levels during metabolic alkalosis after restoration of nearly normal rates of single nephron glomerular filtration with ANF.

Ten Munich-Wistar rats were rendered alkalotic as described (*1*). For 11 to 14 days, they were fed an electrolyte-deficient diet supplemented with Na_2SO_4 (2.6 meq/day), injected with deoxycorticosterone acetate (0.5 mg/day, intramuscularly), and allowed free access to drinking water containing sodium bicarbonate (80 m*M*). Rats prepared in this way sustain marked chloride and potassium deficiencies (decrease in plasma volume of 28 percent and reduction in the potassium content of muscle of 46 percent (*1*). Food was withdrawn 24 hours before anesthetization with Inactin (100 mg/kg, intraperitoneal). During the surgical preparation for micropuncture, plasma volume losses were replenished (0.9 percent body weight) with plasma obtained from donor rats maintained on the same diet (*1*). An inulin infusion (*1*) was then started, and, after a stabilization period of 1 hour, free-flow micropuncture samples were obtained from Bowman's space and the end-proximal tubule. Simultaneous urine collections were also made. ANF (25-amino-acid synthetic rat auriculin, California Biotechnology, Inc., Palo Alto) was then administered as a bolus (10 μg/kg) and sustaining infusion (1 μg/kg per minute) in a modified bicarbonate Ringer's solution (40 m*M* $NaHCO_3$, 88 m*M* NaCl) at 30 μl/min. This rate is sufficient to replace urinary volume and electrolyte losses (*7*), and the measured sodium and chloride balances in the present study were not significantly different from zero. After a stabilization period of 15 minutes, the second period of micropuncture and clearance measurements commenced. Total CO_2 concentrations in urine and tubular fluid were measured by microcalorimetry. For convenience, the results were expressed as bicarbonate, the major component of the measured total CO_2. Means ± standard error of the mean are given, and significance was assessed by means of the paired *t* test with $n = 10$ in all cases.

Chronic metabolic alkalosis predictably developed on this regimen: the bicarbonate concentration of the glomerular ultrafiltrate increased to 46.9 ± 1.0 meq/liter (about 75 percent above normal), with associated hypochloremia (98 ± 2 meq/liter), and an arterial *p*H of 7.57 ± 0.01 and arterial pCO_2 of 47 ± 1 mmHg. As in earlier work (*1*, *2*), a reciprocal reduction from normal values (*9*) occurred in the single nephron (32.2 ± 1.3 nl/min) and whole kidney (0.66 ± 0.03 ml/min) GFR. As a result, the animals had normal levels (*9*) of filtered (1502 ± 52 peq/min), reabsorbed (1189 ± 42 peq/min), and distally deliv-

ered (312 ± 24 peq/min) bicarbonate during metabolic alkalosis, and there was no bicarbonaturia (7 ± 4 neq/min).

Despite a drop in mean arterial blood pressure (from 113 ± 3 to 90 ± 3 mmHg, $P < 0.001$), ANF caused a substantial rise in single nephron GFR (to 40.3 ± 1.5 nl/min, $P < 0.001$) and whole kidney GFR (to 0.90 ± 0.04 ml/min, $P < 0.001$) (Fig. 1). This increase in filtration rate to values close to normal (*9*) occurred without alteration in the contracted plasma and extracellular volumes (hematocrit and plasma protein concentration were not significantly changed by ANF administration, 47.9 ± 0.6 versus 48.4 ± 0.8 percent by volume and 4.9 ± 0.1 versus 4.9 ± 0.1 g/dl, respectively) or in the diminished potassium stores (plasma potassium concentration remained constant, 1.5 ± 0.1 versus 1.5 ± 0.1 meq/liter, not significant). The filtered bicarbonate load per nephron increased to 1711 ± 45 peq/min ($P < 0.001$). However, only a small amount of the increment in filtered bicarbonate load was reabsorbed; absolute proximal reabsorption rose only slightly (to 1260 ± 41 peq/min, not significant), and hence fractional proximal bicarbonate reabsorption fell from 79 ± 1 to 74 ± 1 percent ($P < 0.01$). Thus, the amount of bicarbonate leaving the proximal tubule (distal delivery) markedly increased, to 451 ± 23 peq/min ($P < 0.001$). Simultaneously, pronounced bicarbonaturia developed (683 ± 112 neq/min, $P < 0.001$), representing 1.9 ± 0.3 percent of the filtered bicarbonate load. The alkalosis diminished, with a significant drop in the bicarbonate concentration of the glomerular ultrafiltrate to 42.7 ± 1.1 meq/liter ($P < 0.001$).

These results support the contention that proximal bicarbonate reabsorption cannot increase to accommodate the high load of filtered bicarbonate that would occur in the presence of metabolic alkalosis if GFR remained normal. The observed reduction in GFR during metabolic alkalosis is thus critical for maintaining the alkalotic state by preventing an increase in filtered bicarbonate. An increase in distal bicarbonate delivery and hence urinary bicarbonate excretion is thereby prevented. These findings with ANF are consonant with earlier observations in which volume repletion or expansion was used for normalizing single nephron and whole kidney GFR (*1*).

The bicarbonaturic response to ANF in rats with metabolic alkalosis contrasts with that of normal rats, in which the increase in GFR induced by ANF results in virtually no urinary bicarbonate; increased sodium excretion is accompanied almost exclusively by increased excretion of chloride (*6–8*). In normal rats, there is excellent flow dependence for proximal reabsorption of bicarbonate (glomerulotubular balance), as a result of modification of the luminal bicarbonate concentration profile and other factors (*7, 9*). Flow dependence for proximal reabsorption of sodium chloride is poor, however, and, although much of the increment in sodium chloride delivery out of the proximal tubule is reabsorbed by more distal transport segments, a fraction of the augmented salt delivery escapes reabsorption, resulting in natriuresis and chloruresis (*7*).

In contrast, in rats with metabolic alkalosis that were given ANF, the increase in GFR occurs in a setting of an elevated filtered bicarbonate concentration and, in addition, a poor load dependency of proximal bicarbonate reabsorption induced by alkalemia. Little (*10*) or no (*1, 2*) stimulation of proximal acidification in the presence of an increased bicarbonate load during chronic metabolic alkalosis has been observed, and this result is best accounted for by the inhibitory effect of peritubular alkalemia on cellular bicarbonate exit (*4*). Peritubular alkalemia resets the maximal proximal bicarbonate reabsorptive rate and thereby renders the tubule unresponsive to normal acute stimulants, such as higher luminal flow rate and mean luminal bicarbonate concentration (*1, 4*). The results presented here do not preclude the possibility, however, that more prolonged elevation in GFR might have led to stimulation of proximal bicarbonate reabsorption.

As a consequence of the diminished fractional proximal reabsorption of bicarbonate, in concert with the low filtered chloride concentration, fluid delivered out of the proximal tubule is rich in bicarbonate relative to chloride. Distal acidification sites can reabsorb only a portion of the supernormal bicarbonate delivery so that bicarbonaturia ensues. In the present studies, about a fifth of the increment in bicarbonate delivered out of the proximal tubule after administration of ANF was excreted into the urine (*11*). In summary, the urinary anion composition after ANF administration depends on the electrolyte composition of the blood. Such a response contrasts with that evoked by conventional diuretics, which act directly on specific tubular transport processes in discrete nephron segments, so that the anion excreted is invariant (for example, bicarbonate with acetazolamide or chloride with furosemide or thiazide).

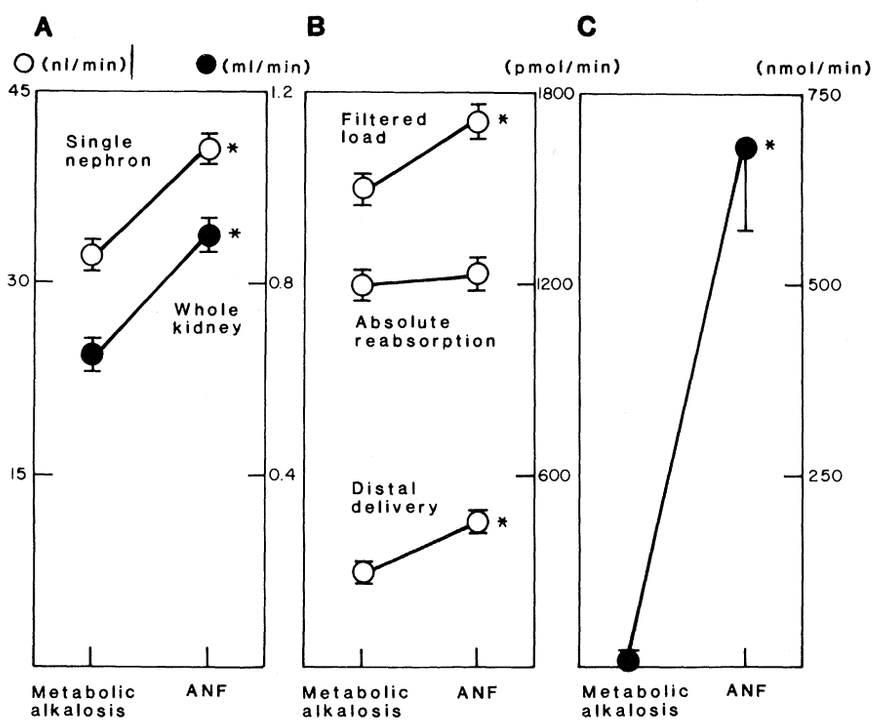

Fig. 1. Changes induced by atrial natriuretic factor (ANF) during chronic metabolic alkalosis in single nephron and whole kidney glomerular filtration rate (A); in proximal filtration, reabsorption, and distal delivery of bicarbonate (B); and in whole kidney urinary excretion of bicarbonate (C). Each symbol denotes the mean value for the period with an error bar representing standard error of the mean. An asterisk denotes a significant difference ($P < 0.001$, paired t test, ten animals).

These results with ANF demonstrate that acute renal repair of chronic chloride-depletion metabolic alkalosis in a rat model system can be initiated without replacement of the chloride or potassium deficits (or without pharmacological inhibition of tubular function). They support the thesis that the resolution of chronic metabolic alkalosis can be achieved by normalizing GFR.

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Deficient Vasoactive Intestinal Peptide Innervation in the Sweat Glands of Cystic Fibrosis Patients

Abstract. *The innervation of acini and ducts of eccrine sweat glands by immunoreactive, vasoactive intestinal peptide–containing nerve fibers was sharply reduced in seven patients with cystic fibrosis compared to eight normal subjects. The decrease in innervation by this neuropeptide, which has been shown to promote blood flow and the movement of water and chloride across epithelial surfaces in other systems, may be a basic mechanism for the decreased water content and relative impermeability of the epithelium to chloride and other ions that characterize cystic fibrosis.*

PETER HEINZ-ERIAN
Department of Medicine, University of Oklahoma Health Sciences Center, and *Veterans Administration Medical Center, Oklahoma City 73190*
RICHARD D. DEY
Department of Cell Biology, University of Texas Health Science Center, Dallas 75235
MARINUS FLUX
Department of Pediatrics, University of Oklahoma Health Sciences Center, and *Veterans Administration Medical Center*
SAMI I. SAID
Department of Medicine, University of Oklahoma Health Sciences Center, and *Veterans Administration Medical Center*

Cystic fibrosis (CF), the most common lethal genetic disease in Caucasians, manifests itself as an exocrinopathy characterized by elevated concentrations of Cl^- and Na^+ in sweat, and other electrolyte abnormalities of exocrine secretions (*1*). The pathogenesis of CF remains poorly understood, but recent investigations have shed new light on the mechanism of the electrolyte disturbance (*2*). In CF, there is an increase in the transepithelial potential difference in sweat gland ducts (*3*) and respiratory mucosa (*4*), which has been attributed to a decreased epithelial permeability to Cl^- (*5*). We report here that CF patients showed a marked deficiency of vasoactive intestinal peptide (VIP)–immunoreactive nerves in both acini and ducts of eccrine sweat glands (*6*). Since VIP promotes blood flow and the movement of water and Cl^- across epithelial surfaces, the deficient VIP innervation may be responsible for the decreased water content and relative impermeability of the epithelium to Cl^- and other ions that characterize CF.

The secretory activity of sweat and other exocrine glands is influenced by adrenergic and cholinergic innervation (*7*) and by the peptidergic system of nerves (*8*). Among the neuropeptides supplying exocrine glands, VIP richly innervates and promotes blood flow to the pancreas and to sweat, salivary, lacrimal, bronchial, and intestinal glands (*9*). This peptide binds to specific membrane receptors in these glands (*10*), stimulates Cl^- and water transport across intestinal (*11*) and tracheobronchial mucosa (*12*), and stimulates HCO_3^- secretion by pancreatic acini (*13*). VIP also reduces the potential difference across the duct of the rabbit submandibular gland (*14*). These observations suggested that VIP exerts a physiologic regulatory influence on exocrine function. Since the effects elicited by VIP are counter to the observed defects in CF, we postulated that the exocrine abnormalities of CF may be caused by a deficiency of VIP innervation. We therefore examined the presence, distribution, and density of VIP-immunoreactive nerves in the sweat glands of normal subjects and CF patients. We selected sweat glands because (i) they reflect one of the principal functional abnormalities of the disease; (ii) unlike other exocrine organs, such as the lungs, they remain free of infection or morphologic changes; and (iii) they are easily accessible through skin biopsy. The study was approved by our Institutional Review Board, and written informed consent was obtained in advance from the patients or their parents.

Skin samples were obtained by biopsy from the chest region in seven CF patients of both sexes, aged 13 to 28 years; control specimens of normal skin (as determined by light microscopy) were removed at mastectomy (from six women aged 36 to 78 years) or leg amputation (from two males aged 7 and 22 years). Skin samples were fixed in picric acid–formaldehyde (*15*) for 3 hours at 4°C, then transferred to 0.1*M* phosphate-buffered saline with 0.3 percent Triton X-100 (PBS-TX) (*p*H 7.8), and rinsed overnight at 4°C. Cryostat sections, 10 μm thick, were incubated for 30 minutes at 37°C with a standardized 40-μl drop of VIP antiserum (rabbit 89N, diluted 1:100 in PBS-TX), washed, and then incubated for 30 minutes at 37°C with goat anti-rabbit immunoglobulin G labeled with fluorescein isothiocyanate (Miles Labs, Elkhart, Indiana). The sections were washed in PBS-TX, mounted on slides in glycerine diluted with PBS-TX (1:4), and examined in a fluorescence microscope (Olympus BH2T) with a mercury vapor lamp.

The specificity of the VIP antiserum in immunocytochemical investigations has been demonstrated (*16*). In our study, specific immunofluorescence was confirmed by absorption of the antiserum with synthetic VIP (100 g per milliliter of diluted antiserum) 24 hours before use and by the substitution of normal rabbit serum for the immune antiserum; no fluorescence was present in either control section.

The extent of VIP innervation to acinar and ductal regions of sweat glands