Secretion of $\text{HCO}_3^-/\text{OH}^-$ in Cortical Distal Tubule of the Rat

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Abstract. Secretion of bicarbonate has been described for distal nephron epithelium and attributed to apical $\text{Cl}^-/\text{HCO}_3^-$ exchange in beta-intercalated cells. We investigated the presence of this mechanism in cortical distal tubules by perfusing these segments with acid (pH 6) 10 mM phosphate Ringer. The kinetics of luminal alkalinization was studied in stationary microperfusion experiments by double-barreled pH (ion-exchange resin)/$\text{KCl}$ reference microelectrodes. Luminal alkalinization may be due to influx (into the lumen) of $\text{HCO}_3^-$ or $\text{OH}^-$, or efflux of $\text{H}^+$. The magnitude of the $\text{Cl}^-/\text{HCO}_3^-$ exchange component was measured by perfusing the lumen with solutions with or without chloride, which was substituted by gluconate. This component was not different from zero in control and alkalotic (chronic plus acute) Wistar rats. Homozygous Brattleboro rats (BRB), genetically devoid of antidiuretic hormone, were used since this hormone has been shown to stimulate $\text{H}^+$ secretion, which could mask bicarbonate secretion. In these rats, no evidence for $\text{Cl}^-/\text{HCO}_3^-$ exchange was found in control BRB and in early distal segments of alkalotic animals, but in late distal tubule a significant component of $0.14 \pm 0.033$ nmol/cm$^2$ . sec was observed, which, however, is small when compared to the reabsorptive flux found in control Wistar rats, of $0.95 \pm 0.10$ nmol/cm$^2$ . sec. In addition, $5 \times 10^{-4}$ M SITS had no effect on distal bicarbonate reabsorption in controls as well as on secretion in alkalotic Wistar and Brattleboro rats, which is compatible with the absence of effect of this drug on the apical $\text{Cl}^-/\text{HCO}_3^-$ exchange in other tissues. It is concluded that most distal alkalinization is not $\text{Cl}^-$ dependent, and that $\text{Cl}^-/\text{HCO}_3^-$ exchange may be found in cortical distal tubule, but its magnitude is, even in alkalosis, markedly smaller than the reabsorptive flux, which predominates in the rats studied in this paper, keeping luminal pH lower than that of blood.

Key words: Bicarbonate secretion — $\text{Cl}^-/\text{HCO}_3^-$ exchange — SITS — Stationary microperfusion

Introduction

The observation that the luminal bicarbonate concentration in cortical distal tubule remains almost constant while inulin concentration increases at least threefold [5, 23] is evidence for the presence of significant net bicarbonate reabsorption at this site. Estimates of net bicarbonate reabsorption, based on “in vivo” micropuncture techniques, have yielded a reabsorption rate of 5 to 8% of filtered bicarbonate along this segment.

The only study in which modest net secretion of bicarbonate was observed in free-flow experiments was in animals undergoing hypotonic volume expansion, a situation which reduced plasma levels of ADH [1]. The situation is more complicated when bicarbonate transport is studied in perfused distal tubules. Net bicarbonate reabsorption or secretion in pump-perfused tubules depends critically on experimental conditions such as protein content of the diet, the acid-base status and luminal flow-rate [12, 34]. In tubules that under normal conditions secreted bicarbonate, fasting induced bicarbonate reabsorption [18]. On the other hand, both net reabsorption [34, 35] and net secretion of bicarbonate [17] have been reported in metabolic and in chloride depletion alkalosis.

A satisfactory explanation for the apparent discrepancy between the demonstration of significant net reabsorption of bicarbonate under free-flow conditions and net bicarbonate secretion in some tubule perfusion studies is presently not available. Capasso et al. [4] attributed this difference to the absence of some transport
stimulating substance in artificial perfusion studies, present either in the filtrate or added to the tubule fluid in vivo. Evidence for the presence of a transport stimulating substance has been obtained in perfusion studies of distal tubules with native proximal tubule fluid [22].

Replacing luminal chloride with gluconate inhibits bicarbonate secretion and enhances bicarbonate reabsorption, while high chloride perfusates elicit net bicarbonate secretion in usually reabsorbing distal tubules [19]. These results are evidence for Cl/\(\text{HCO}_3\) exchange modulating bicarbonate secretion in this segment. On the other hand, studies with stationary microperfusion and direct recording of lumen pH do not demonstrate bicarbonate secretion in control rats [10].

The purpose of the present investigation was to study the presence of net bicarbonate secretion in distal tubule of rats subjected to situations in which this secretion is stimulated: chronic metabolic alkalosis and reduced levels of ADH. The flux of bicarbonate was derived from the continuous record or lumen pH in experiments of stationary microperfusion.

Materials and Methods

Two populations of male rats were used in the experiments: Wistar and homozygous Brattleboro. Wistar rats were obtained from Instituto de Ciencias Biomédicas, and Brattleboro rats from Escola Paulista de Medicina. They were divided into two groups: control and chronic metabolic alkalosis. Control rats received tap water and a standard pellet diet containing 10% protein up to the time of the experiment. The urine was collected under oil. It was superfused with mammalian Ringer solutions at 37°C containing 0.05% FDC-green, which was used to detect early and late distal tubule loops. Further downstream in the proximal tubule a single micropipette containing the perfusion solution with the test substance was inserted. A double-barreled microelectrode was then inserted into an early or late distal loop. Perfusions were performed by applying pressure to the double-barreled micropipettes via hand-held oil-filled syringes. This method allowed for perfusions at a rate sufficient to achieve luminal pH near that of the original solution, minimizing the function of the segments between site of perfusion and measurement. Following the manual perfusion, a column of oil was injected into the late proximal or early distal tubule lumen, blocking the flow of fluid.

After control perfusions, the experimental solution without Cl⁻ was used, followed again by the control solution. pH was measured as the voltage difference between the two barrels of an asymmetric double-barreled microelectrode made out of Hilgenberg (Malsfeld, Germany) double-barreled glass capillaries. The larger barrel contained an H⁺-ion-sensitive ion-exchange resin (Fluka, Buchs, SW), and the smaller one, 1 mKCl colored by FDC green (reference barrel). With this technique, the slope of the electrode in the pH range 6.5 to 7.5 was 58.4 ± 1.85 mV per pH unit in phosphate-buffered solutions [21]. Trans epithelial electrical potential difference (Vt) was the difference between the reference barrel and ground. Voltages were read by a WPI (New Haven, CT) model FD 223 differential electrometer, the output of which was recorded on a Beckman model R511A Dynograph, and digitized in 1 sec intervals by means of an AD conversion board (Data Translation model DT 2801, Marlborough, MS) mounted on a 386 DX (DELL 333D) IBM-PC compatible microcomputer.

The tubules were perfused with phosphate or bicarbonate Ringer solutions at pH 6 or 8, respectively. After blocking fluid in the lumen, the pH of the perfusate approached the stationary value (pHs) due to fluxes of H⁺, OH⁻ or \(\text{HCO}_3\) across the tubule epithelium.

From the time course of the pH change the concentration of NaH₂PO₄ or NaHCO₃ was calculated by the Henderson-Hasselbalch equation using a \(pK_a\) of 6.8 or 6.1. These concentrations are obtained assuming either constant total phosphate content in the lumen, or a \(pCO_2\) equivalent to that in systemic blood [24a]. To calculate alkalization rates, the log of \((\text{NaH}_2\text{PO}_4)_{\text{s}} - (\text{NaH}_2\text{PO}_4)_{\text{o}}\) or \((\text{HCO}_3)_{\text{s}} - (\text{HCO}_3)_{\text{o}}\) was plotted against time in seconds. The subscript \(t\) refers to time, \(s\) to the stationary situation. In contrast to pH changes, such a plot generated a straight line, indicating that the buffer concentrations approach their steady-state value in an exponential manner. The halftime \((t/2)\) of the approach of acid phosphate or bicarbonate concentrations to their steady-state value was calculated from the slope of these lines.

The rationale for the detection of bicarbonate secretion was to measure the rate of alkalization of this fluid during stationary perfusion when Cl⁻ was the dominant anion, and after its substitution by gluconate. Bicarbonate secretion dependent on Cl⁻/\(\text{HCO}_3\) exchange is the difference between the alkalization rates obtained during these two perfusions. Bicarbonate flux (\(\text{JHCO}_3\)) was calculated by [6]:

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\text{J}_{\text{HCO}_3} = \lim_{t \to \infty} \frac{\text{d} [\text{NaH}_2\text{PO}_4]}{\text{d} t} = \frac{\text{d} [\text{NaH}_2\text{PO}_4]}{\text{d} t} = \left(\frac{\text{d} [\text{NaH}_2\text{PO}_4]}{\text{d} t}\right) - \left(\frac{\text{d} [\text{NaH}_2\text{PO}_4]}{\text{d} t}\right) = r \left(\frac{\text{d} [\text{NaH}_2\text{PO}_4]}{\text{d} t}\right)
\]

where \(t/2\) is the alkalization half-time, \(r\) is the tubule radius, NaH₂PO₄ and NaH₂PO₄ are initial and stationary acid phosphate concentrations. The decrease in these concentrations is equivalent to elevation in \(\text{OH}^-\) or \(\text{HCO}_3^-\) concentrations. Bicarbonate reabsorption is calculated in a similar manner, based on initial (\(\text{HCO}_3^-\)) and stationary (\(\text{HCO}_3^-\)) concentrations.

Solutions

The control luminal perfusion solution contained 80 mM NaCl, 5 mM KCl, 1 mM CaCl₂, 1.2 mM MgSO₄, raffinose to isoncoticity and was...