Kidney oxygen consumption, carbonic anhydrase, and proton secretion

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The kidney consumes oxygen, mainly to generate potential energy for tubular reabsorption (6, 20, 32, 41). The renal blood flow (RBF) is normally high relative to the kidney’s demand for oxygen, so the kidney extracts only a small fraction of the oxygen that is supplied to it. However, due to countercurrent exchange between the arterial and venous circulations, the partial pressure of oxygen in the renal tissue is low, ranging from 40–45 mmHg in the cortex to 25–30 mmHg in the medulla (7, 35, 48). Most of the glomerular filtrate is reabsorbed and the work of reabsorption is closely tied to sodium reabsorption by the kidney. This is not purely due to shifting the burden of reabsorption to a more expensive site downstream from the proximal tubule. Instead, increased cost may be incurred within the proximal tubule as the result of increased active chloride transport.

NaCl reabsorption; transport efficiency; proximal tubule; benzolamide; adenosine; sodium-hydrogen exchange

The kidney consumes oxygen, mainly to generate potential energy for tubular reabsorption (6, 20, 32, 41). The renal blood flow (RBF) is normally high relative to the kidney’s demand for oxygen, so the kidney extracts only a small fraction of the oxygen that is supplied to it. However, due to countercurrent exchange between the arterial and venous circulations, the partial pressure of oxygen in the renal tissue is low, ranging from 40–45 mmHg in the cortex to 25–30 mmHg in the medulla (7, 35, 48). Most of the glomerular filtrate is reabsorbed and the work of reabsorption is closely tied to sodium transport. Therefore, if a given amount of energy is required to absorb each sodium ion, then oxygen tension in the kidney must be determined, principally, by the filtration fraction. However, some studies have shown that the amount of oxygen consumed per amount of filtrate reabsorbed can vary significantly depending on a variety of conditions other than filtration fraction (11, 25, 28, 37). This observation raises the possibility that the kidney could suffer “relative ischemia” without an actual reduction in RBF if reabsorption were to become more expensive. For example, endogenous neurohumoral effectors such as nitric oxide and ANG II have been shown to influence respiration (25) and the efficiency of sodium reabsorption in the kidney. Welch and co-workers (48) reported higher ratio of renal oxygen consumption QO2 to sodium reabsorption (QO2/TNa) in the spontaneously hypertensive rat than Wistar-Kyoto rats and postulated that an imbalance between nitric oxide generation and ANG II may account for this. In addition, ANG II has been shown to contribute to oxidative stress which may parallel changes in oxidative efficiency (19, 21, 48). We recently examined this issue in normal rats. Blocking the angiotensin AT-1 receptor with losartan did not affect QO2/TNa, but infusing NOS-1 inhibitor, SMTC, increased QO2/TNa. This effect of SMTC occurred both at a dose sufficient to cause renal vasoconstriction and at a lower dose that had no hemodynamic effects (11). Exposure to SMTC also produced a significant increase in oxygen consumption in freshly harvested proximal tubules, indicating that the regulation of QO2 by NOS I (5) in the proximal tubule is an autocrine event. Changes in QO2/TNa might occur for multiple reasons. For example, shifting the burden of reabsorption from the proximal tubule to the loop of Henle increases the average cost of reabsorbing a sodium ion (20, 22). Theoretically, the efficiency of reabsorption might also be altered within a single nephron segment by changing the permeability of intercellular tight junctions, changing the number of ATP molecules formed per oxygen molecule consumed in mitochondria, or by switching to a different transport mechanism. Among all the nephron segments, the proximal tubule is most likely to manifest variable QO2/TNa because it employs more than one mechanism for active sodium transport, and because it engages in both active and passive transport. We presently examined whether the efficiency of proximal tubular reabsorption might be altered by manipulating the fraction of proximal reabsorption that depends on carbonic anhydrase (10, 16, 42). Prior studies in the literature have demonstrated an increase in the ratio of oxygen consumed per Na ion reabsorbed during carbonic anhydrase inhibition (30, 47). To accomplish this, we used inhibitors of carbonic anhydrase, sodium-hydrogen exchange, and sodium-bicarbonate cotransport in rats or freshly harvested rat proximal tubules. We discovered a substantial overall increase in QO2 and reduced transport efficiency during carbonic anhydrase inhibition that could result from the sum of a diminished driving force for passive reabsorption of NaCl (17, 30, 34) and an increase in active chloride transport (3, 33).

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Am J Physiol Renal Physiol 290: F1009–F1015, 2006. First published December 13, 2005; doi:10.1152/ajprenal.00343.2005.—Oxygen consumed by the kidney (QO2) is primarily obligated to sodium reabsorption (TNa). The relationship of QO2 to TNa (QO2/TNa) may be altered by hormones and autacoids. To examine whether QO2/TNa depends on the mechanism of sodium reabsorption, we first evaluated the effects on QO2 and QO2/TNa of benzolamide (BNZ), a proximal diuretic that works by inhibiting membrane carbonic anhydrase. During BNZ infusion in anesthetized rats, QO2 increased by 50% despite a 25% decline in TNa. However, BNZ failed to increase QO2/TNa when given along with the adenosine A1 receptor blocker, DPCPX, which inhibits basolateral Na-bicarbonate cotransport (NBC1), or EIPA, which inhibits sodium-hydrogen exchange (NH3). Incubating freshly harvested rat proximal tubules with BNZ also caused QO2 to increase by 62%, an effect that was prevented by blocking the apical NHE3 with S3226. Blocking NBC1 or NHE3 in the proximal tubule will have opposite effects on cell pH, but both maneuvers should reduce active chloride transport. In conclusion, inhibiting membrane carbonic anhydrase in the proximal tubule increases QO2 and reduces the energy efficiency of sodium reabsorption by the kidney. This is not purely due to shifting the burden of reabsorption to a more expensive site downstream from the proximal tubule. Instead, increased cost may be incurred within the proximal tubule as the result of increased active chloride transport.

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METHODS

Animal experiments described herein had protocol approval from the Institutional Animal Care and Use Committee and were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Animals in Research. Adult male Wistar rats (Harlan Sprague Dawley, Indianapolis, IN) weighing 300–350 g were maintained on standard rat chow and tap water until the day of the experiment.

Renal $Q_O2$ and Sodium Reabsorption in Anesthetized Rats

Overview. $Q_O2$ and $T_{Na}$ were measured before and during pharmacological manipulation of proximal reabsorption in four groups of inactin-anesthetized male Wistar rats (n = 5–6). Group I received the membrane carbonic anhydrase inhibitor benzolamide (BNZ; n = 6). Group II received BNZ plus the inhibitor of sodium hydrogen exchange, 5-(N-ethyl-N-isopropyl) amiloride (BNZ + EIPA; n = 6). Group III received BNZ plus the adenosine A1 receptor blocker 8-cyclopentyl-1,3-dipropylxanthine (BNZ + DPCPX; n = 6). Group IV received no drugs and served as a time control (n = 5). The purpose of BNZ was to trap filtered bicarbonate in the proximal tubular lumen, thereby preventing the active reabsorption of NaHCO3, but not active chloride transport. The purpose of EIPA was to prevent luminal acidification, which is necessary for active chloride transport (2, 3). The purpose of DPCPX was to block adenosine-mediated exit of NaHCO3 through the basolateral cotransporter NBC1 (24, 39). All drugs were given intravenously in Ringer saline except for BNZ, which was given in 300 mM NaHCO3. The respective drug doses were: BNZ, 5 mg/kg bolus then 5 mg·kg$^{-1}$·h$^{-1}$; EIPA, 1.2 mg/kg bolus followed by 2.6 mg·kg$^{-1}$·h$^{-1}$; DPCPX, 90 μg/kg bolus followed by 547 μg·kg$^{-1}$·h$^{-1}$.

Animal preparation and renal function measurements. Surgical preparation has been described previously (10, 11, 13). Briefly, the animals were anesthetized with inactin (100 mg/kg ip; Research Biochemical, Natick, MA) and placed on a thermostatically controlled surgical table to maintain body temperature at 37°C. Catheters were placed in the trachea (PE 240) to facilitate breathing, in left jugular vein (PE50) for recording mean arterial pressure and collecting blood. Urine was collected via a PE50 catheter inserted into the bladder. The left kidney was exposed through a midline abdominal incision and the renal artery was dissected apart from the renal vein. A perivascular transit time flow probe (Transonics T206, Ithaca, NY) was placed around the renal artery to monitor RBF. A 23-gauge needle was bent at 90° and inserted in the proximal left renal vein for sampling renal venous blood (10, 11, 13). Constant infusion was begun of Ringer saline at 1.5 ml/h containing 13 μCi per ml of $^3$H-inulin as a marker of glomerular filtration rate (GFR). Thereafter, 120 min was allowed for blood pressure and RBF to stabilize. Then, 200 μl of blood were slowly withdrawn from the renal vein and femoral artery to measure hematocrit, plasma $^3$H-inulin concentration, and oxygen content. A corresponding 20-min timed urine collection was also made. Subsequently, drug infusions were begun and all measurements were repeated once 10 min after the quick prompt increase in urine flow rate and again after 60 min. A timed urine collection lasting 10 min was made corresponding to the 10-min blood sampling and a timed urine collection lasting 20 min was made corresponding to the 60-min blood sampling. $^3$H-inulin was assayed with a beta scintillation counter. $P_O2$, $P_Na^+$, and $HCO_3^{-}$ content of blood or plasma were measured with a spectrophotometer and co-oximeter (682 CO-Oximeter Instrumentation Laboratory, Lexington, MA). Oxygen content of blood ($O_{ct}$, ml/dl) was computed from its partial pressure ($P_O2$, mmHg), total hemoglobin ($Hb$, g/dl), and hemoglobin saturation ($O_2 Hb%$) as

$$O_{ct}(ml/dl) = 1.39 \times Hb \times O_2 Hb\% + P_O2 \times 0.003$$

The units for oxygen content can be converted from milliliters to micromoles by the relationship, 1 ml $O_2 = 45$ μmol $O_2$.

Left kidney GFR was computed from RBF, hematocrit, and arteriovenous $^3$H-inulin ratio according to the Fick principle. Left kidney $Q_O2$ was obtained by multiplying the $A-V O2$ difference × RBF. Left kidney salt excretion ($U_{Na} \times V$) was estimated by halving the urinary salt excretion. Left kidney $T_{Na}$ was obtained by subtracting left kidney salt excretion from the product of GFR × plasma $[Na^+]$.

$Q_O2$. By Freshly Isolated Renal Proximal Tubules

Isolation of rat renal proximal tubules. Isolation and purification of rat renal proximal tubules were accomplished according to previously published methods (11, 14, 31, 44) with some modifications. All solutions were gassed with 95% $O_2$ and 5% CO2 before use. Male Wistar rats weighing 300 g were anesthetized with inactin (100 mg/kg ip). After the abdominal aorta was cannulated, the kidneys were first flushed with 40 ml cold solution A (in mM, pH 7.1) 112 NaCl, 20 NaHCO3, 5 KCl, 1.6 CaCl2, 2 Na2HPO4, 1.2 MgSO4, 5 glucose, 10 HEPES, 10 mannitol, 1 glutamine, 1 sodium butyrate, and 1 sodium lactate within 5 min and then perfused with 30 ml of warm buffer (37°C) containing 60 mg collagenase (type II, Worthington, cat. no. S3N6800) within 10 min. After in situ digestion, the kidneys were decapsulated and renal cortices were dissected, chopped, and placed into 15 ml solution A containing 30 mg collagenase at 37°C for 5 min. The preparation was pressed through a sieve (125-μm opening, U.S.A. Standard Sieve Series, Sieve No. 120, wire cloth, Newark, NJ) and 20 ml of cold solution A containing 3.5 ml fetal bovine serum were added to terminate collagenase activity. The tubules were washed with DMEM/F12 media and gassed with 95% O2-5% CO2 during preparation (Invitrogen, cat. no. 11330032) three times by centrifugation at 50 g for 2 min. The tubule preparation was suspended in DMEM/F12 medium containing 3.3% bovine serum albumin on ice for 20 min to recover from the digestion/sieving procedure. Proximal tubules were purified from other nephron segments and glomeruli by centrifugation on 45% Percoll at 15,000 g for 30 min at 4°C in Sorvall RC 5B plus centrifuge (SA600 Rotor). One hundred percent Percoll was prepared by mixing nine parts of the pure Percoll (Pharmacia Biotech, cat. no. 17–0891-01) with one part of 10× PBS. One hundred percent Percoll was then diluted with 1× PBS into 45% Percoll. The proximal tubules were recovered from the lowest band. As three washes with DMEM/F12 media to remove the Percoll, the proximal tubules were resuspended in 4 ml of cold chamber buffer [DMEM/F12 supplemented with 1× Insulin-Transferrin-Selenium (GIBCO, cat. no. S1500–056)], 2 mM butyrate, 1 mM glutamine, 1 mM malate, and 1 mM pyruvate for oxygen consumption studies.

Measuring $Q_O2$ in isolated tubules. $Q_O2$ (μmol·min$^{-1}$·mg protein$^{-1}$) was measured polarographically in a 0.6-ml chamber (Instech Laboratories) with a water jacket maintained at 37°C by using a Clarke-type oxygen electrode and an YSI model 5300 oxymeter (Yellow Spring Instrument, Yellow Spring, OH). The chamber was filled with buffer and a stir bar maintained a constant agitation of the solution and a flat line of slope (changes in $O_2$ over time) was recorded. Twenty five to one hundred microliters of the proximal tubule suspension were then added to the chamber for evaluation of slope change. Once a stable slope was confirmed (≥3 min was required for this), drug(s) were deposited at the bottom of the chamber through the port on the top using a pipette with an extra long tip (Myriada Industries, cat. no. 0062–0150) and recording continued for an additional 3 to 6 min. Drugs used in these experiments included BNZ (final concentration 67 μg/ml) or BNZ plus the NHE3 specific blocker, S3226 (36) (final concentration 1 mM). After completion of the recording, 0.2 ml of tubular suspension were taken from the chamber for measurement of protein content using a kit from BioRad.

$Q_O2$ was calculated as follows: oxygen saturated condition is considered at 100 U. Under this condition, the $O_2$ dissolved in the
solution is 544 nmol/ml. The chamber volume is 0.6 ml. Hence, 544 (nmol/ml) \times 0.6 (ml) + 100 (U) = 3264 (nmol/U) = 3264 (pmol/U). Therefore, QO₂ (pmol/min⁻¹·μg protein⁻¹) = 3264 (pmol/U) \times \text{slope} (\text{Us}) \times 60/\text{total protein} (μg) (11).

**Statistical Analysis**

Systat 6.0 for Windows (SPSS, 1996) was used to perform ANOVA with repeated-measures ANOVA as appropriate. Bonferroni correction was used for multiple comparisons as appropriate. Statistical significance was defined as *P* < 0.05. Results are presented as means ± SE.

**RESULTS**

*In Vivo Studies*

Baseline RBF, GFR, QO₂, QO₂/TNa, and UNaV and time controls. Baseline values for RBF, GFR, UNaV, QO₂, and QO₂/TNa were not different across the groups (Table 1). In group IV (time controls not included in Table 1), RBF, GFR, QO₂, and QO₂/TNa remained unchanged over a period exceeding 60 min (Fig. 1, A and B).

Effect of BNZ on RBF, GFR, QO₂, QO₂/TNa, and UNaV. BNZ alone (group I) caused immediate reductions in GFR (25%) and RBF (16%) commensurate with the known action of this drug to reduce proximal reabsorption, thereby activating tubuloglomerular feedback (TGF) (42) (Fig. 2A). BNZ also increased UNaV by 30-fold \(P < 0.01\); Table 1). Over the ensuing 60 min, RBF drifted back nearly to its original value, whereas GFR increased minimally from its 10-min nadir, and UNaV increased a bit further. During the 60-min infusion, BNZ stably reduced TNa by 30% \(P < 0.01\). Because the initial fractional reabsorption of sodium was quite high (0.9987), most of the impact of BNZ on TNa resulted from the 25% decrease in the filtered load of sodium, rather than the 30-fold increase in UNaV. Despite eliciting a major reduction in TNa, BNZ caused a 40% increase in QO₂, an effect that showed no sign of abating during the next hour \(P < 0.01\). These combined effects of BNZ on QO₂ and TNa netted a doubling in the amount of oxygen consumed per sodium ion reabsorbed \(P < 0.01\); Fig. 2B).

Effect of EIPA + BNZ on RBF, GFR, QO₂, QO₂/TNa, and UNaV. In the proximal tubule, carbonic anhydrase is necessary for bicarbonate reabsorption, but not for active chloride reabsorption (27, 30, 32), whereas apical NHE is required for both bicarbonate and active chloride reabsorption (3, 23, 45). Coadministration of the carbonic anhydrase inhibitor, BNZ, and the NHE blocker, EIPA, caused immediate declines in GFR (37%) and RBF (16%) that persisted throughout the 60-min experiment (Table 1). Presumably, these effects were due to activation of TGF. BNZ + EIPA also increased UNaV by 54-fold within 10 min and the natriuresis appeared to increase further by 60 min (Table 1). It is not possible to determine how much of the natriuresis contributed by EIPA occurred in the proximal tubule vs. collecting duct. However, because the impact on GFR of BNZ + EIPA was 50% greater than the effect of BNZ alone, it is likely that EIPA did further reduce proximal reabsorption, thereby augmenting the stimulus for TGF (42). There was no apparent effect of BNZ + EIPA on QO₂ after 10 min. However, within 60 min of infusion, QO₂ quantitatively declined by 34% and QO₂/TNa increased transiently, then returned to the control level (Fig. 3). In other words, adding EIPA augmented the renal hemodynamic and natriuretic effects of BNZ, but prevented BNZ from increasing QO₂ or QO₂/TNa.

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**Table 1. Changes in RBF, GFR, QO₂, QO₂/TNa, and UNaV before and after BNZ in three groups**

<table>
<thead>
<tr>
<th>Group</th>
<th>Period</th>
<th>RBF, ml/min</th>
<th>GFR, ml/min</th>
<th>QO₂, μmol/min</th>
<th>TNa, μmol/min</th>
<th>UNaV, μmol/min</th>
<th>QO₂/TNa, μmol/mmol</th>
<th>TNa/QO₂, μmol/μmol</th>
</tr>
</thead>
<tbody>
<tr>
<td>BNZ</td>
<td>Baseline 7.7±1.4</td>
<td>1.21±0.08</td>
<td>7.17±0.92</td>
<td>166±12</td>
<td>0.21±0.05</td>
<td>43.28±4.50</td>
<td>24.67±3.12</td>
<td></td>
</tr>
<tr>
<td>10 min</td>
<td>6.6±0.4</td>
<td>0.91±0.07</td>
<td>10.27±1.67</td>
<td>125±10</td>
<td>5.74±0.06</td>
<td>84.62±15.47</td>
<td>13.20±1.57</td>
<td></td>
</tr>
<tr>
<td>60 min</td>
<td>7.5±0.4</td>
<td>0.97±0.07</td>
<td>11.33±1.39</td>
<td>134±11</td>
<td>6.35±0.50</td>
<td>83.42±7.42</td>
<td>12.39±1.25</td>
<td></td>
</tr>
<tr>
<td>BNZ + EIPA</td>
<td>Baseline 7.0±0.3</td>
<td>1.19±0.04</td>
<td>8.94±1.38</td>
<td>163±5</td>
<td>0.16±0.04</td>
<td>54.15±7.14</td>
<td>20.04±3.08</td>
<td></td>
</tr>
<tr>
<td>10 min</td>
<td>5.9±0.2</td>
<td>0.75±0.02</td>
<td>8.95±1.19</td>
<td>95±3</td>
<td>8.65±1.03</td>
<td>94.53±11.67</td>
<td>11.21±1.31</td>
<td></td>
</tr>
<tr>
<td>60 min</td>
<td>5.8±0.2</td>
<td>0.75±0.06</td>
<td>5.86±0.46</td>
<td>94±7</td>
<td>9.87±0.49</td>
<td>63.51±5.63</td>
<td>16.23±1.40</td>
<td></td>
</tr>
<tr>
<td>BNZ + DPCPX</td>
<td>Baseline 6.8±0.03</td>
<td>1.04±0.08</td>
<td>7.89±0.62</td>
<td>147±9</td>
<td>0.39±0.08</td>
<td>59.14±6.27</td>
<td>16.72±1.74</td>
<td></td>
</tr>
<tr>
<td>10 min</td>
<td>6.2±0.2</td>
<td>0.87±0.06</td>
<td>8.53±0.56</td>
<td>136±10</td>
<td>8.87±0.75</td>
<td>72.78±1.64</td>
<td>13.17±0.65</td>
<td></td>
</tr>
<tr>
<td>60 min</td>
<td>5.7±0.2</td>
<td>0.79±0.04</td>
<td>6.59±0.40</td>
<td>117±6</td>
<td>8.74±0.57</td>
<td>67.20±1.39</td>
<td>15.30±0.60</td>
<td></td>
</tr>
</tbody>
</table>

Data are means ± SE. Drug infusion started at time 0 min. RBF, left kidney blood flow rate; GFR, left glomerular filtration rate; QO₂, left kidney oxygen consumption; TNa, left kidney sodium transport; BNZ, benzamilide; UNaV, left kidney urinary sodium excretion. *P < 0.01 vs. control, paired t-test. \(t^*P < 0.05\) vs. BNZ alone, t-test. \(t^*P < 0.01\) vs. BNZ alone, t-test. \(t^*P < 0.05\) vs. control, paired t-test. \(t^*P < 0.05\) vs. 10 min, paired t-test.
Effect of BNZ

In group III, adding the adenosine A1 blocker, DPCPX, to BNZ augmented the natriuresis commensurate with the known effects of A1 adenosine blockers as proximal diuretics (4, 24, 26, 49). DPCPX scaled up BNZ had no significant effect on GFR, although the quantitative values were similar to BNZ alone (Table 1), possibly due to interference by DPCPX with the TGF effector response (8, 38, 40, 43). Adding DPCPX abolished the effect of BNZ on QO2 and greatly reduced the impact of BNZ on QO2/\( \text{TNa} \) (\( P < 0.02 \); Fig. 3).

In Vitro Studies

Effects of BNZ and S3226 on freshly isolated renal proximal tubule QO2. The effects of BNZ and the NHE3 blocker, S3226, on QO2 were evaluated in freshly isolated rat renal proximal tubules. Under control conditions, chamber oxygen tension declined at a constant rate, indicating stable QO2. As shown in Fig. 4, BNZ increased QO2 by 63% (\( P < 0.01 \)). Adding S3226 immediately thereafter abolished the effect of BNZ (\( P < 0.001 \) for BNZ + S3226 vs. BNZ, \( n = 10 \) runs from 3 rats). In other words, the increase in QO2 that occurred with carbonic anhydrase inhibition in vivo and the reversal of that effect by NHE blockade also occurred in isolated proximal tubules. S3226 given alone reduced tubular QO2 by 56% and did so in a stable fashion over time (\( P < 0.01 \), \( n = 7 \) runs from 3 rats). The sequence in order of addition of agents did affect the overall results. When S3226 was added first in sequence, the addition of BNZ no longer increased QO2 and values decreased further as a result of inhibition of carbonic anhydrase well below control values (Fig. 5). The latter results suggest that inhibition of proton secretion does prevent the BNZ-induced increase in oxygen consumption. Application of BNZ under conditions of NHE3 blockade actually causes oxygen consumption and presumably transport to decrease further. These findings are not just the consequence of independent positive and negative effects.

Fig. 2. Inhibition of carbonic anhydrase by benzolamide (BNZ) at 5 mg/kg caused a significant decrease in GFR and an increase in QO2 (A), resulting in a marked increase in oxygen consumption per Na+ reabsorbed (QO2/TNa) (B). *\( P < 0.01 \) vs. control, paired \( t \)-test.

Fig. 3. Inhibition of proximal tubular reabsorption by BNZ increased cost of Na+ reabsorption (QO2/TNa). *\( P < 0.01 \). Concurrent application of DPCPX, an adenosine 1 receptor antagonist, and EIPA, an NHE blocker, with BNZ prevented the BNZ-induced increase in QO2/TNa, suggesting that BNZ-induced increase in oxygen consumption requires proton secretion and luminal acidification.

Fig. 4. Effects of BNZ on oxygen consumption (QO2) in freshly isolated proximal tubules and further influence of concurrent application of S3226, a blocker of NHE3 and proton secretion. BNZ increased QO2 significantly in vitro in a fashion quantitatively similar to in vivo observations. Application of S3226 and consequent blockade of proton secretion prevented the increase in QO2. *\( P < 0.01 \) vs. control. **\( P < 0.01 \) vs. BNZ.

Fig. 5. Effects of prior administration of S3226, the NHE3 blocker, on oxygen consumption and the addition of BNZ in freshly harvested proximal tubules. S3226 causes a major reduction in QO2 as shown in middle. The addition of BNZ no longer causes an increase in oxygen consumption. QO2 continues to decrease with addition of BNZ suggesting an additive negative effect of carbonic anhydrase inhibition. These results demonstrate that proton secretion is an absolute requirement for the increase in oxygen consumption during BNZ in freshly harvested tubules. *\( P < 0.01 \) vs. control. **\( P < 0.01 \) vs. S3226.
DISCUSSION

The kidney consumes oxygen mainly to generate chemical potential (ATP), which is needed for sodium reabsorption (6, 22, 41). Approximately two-thirds of this sodium reabsorption normally occurs in the proximal tubule (1, 17, 34). Presently, we have discovered that blocking carbonic anhydrase in the proximal tubule not only reduces the oxygen efficiency of sodium reabsorption, but also increases overall kidney oxygen consumption, despite also effecting a major decrease in net sodium reabsorption. Then, we discovered that these effects of carbonic anhydrase inhibition are preventable by superimposing drugs that suppress sodium-hydrogen exchange or adenosine-mediated proximal reabsorption.

Unobvious explanations are required to account for the finding that a diuretic can increase $Q_O_2$ and for the finding that a major effect of one proximal diuretic can be prevented by two other proximal diuretics. To explain these findings, and to argue their relevance, we insert a synopsis of proximal tubular reabsorption. Most sodium reabsorbed by the nephron passes through tubular epithelial cells and the majority of this reabsorbed sodium exits the basolateral cell membrane via the Na-K-ATPase, which expels three sodium ions from the cell per each ATP molecule that it consumes (22). Hence, in most nephron segments, the ratio of ATP consumed to sodium reabsorbed (and likewise $Q_O_2/T_{Na}$) should be constant and it has been estimated that 18 sodium ions pass through the Na-K-ATPase for each oxygen molecule that is consumed to make ATP (37).

However, in the proximal tubule, some sodium exits the cell by basolateral Na$_3$HCO$_3$ cotransport (NBC1) (39) and even more is reabsorbed passively along a paracellular route (1, 17, 34). Estimates vary as to how much passive reabsorption occurs in the proximal tubule (17, 34, 46), but a typical estimate is that passive reabsorption increases, from 18 to 48, the number of sodium ions reabsorbed per oxygen molecule consumed in the proximal tubule (30). The potential energy for this passive paracellular NaCl reabsorption comes from carbonic anhydrase-dependent active reabsorption of NaHCO$_3$ early in the S1 segment followed by osmotic water reabsorption, which enriches the remaining tubular fluid in chloride (1, 9, 17, 32, 34). This separating of bicarbonate from chloride drives entropy from the system in exchange for free energy that subsequently powers paracellular NaCl reabsorption. This free energy is augmented by the higher Staverman reflection coefficient for bicarbonate (1.0) relative to chloride (0.8) (3, 33, 34).

The proximal tubule also performs active NaCl transport, but this does not contribute extra potential for passive reabsorption. Active NaCl reabsorption in the proximal tubule begins when chloride crosses the apical membrane in exchange for a small organic anion (usually formate), while sodium enters by NHE. At the same time, NHE secretes a proton that is necessary to recycle the secreted formate ion (2, 23). Basolateral chloride exit is via a chloride conductance or chloride-bicarbonate exchanger (33). Sodium exits the basolateral membrane via Na-K-ATPase or NBC1, the latter of which uses electrochemical potential from the bicarbonate gradient. Unlike active NaHCO$_3$ reabsorption, this active mode of NaCl transport does not contribute potential for paracellular reabsorption of other solutes because the reflection coefficient for chloride is relatively low and because most of the osmotically important solutes (bicarbonate and glucose) are reabsorbed upstream from the site where active NaCl transport predominates (34, 46).

Hence, the oxygen cost of active NaCl reabsorption by the proximal tubule should be roughly the same as in other nephron segments where all sodium ions that are reabsorbed must pass through a Na-K-ATPase. Based on this understanding of proximal tubule physiology, $Q_O_2/T_{Na}$ in the proximal tubule is expected to increase along with the amount of active NaCl reabsorption relative to NaHCO$_3$ reabsorption. Lately, there has been renewed interest in $Q_O_2/T_{Na}$ mainly due to the contemporary preoccupation with oxidative stress and the formation of superoxide as a byproduct of electron transport in mitochondria (18, 19, 21, 48). In this context, a nefarious role has been ascribed to ANG II, for provoking superoxide formation and increasing $Q_O_2/T_{Na}$ (19, 48). ANG II also stimulates proximal reabsorption by increasing apical sodium-hydrogen exchange (29).

Because NHE3 activity is prerequisite to both NaHCO$_3$ and active NaCl transport in the proximal tubule (15, 45), it is not trivial to predict how $Q_O_2/T_{Na}$ will change in the proximal tubule, much less in the whole kidney, when NHE3 activity increases in the proximal tubule. Here, we showed that allowing NHE3 to operate freely while using a carbonic anhydrase inhibitor to suppress NaHCO$_3$ reabsorption not only reduces the efficiency of salt reabsorption, but actually increases overall $Q_O_2$ by the kidney and by isolated proximal tubules (12). Starting from the supposition that most renal oxygen consumption provides for sodium reabsorption, it is not immediately apparent that $Q_O_2$ and $T_{Na}$ could ever change in opposite directions. Yet inhibiting carbonic anhydrase caused this to happen. A workable explanation for this seeming paradox follows from the general principle of glomerulotubular balance according to which selectively reducing reabsorption by the S1 segment will lead to increased reabsorption along the remainder of the nephron. First, let us allow that the S1 segment reabsorbs 40% of the filtered sodium and is threefold more efficient at sodium reabsorption than the rest of the nephron, which reabsors 59.9% of the filtered sodium. Now if one eliminates bicarbonate reabsorption in the S1 segment and within the proximal tubule, one would predict that passive NaCl reabsorption would be largely eliminated, in major part due to loss of the created chloride gradient. However, this effect alone would only reduce the efficiency of NaCl reabsorption and not increase oxygen consumption unless this effect was somehow linked to induction of active NaCl reabsorption within the latter segments of the proximal tubule. Second, allow that 90% of the new burden on the downstream nephron will be compensated by glomerulotubular balance. This will result in a major increase in sodium excretion and an increase in $Q_O_2$. Downstream segments exhibit lesser metabolic efficiency of NaCl reabsorption. Candidate mechanisms to incur this oxygen cost include all the reabsorptive machinery that operates in the loop of Henle, distal tubule, and collecting duct where all reabsorbed sodium ions pass through the Na-K-ATPase.

We recognize and credit the much earlier observations of Weinstein and co-workers (47) who demonstrated a dissociation of kidney NaCl reabsorption and oxygen consumption after administration of BNZ, the only other such observation.
In these studies, sodium reabsorption decreased significantly after BNZ, due to both inhibition of proximal reabsorption and a reduction in GFR, while \( Q_{O_2} \) remained constant. These investigators attributed this change in the relationship of oxygen consumption to \( \text{NaCl} \) reabsorption to removal of the \( \text{HCO}_3^-/\text{chloride} \) gradients after BNZ with resulting loss of passive \( \text{NaCl} \) reabsorption in the proximal tubule, due to both loss of osmotic gradients and chloride gradients generating passive \( \text{NaCl} \) reabsorption. This interpretation is likely correct and explains the constancy of oxygen consumption while \( \text{NaCl} \) reabsorption decreased. However, in the present study, oxygen consumption actually increased while \( \text{NaCl} \) reabsorption decreased, a quite different result, possibly as a consequence of higher doses of administered BNZ. Therefore, we postulate that not only is there loss of passive \( \text{NaCl} \) reabsorption, but also recruitment of active \( \text{NaCl} \) transport as a consequence of some elements associated with inhibition of carbonate anhydrase.

The present data in freshly harvested proximal tubules reveal that blocking carbonic anhydrase in the proximal tubule will increase oxygen consumption in the proximal tubule itself. So, while carbonic anhydrase inhibition should invoke compensatory reabsorption in Henle’s loop and the distal nephron, this is not necessary or sufficient to explain the entire increase in \( Q_{O_2} \) that occurs during BNZ. It is likely that BNZ causes \( Q_{O_2} \) to increase in the proximal tubule because, by blocking luminal carbonic anhydrase, it removes the most important mechanism for physicochemical buffering of protons secreted into the lumen by NHE3 (9, 15), thereby augmenting the pH gradient across the apical membrane, which causes the chloride-formate exchanger to cycle more frequently (3, 45). In other words, BNZ may increase \( Q_{O_2} \) in the proximal tubule by increasing active \( \text{NaCl} \) transport (2, 23), which is more expensive than the combination of \( \text{NaHCO}_3^- \) and passive \( \text{NaCl} \) transport that it partially replaces. If this explanation is correct, then blocking apical NHE should abrogate the increase in \( Q_{O_2} \), during carbonic anhydrase inhibition by eliminating the pH gradient and reducing the free energy available to drive chloride-formate exchange (45). Indeed, NHE blockers did prevent BNZ from increasing \( Q_{O_2} \) both in vivo and in isolated proximal tubules. In fact, when the NHE blocker was given first in sequence to block proton secretion, oxygen consumption not only no longer increased after BNZ, but remained significantly below control values (Fig. 5).

We did not actually measure active chloride transport to confirm that this accounts for the increase in \( Q_{O_2} \) during BNZ, and one could argue that NHE blockade is too nonspecific a method for interfering with active chloride transport to accept reversal of the BNZ effect by NHE blockade as proof that increased active chloride reabsorption explains the increase in \( Q_{O_2} \). Perhaps, NHE blockade reduces \( Q_{O_2} \) by some unknown mechanism tied to cell pH? The comparable ability of the adenosine A1 receptor blocker, DPCPX, or NHE blockers to prevent an increase in \( Q_{O_2} \) argues against this possibility, since A1 blockers and NHE blockers are proximal diuretics that should both interfere with active chloride transport while having opposite effects on cell pH (39, 45). Hence, the available information, while circumstantial, points toward active chloride transport as a major determinant of \( Q_{O_2} \) in the proximal tubule. More direct testing of this hypothesis awaits the application of better tools to manipulate active chloride transport.

In summary, these data are the first to demonstrate that oxygen consumption can increase while \( \text{NaCl} \) reabsorption is inhibited by interfering with carbonic anhydrase. This likely results from both a loss of the driving force for passive salt reabsorption and may involve a compensatory increase in active chloride transport, which is less efficient and more costly of oxygen.\(^1\)

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DISCLOSURES

The authors declare no conflicts of interest.

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\(^1\) Many textbooks contain figures in which paracellular sodium reabsorption is invoked in the thick ascending limb of Henle’s loop (TALH) to compensate for the 2:1 ratio of chloride to sodium uptake by the sodium-potassium-2 chloride cotransporter (NKCC). However, it has been pointed out that passive sodium reabsorption in the TALH is forbidden due to an unfavorable Nernst potential and that the overall electroneutrality of TALH transport is maintained by chloride backleak, not by passive sodium reabsorption (20).


