Different Mechanisms of Action of Acetazolamide and Parathyroid Hormone on Proximal Tubular Absorption of Fluid and 5,5-Dimethyl-2,4-Oxazolidinedione1, 2

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ABSTRACT

The effects of acetazolamide (Diamox), parathyroid hormone (PTH) and dibutyryl cyclic adenosine 3':5'-monophosphate (db-cAMP) on fluid and 5,5-dimethyl-2,4-oxazolidinedione (DMO) absorption in the rat proximal convoluted tubule were studied by using microperfusion methods. The rate of tubular absorption of DMO was used to estimate the rate of hydrogen ion secretion. When the tubular and the peritubular capillaries were perfused simultaneously with bicarbonate-free Ringer's solution containing DMO, the rate of DMO absorption (JDMO) was 140 ± 15.7 pmol/min-mm, a value comparable to the rate of absorption of bicarbonate and glycocidzine, and net fluid absorption (JL) was 2.20 ± 0.19 nl/min-mm. Administration of PTH (10^-6 M) to the capillary perfusate caused a decrease of JDMO by 23% and a decrease of J, by 28%. Similar results were observed when db-cAMP (10^-4 M) was administered to the luminal perfusate. Addition of acetazolamide (10^-4 M) to the luminal perfusate caused a decrease of JDMO by 66% and a decrease of J, by 45%. The effect of either PTH or db-cAMP was additive to the maximal effect of acetazolamide. However, the effect of PTH was not additive to the effect of db-cAMP. Thus, the results suggest that PTH and acetazolamide have different mechanisms of action on fluid and DMO absorption by proximal tubule and that cAMP mediates the effect of PTH.

Administration of parathyroid hormone (PTH) generally causes inhibition of fluid, bicarbonate and phosphate reabsorption in the kidney (Agus et al., 1971; Arruda et al., 1977; Kleeman and Cooke, 1951; Nordin, 1960). Acetazolamide administration also depresses renal fluid, bicarbonate and phosphate reabsorption by decreasing hydrogen ion secretion through the mechanisms of inhibiting carbonic anhydrase (Beck and Goldberg, 1973; Maren, 1974; Nascimento et al., 1977). The similar effects of PTH and acetazolamide led to the postulate that bicarbonate and phosphate reabsorption were depressed by both agents as a consequence of carbonic anhydrase inhibition (Beck et al., 1974; Peraino, 1978). However, several authors have suggested that PTH depresses bicarbonate and phosphate reabsorption by a mechanism other than carbonic anhydrase inhibition (Beck et al., 1973; Nascimento et al., 1977; Puschett and Goldberg, 1969). The differing suggestions may result from the limitations of clearance experiments which do not clearly demonstrate the tubular segment responsible for fluid and electrolyte reabsorption and for drug action. Moreover, PTH has been shown to exert different effects on electrolyte reabsorption along the various nephron segments (Harris et al., 1979; Iino and Burg, 1979; Knox et al., 1976).

The present study was designed to re-evaluate the mechanism of action of PTH and acetazolamide on fluid and hydrogen ion secretion at the proximal convoluted tubule by using microperfusion methods. The rate of tubular absorption of 5,5-dimethyl-2,4-oxazolidinedione (DMO), a compound that has been extensively used in the measurement of intracellular pH (Butler et al., 1967; Waddel and Bates, 1969), was used to estimate the rate of hydrogen ion secretion based on the assumption that secreted hydrogen ion combines with the dissociated acid resulting in an undissociated acid which can then diffuse across the luminal membrane. A similar approach has been reported previously in a study of glycodiazine transport (Ullrich et al., 1975). The present study also compares the rates of tubular absorption of DMO to those of bicarbonate and glycocidzine.

Methods
Animal preparation. Male Spague-Dawley rats weighing 200 to 250 g were maintained on a regular rat pellet diet and tap water. The rats were anesthetized with 5-ethyl-5-(1-methylpropyl)-2-thiobarbituric acid (Inacin), 100 mg/kg b.wt. i.p. They were placed on a thermostatically controlled animal table where their body temperatures were maintained at 37°C. The surgical procedures for microperfusion studies have been described previously (Chan, 1976; Baumann et al., 1977).

Microperfusion of proximal tubule and peritubular capillaries. The technique of simultaneous microperfusion of proximal tubule and peritubular capillaries has been described in detail elsewhere.
above procedures were repeated at two different pCO2 tensions, namely 5 and 11% CO2. The mean value of bicarbonate concentration obtained from the determination was used. Routine differences of these bicarbonate solutions were measured. When anions.

The concentration of buffer in the original perfusion solution

Sb = concentration of buffer in the original perfusion solution

St = concentration of buffer in the collected fluid

These quantities were expressed per millimeter of tubular length.

The data were presented as mean ± S.E. Only absolute values were used in the statistical analysis. Statistical significance of differences between means were evaluated by Students’ t test.

Material. [3H]Inulin, [3H]inulin and [3H]D MO were purchased from New England Nuclear (Boston, MA). Acetazolamide (Diamox) was obtained from Lederle Laboratories Division, American Cyanamid Co. (Pearl River, NY). PTHs were obtained from Wilson Laboratories (Chicago, IL). Dibutyryl-cyclic adenosine 3’:5’-monophosphate (db-cAMP) and D MO were purchased from Sigma Chemical Co., (St. Louis, MO).

Results

Proximal tubular absorption of buffers and fluid. A total of 51 proximal tubules were perfused to study the rate of absorption of D MO, bicarbonate and glycodiazine. For each buffer studied, four to five rats were used. The results are presented in figure 1. When the tubules were perfused with D MO, the rate of absorption of D MO (J D MO) was 140 ± 15.7 pmol/min-mm and the rate of net fluid absorption (J) was 2.20 ± 0.19 nl/min-mm. J D MO was slightly less than the rate of bicarbonate absorption (not significantly different, P > .05) and was 82% of the rate of glycodiazin absorption (P < .05). J was significantly less (P < .05) when D MO was in the perfusate than when other of the other two buffers was in the perfusate. In order to examine whether the rate of D MO reabsorption was saturable, concentrations in the perfusate were varied from 10 to 50 mM. The results are presented in figure 2. Increase in J D MO was linearly related to the increase of D MO concentration. There was no saturation of D MO absorption observed within the range of D MO concentration studied.

Effects of acetazolamide and PTH on D MO absorption. Experiments were performed in 77 proximal tubules in 17 rats. Results are presented in table 1 and figure 3. Addition of 10⁻⁴ M acetazolamide to both the luminal and the capillary perfusate reduced J D MO by 65.7% (P < .001) and J, by 45% (P < .001). Increase of the acetazolamide concentration to 10⁻³ M did not reduce J D MO and J, further. Addition of 10⁻⁶ M PTH to the capillary perfusate reduced J D MO by 23.6% (P < .01) and J, by 28.2% (P < .01). Increase of PTH concentration to 10⁻⁴ M did not reduce J D MO and J, further. When 10⁻⁶ M PTH was added together with 10⁻⁴ M acetazolamide, J D MO was decreased by 85% and J, was reduced by 60.9%. The effect of PTH was additive to that of acetazolamide.

The role of db-cAMP. The effects of db-cAMP on J D MO and J, were examined in 28 proximal tubules of six rats. Results are
DM0 Concentration (mM)

![Graph](image)

**Fig. 2.** The relationship between the rate of DM0 absorption in the rat proximal tubule and DM0 concentration in the perfusate. $J_{DMO}$ = the rate of DM0 absorption. Each point and bar represents mean ± S.E. of 11 to 14 tubules in three to four rats. Linear regression line is calculated by the least-squares method. $Y = 4.67x + 7.69$ ($r^2 = 0.96$).

**Discussion**

DM0 is a weak organic acid with pKa of 6.1. Since it is not metabolized and is nonvolatile, it offers some advantages over the carbonic acid bicarbonate system as an indicator of intracellular pH, based on the theory that cellular membranes are only permeable to undissociated forms (Waddell and Bates, 1969). The results of the present study clearly demonstrate that DM0 is absorbed by the proximal tubule at a rate approximately equal to that of bicarbonate. Evidence has been accumulated to support the view that hydrogen ion secretion is the primary process responsible for bicarbonate absorption (Burg and Green, 1977; Lucci et al., 1979; Malnic and Giebisch, 1972).

Thus, the rate of DM0 or bicarbonate absorption is an estimate of the rate of hydrogen ion secretion based on the assumption that secreted hydrogen ion combines with a dissociated buffer becoming an undissociated buffer which can then diffuse across the luminal membrane. A similar approach has been reported previously in which the secretory rate of hydrogen ion in the proximal tubule was evaluated by measuring the rate of glycodiazin absorption (Ullrich et al., 1975). This study shows that the rate of DM0 absorption is comparable to that of glycodiazine and bicarbonate absorption and supports the view that hydrogen ion secretion in the proximal tubule can proceed in the presence of appropriate buffer other than bicarbonate. However, it should be noted that fluid absorption is reduced somewhat when bicarbonate is replaced by DM0 (fig. 1), indicating that sodium absorption is somehow dependent upon bicarbonate absorption.

In order to investigate the possibility that a carrier-mediated transport process could be involved in the DM0 absorption, a kinetic study on DM0 absorption was performed by varying DM0 concentrations in the perfusate from 10 to 50 mM. Our results show that no saturation can be observed (fig. 2). On the other hand, saturation phenomena have been observed within the same range of concentrations with most of the other organic substances which are considered to be subject to carrier-mediated transport (Chan and Huang, 1971; Mudge et al., 1973; Weiner, 1973). The present results are consistent with the view that DM0 transport across cellular membrane is not a carrier-mediated process (Butler et al., 1967).

It appears, therefore, that DM0 is suitable for the study of hydrogen ion secretion in the proximal tubule.

Our data show that acetazolamide maximally inhibits DM0 absorption at a concentration ($10^{-4}$ M) which can completely inhibit carbonic anhydrase in vitro (Maren, 1967, 1974, 1977). The same maximum inhibitory concentration of acetazolamide has been reported recently in the study of bicarbonate absorption in the proximal tubule (Cogan et al., 1979; Lucci et al.,...
TABLE 1

Effects of PTH, Diamox and db-cAMP on proximal tubular transport of fluid and DMO

<table>
<thead>
<tr>
<th>Agents</th>
<th>No. of Tubules</th>
<th>J_0</th>
<th>Δ %</th>
<th>J_DMO</th>
<th>Δ %</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>17</td>
<td>2.20 ± 0.19</td>
<td>0</td>
<td>140 ± 15.7</td>
<td>0</td>
</tr>
<tr>
<td>PTH, 10^{-6} M</td>
<td>16</td>
<td>1.58 ± 0.17**</td>
<td>-28.2</td>
<td>107 ± 9.6**</td>
<td>-23.6</td>
</tr>
<tr>
<td>PTH, 10^{-5} M</td>
<td>12</td>
<td>1.59 ± 0.23**</td>
<td>-27.7</td>
<td>109 ± 8.5**</td>
<td>-22.2</td>
</tr>
<tr>
<td>db-cAMP, 10^{-4} M</td>
<td>15</td>
<td>1.63 ± 0.21**</td>
<td>-25.9</td>
<td>113 ± 8.2**</td>
<td>-19.3</td>
</tr>
<tr>
<td>db-cAMP, 10^{-3} M</td>
<td>13</td>
<td>1.56 ± 0.16**</td>
<td>-29.1</td>
<td>108 ± 7.9**</td>
<td>-22.9</td>
</tr>
<tr>
<td>Diamox, 10^{-4} M</td>
<td>18</td>
<td>1.21 ± 0.13*</td>
<td>-45.0</td>
<td>48 ± 9.4*</td>
<td>-65.7</td>
</tr>
<tr>
<td>Diamox, 10^{-3} M</td>
<td>12</td>
<td>1.18 ± 0.16*</td>
<td>-46.4</td>
<td>45 ± 8.6*</td>
<td>-67.8</td>
</tr>
<tr>
<td>PTH, 10^{-6} M + db-cAMP, 10^{-4} M</td>
<td>17</td>
<td>1.64 ± 0.20**</td>
<td>-25.5</td>
<td>109 ± 10.5**</td>
<td>-22.1</td>
</tr>
<tr>
<td>Diamox, 10^{-4} M + db-cAMP, 10^{-4} M</td>
<td>18</td>
<td>0.84 ± 0.16*</td>
<td>-61.8</td>
<td>23 ± 3.8*</td>
<td>-83.6</td>
</tr>
<tr>
<td>PTH, 10^{-6} M + Diamox, 10^{-4} M</td>
<td>19</td>
<td>0.86 ± 0.11*</td>
<td>-60.9</td>
<td>21 ± 3.5*</td>
<td>-85.0</td>
</tr>
</tbody>
</table>

* Significantly different (P < .001) from control value; ** significantly different (P < .01) from control value.

Fig. 3. Effects of PTH and acetazolamide (Diamox) on fluid and DMO absorption in the rat proximal tubule. J = the rate of net fluid absorption; J_DMO = the rate of DMO absorption; Control = without PTH and acetazolamide in the perfusate. Data are presented as mean ± S.E. of 16 to 19 tubules in four to five rats.

Fig. 4. Effects of db-cAMP and acetazolamide (Diamox) on fluid and DMO absorption in the rat proximal tubule. J = the rate of net fluid absorption; J_DMO = the rate of DMO absorption; Control = without db-cAMP and acetazolamide in the perfusate. Data are presented as mean ± S.E. of 15 to 18 tubules in four to five rats.

1979). It is of interest to point out that the degree of inhibition of acetazolamide is greater on bicarbonate absorption than on DMO absorption. This can be explained by the fact that DMO absorption is influenced by hydrogen ion supply which depends upon cellular carbonic anhydrase; however, bicarbonate absorption in the proximal tubule requires both cellular hydrogen ion supply and a luminal hydration and dehydration step which depends upon luminal carbonic anhydrase and, therefore, is more susceptible to acetazolamide inhibition. Inhibition of cellular carbonic anhydrase results in reduction of hydrogen ion supply to the proton pump. On the other hand, inhibition of the luminal enzyme causes a delay in the decomposition of carbonic acid into CO_2 which presumably diffuses across the luminal membrane.

Our data clearly demonstrate that PTH can inhibit DMO and fluid absorption further in the presence of the maximum inhibitory effect of acetazolamide, indicating that the effects of PTH and acetazolamide on DMO and fluid absorption are additive. The results suggest that PTH inhibits DMO and fluid absorption by mechanisms other than carbonic anhydrase inhibition and support the view that PTH and acetazolamide have different mechanisms of action on bicarbonate and other electrolyte excretions (Beck and Goldberg, 1973; Nascimento et
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Fig. 5. Effects of PTH and db-cAMP on fluid and DMO absorption in the rat proximal tubule. J = the rate of net fluid absorption; \( J_{\text{DMO}} \) = the rate of DMO absorption. Data are presented as mean ± S.E. of 15 to 17 tubules in four to five rats.

al., 1977; Puschett and Goldberg, 1969). Moreover, neither PTH nor cAMP affects carbonic anhydrase activity in vitro (Garg, 1975). Our data also demonstrate that db-cAMP and acetazolamide have different mechanisms of action on DMO and fluid absorption since the effects of both are also additive (fig. 4). A recent study in the toad urinary bladder also has shown that the effect of cAMP was additive to the effect of benzolamide, a specific renal carbonic anhydrase inhibitor, on hydrogen ion secretion and that cAMP did not inhibit carbonic anhydrase in this tissue (Lief et al., 1979). It was also suggested that cAMP inhibited active but not passive hydrogen secretion by directly affecting the proton pump. It is of interest to note that acetazolamide can increase cyclic guanosine 3′,5′-monophosphate (cGMP) but not cAMP production in the rat kidney (Oswald and Hawlina, 1979). How cGMP and cAMP participate in the regulation of acid-base balance and renal effect of acetazolamide requires further study.

Considerable evidence suggests that PTH inhibits fluid and bicarbonate absorption by stimulating membrane-bound adenylate cyclase which increases cAMP concentration; and cAMP, in turn, inhibits the transport processes by undefined mechanisms (Baumann et al., 1977; Chase and Aurbach, 1968; Karlinsky et al., 1974; Slats et al., 1975). The present study also supports the previous suggestions that cAMP mediates the effect of PTH, since the data (fig. 5) fail to show any additive effects of PTH and cAMP on fluid and DMO absorption at the concentrations which have been shown by our previous study to have maximum inhibitory effect on fluid absorption (Baumann et al., 1977).

In conclusion, the present study suggests that PTH and db-
cAMP exert their effects on fluid and hydrogen ion secretion in the proximal tubule through the mechanisms other than carbonic anhydrase inhibition and that cAMP mediates the effects of PTH.

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References


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