The Effects of the Potassium Channel Opener Minoxidil on Renal Electrolytes Transport in the Loop of Henle

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ABSTRACT

ATP-sensitive potassium channels (K\textsubscript{ATP}) in the thick ascending limb of the loop of Henle play an important role in apical K\textsuperscript{+} recycling, a mechanism essential for maintaining the activity of the Na/2Cl/K-cotransporter. We have previously demonstrated that inhibition of K\textsubscript{ATP} decreases Na\textsuperscript{+} and K\textsuperscript{+} absorption in the loop of Henle and induces diuretic and natriuretic effects. In the present study, we used renal clearance and in vivo microperfusion techniques to evaluate the effects of the K\textsubscript{ATP} opener minoxidil on the urinary excretion and absorption in the loop of Henle of Na\textsuperscript{+}, K\textsuperscript{+}, Ca\textsuperscript{2+}, and Mg\textsuperscript{2+}. Intravenous injection of minoxidil (1.5 mg/kg) significantly decreased fractional Na\textsuperscript{+} (FENa) and Mg\textsuperscript{2+} (FEMg) excretion and urine volume with a moderate decrease in blood pressure (12%) and glomerular filtration rate (15%). Urine volume decreased 63%, and FENa and FEMg decreased 58 and 37%, respectively. In contrast, K\textsuperscript{+} and Ca\textsuperscript{2+} excretion did not change significantly. In the microperfusion of the loop of Henle, addition of minoxidil to the perfusion fluid significantly increased fluid (J\textsubscript{V}), Na\textsuperscript{+} (J\textsubscript{Na}), Cl\textsuperscript{−} (J\textsubscript{Cl}), and K\textsuperscript{+} (J\textsubscript{K}) absorption. J\textsubscript{V} increased 44% (from 8.32 to 11.95 nl/min), J\textsubscript{Na} increased 14% (from 1.96 to 2.34 nmol/min), J\textsubscript{Cl} increased 21% (from 1.72 to 2.08 nmol/min), and J\textsubscript{K} increased 57% (from 35.8 to 56.4 pmol/min). We conclude that the activation of K\textsubscript{ATP} leads to stimulation of Na/2Cl/K-cotransporter activity and increases the rates of Na\textsuperscript{+}, Cl\textsuperscript{−}, and K\textsuperscript{+} absorption in the loop of Henle, an effect contributing to the antidiuretic and antinatriuretic action of this K channel opener.

ATP-sensitive potassium channels (K\textsubscript{ATP}) maintain the large apical K\textsuperscript{+} conductance in the thick ascending limb (TAL) of the loop of Henle and permit extensive recycling of K\textsuperscript{+} from the lumen to cell. This large K\textsuperscript{+} conductance is necessary to supply K\textsuperscript{+} to the Na/2Cl/K-cotransporter and for the generation of the lumen-positive transepithelial potential difference (Hebert and Andreoli, 1984; Greger, 1985; Bleich et al., 1990). The importance of apical K\textsuperscript{+} channels has been underscored by the observation that inactivating mutations of K\textsubscript{ATP} cause a type II Bartter’s syndrome with salt wasting, hypotension, and hypokalemic alkalosis (Simon, 1998). These transport abnormalities are best explained by a lack of K recycling and decreased activity of the Na/2Cl/K-cotransporter (Hebert and Andreoli, 1984). Recent studies also indicate that K\textsuperscript{+} recycling by K\textsubscript{ATP} in macula densa cells is important for mediating the full tubuloglomerular feedback response.

Portions of the study were previously published in abstract form (Wang and Giebisch, 2000). This work was supported by National Institutes of Health Grant DK-17433.

ABBREVIATIONS: K\textsubscript{ATP}, ATP-sensitive potassium channels; TAL, thick ascending limb of the loop of Henle; GFR, glomerular filtration rate; ENa, excretion of Na\textsuperscript{+}; EK, excretion of K\textsuperscript{+}; ECa, excretion of Ca\textsuperscript{2+}; EMg, excretion of Mg\textsuperscript{2+}; FENA, fractional excretion of Na\textsuperscript{+}; FEK, fractional excretion of K\textsuperscript{+}; FECA, fractional excretion of Ca\textsuperscript{2+}; FEMg, fractional excretion of Mg\textsuperscript{2+}; J\textsubscript{V}, rate of fluid absorption; U-37883A, 4-morpholinecarboximidined-N-1-adamantyl-N’-cyclohexylhydrochloride.
Materials and Methods

**Animal Preparation.** Male Sprague-Dawley rats (Harlan, Indianapolis, IN) weighing 200 to 250 g were used for the renal clearance and tubule microperfusion experiments. Animals were kept on a standard rat chow diet and given tap water until the time of the experiment. The animals were anesthetized by the intravenous injection of Inactin (100 mg/kg, Byk-Gulden, Constance, Germany) and placed on a thermostatically controlled surgical table to maintain body temperature at 37°C. The left jugular vein and the carotid artery were cannulated for the infusion of saline and for collection of arterial blood samples, respectively.

**Renal Clearance Studies.** Renal clearance techniques were used as previously described (Wang et al., 1995) to investigate the effects of minoxidil on the glomerular filtration rate (GFR) and absolute (E\Na, E\K) and fractional excretion rates of \Na, \K, \Ca, and \Mg (F\Na, F\K, F\Ca, and F\Mg). After replacement of surgical fluid losses with isotonic saline, a priming dose of 25 \muCi of [\H]methoxy-inulin was given in 0.5 ml of isotonic saline, followed by a maintenance infusion of 0.9% NaCl, which contained 25 \muCi/h, at a rate of 4.6 ml/h. Collections of blood and urine samples were made after a 45-min equilibration period. Urine collections lasted 30 min, and blood samples were taken at the beginning and end of each collection period. After two control periods, minoxidil was given by bolus injection i.v. at a concentration of 1.5 mg/kg. A similar amount of vehicle was received in the control group. * signifies significantly different from control values (P < 0.05).

**Fig. 1.** Effects of minoxidil on urine volume and GFR. Data presented as the means ± S.E. from eight animals. Minoxidil was given by bolus injection i.v. at a concentration of 1.5 mg/kg. A similar amount of vehicle was received in the control group. * significantly different from control values (P < 0.05).
Fig. 2. Effects of minoxidil on fractional excretion of Na⁺ (FENa; top) and K⁺ (FEK; bottom). Minoxidil was given by bolus i.v. at a concentration of 1.5 mg/kg. A similar amount of vehicle was received in the control group. *, significantly different from control values ($P < 0.05$).

**TABLE 1**

Effect of minoxidil on GFR, urine volume, and plasma electrolytes

Values are means ± S.E. from eight animals of each group. Control periods are the mean of two collection periods before administration of minoxidil or vehicle; experimental periods are the mean of four collection periods after i.v. minoxidil or vehicle.

<table>
<thead>
<tr>
<th></th>
<th>GFR (ml/min/100 g)</th>
<th>UV (ml/min)</th>
<th>PNa (mM/l)</th>
<th>PK (mM/l)</th>
<th>PMg (mM/l)</th>
<th>PCa (mM/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control periods</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Control</td>
<td>0.734 ± 0.04</td>
<td>0.011 ± 0.001</td>
<td>142.1 ± 1.20</td>
<td>4.15 ± 0.18</td>
<td>0.68 ± 0.03</td>
<td>2.24 ± 0.05</td>
</tr>
<tr>
<td>Minoxidil</td>
<td>0.762 ± 0.05</td>
<td>0.013 ± 0.002</td>
<td>143.0 ± 0.94</td>
<td>4.51 ± 0.20</td>
<td>0.68 ± 0.02</td>
<td>2.06 ± 0.03</td>
</tr>
<tr>
<td><strong>Experimental periods</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>0.690 ± 0.02</td>
<td>0.019 ± 0.002</td>
<td>141.0 ± 0.43</td>
<td>4.12 ± 0.09</td>
<td>0.66 ± 0.02</td>
<td>2.17 ± 0.03</td>
</tr>
<tr>
<td>Minoxidil</td>
<td>0.584 ± 0.03*</td>
<td>0.007 ± 0.0008**</td>
<td>144.3 ± 1.33</td>
<td>4.44 ± 0.14</td>
<td>0.68 ± 0.01</td>
<td>2.10 ± 0.02</td>
</tr>
</tbody>
</table>

UV, urine volume; PNa, plasma Na⁺; PK, plasma K⁺; PMg, plasma Mg²⁺; PCa, plasma Ca²⁺.

* Significant difference from control ($P < 0.05$).

** Significant difference from control ($P < 0.01$).
intravenously as a bolus injection (1.5–3 mg/kg) followed by four additional collection periods. In the control group, a similar amount of vehicle was administered. Urine and plasma Na⁺ and K⁺ concentrations were measured by flame photometry (type 480 Flame Photometer; Corning Medical and Scientific, Corning, NY), and absolute and fractional renal excretions were calculated by standard methods (Giebish et al., 1993).

**Microperfusion of the Loop of Henle.** The methods of in vivo microperfusion of superficial loops of Henle were similar to those described previously (Wang et al., 1995a). First, a loop of Henle was selected by microperfusing a proximal tubule to locate its last loop on the kidney surface. Then, the loop of Henle was perfused from the last loop of the proximal tubule with a microperfusion pump at a rate of 20 nl/min. Tubule fluid was collected from the first segment of the early distal tubule with an oil block placed distally from the collection site. The rate of fluid Na⁺, Cl⁻, and K⁺ absorption in the loop of Henle was expressed as absorption rate per loop since the length of individual loops of Henle in the rat has been found to vary little (Wahl and Schnerrmann, 1989). Na⁺ and K⁺ concentrations in the perfusate and collected tubule fluid were measured by ultramicroscopic absorption spectrophotometry, as described by Good and Wright (1979) and Wingo et al. (1987). The Cl⁻ concentrations were measured by a Cl⁻ microelectrometric method (Wang et al., 1993), and net fluxes were calculated by standard methods (Giebish et al., 1993).

The composition of the perfusion fluids was as follows: 115 mM NaCl, 25 mM NaHCO₃, 4 mM KCl, 1 mM CaCl₂, 5 mM Na-acetate, 5 mM glucose, 5 mM L-alanine, 2.5 mM Na₂HPO₄, 0.5 mM NaH₂PO₄ (pH was adjusted to 7.4, and the osmolality was at 295 mOsm).

Data are presented as means ± S.E. Control and experimental values were compared using the unpaired Student’s t test. Dunnett’s test (Dunnett, 1964) was used for comparison of several treatment groups with a single control group. Differences between groups are reported as significant at P < 0.05.

**Materials.** [³H]Methoxy insulin was obtained from New Research Products (Boston, MA), and minoxidil was purchased from Sigma-Aldrich (St. Louis, MO).

### Results

**Renal Clearance Studies**

**Effects of Minoxidil on Urine Volume and GFR.** Minoxidil is a potent inhibitor of vascular tone and known to lower blood pressure (Cotorruelo et al., 1982). We have found that an intravenous injection of 3 mg/kg minoxidil significantly decreased mean blood pressure (by 40%). Minoxidil at the concentration of 1.5 mg/kg, however, lowered blood pressure only by 12%, and the change did not reach statistical significance. Therefore, we used the lower concentration of minoxidil in the clearance studies to minimize hemodynamic changes.

Figure 1 shows the time course of the changes in urine flow rate and GFR. The urine volume decreased significantly from 0.014 ± 0.003 to 0.01 ± 0.002, 0.0056 ± 0.001 (P < 0.05), 0.006 ± 0.001 (P < 0.05), and 0.0065 ± 0.001 ml/min (P < 0.05); GFR decreased from 0.77 ± 0.08 to 0.49 ± 0.04 (P < 0.05), 0.57 ± 0.05 (P < 0.05), 0.58 ± 0.07 (P > 0.05), and 0.70 ± 0.06 ml/min/100 g b.wt. after a 30-, 60-, 120-, and 150-min bolus injection of Minoxidil, respectively. Table 1 shows the effects of minoxidil on urine volume, GFR, and plasma Na⁺, K⁺, Mg²⁺, and Ca²⁺; data was calculated from the mean of two control periods and four experimental periods. The plasma concentrations of Na⁺, K⁺, Mg²⁺, and Ca²⁺ were similar in control and experimental groups.

**Effects of Minoxidil on Urine Na⁺ and K⁺ Excretion.** As shown in Fig. 2, fractional excretion of Na⁺ was decreased from 0.57 ± 0.22 to 0.29 ± 0.1, 0.26 ± 0.07 (P < 0.05), 0.278 ± 0.08 (P < 0.05), and 0.28 ± 0.09% (P < 0.05) after a 30-, 60-, 120-, and 150-min bolus injection of minoxidil, respectively. The lower panel of Fig. 2 shows the effect of minoxidil on K⁺ excretion. FEK increased slightly after intravenous administration of minoxidil and then decreased to a level similar to the control group. These changes did not reach statistical significance. Because sodium and potassium excretion rose modestly in control experiments over the time period of the clearance experiments, the excretion rates of Na⁺ and K⁺ in the experimental groups were corrected for these changes in ion excretion. Mean excretion rates during two controls and four experimental collection periods are summarized in Table 2. Both ENa and FENa decreased significantly after minoxidil treatment, but EK and FEK remained unchanged.

**Effects of Minoxidil on Urine Mg²⁺ and Ca²⁺ Excretion.** As shown in the top panel of Fig. 3, minoxidil also induced a significant decrease in fractional urinary Mg²⁺ excretion. FEMg decreased from 12.63 ± 1.44 to 9.83 ± 2.08, 7.97 ± 0.97 (P < 0.05), 7.97 ± 0.94 (P < 0.05), and 8.56 ± 0.91% (P < 0.05) after 30-, 60-, 120-, and 150-min administration of the K⁺ channel opener. The lower panel of Fig. 3 shows the effect of minoxidil on Ca²⁺ excretion. Although FECa decreased modestly, the decline was not significant. Table 3 summarizes the mean of Mg²⁺ and Ca²⁺ excretion of two controls and four experimental collection periods. Both EMg and FEMg were decreased significantly in the minoxidil-treated group, whereas the ECa and FECa did not change significantly. Unlike sodium and potassium excretion, Mg²⁺ and Ca²⁺ excretion did not increase over the time period of the control experiments, indicating that Mg²⁺ and Ca²⁺ excretion in the present studies was not sensitive to the infusion of saline.

<table>
<thead>
<tr>
<th>ENa µM/min/100 g</th>
<th>EK</th>
<th>FENa%</th>
<th>FE%</th>
</tr>
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<tbody>
<tr>
<td><strong>Control periods</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.451 ± 0.10</td>
<td>0.678 ± 0.06</td>
<td>0.529 ± 0.16</td>
</tr>
<tr>
<td>Minoxidil</td>
<td>0.446 ± 0.11</td>
<td>0.849 ± 0.06</td>
<td>0.513 ± 0.14</td>
</tr>
<tr>
<td><strong>Experimental periods</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>0.601 ± 0.08</td>
<td>0.728 ± 0.06</td>
<td>0.662 ± 0.09</td>
</tr>
<tr>
<td>Minoxidil</td>
<td>0.266 ± 0.05**</td>
<td>0.766 ± 0.05</td>
<td>0.276 ± 0.04**</td>
</tr>
</tbody>
</table>

* Significant difference from normal control mouse (P < 0.05).
** Significant difference from normal control mouse (P < 0.01).
Microperfusion Studies

Effects of Minoxidil on Na⁺, Cl⁻, and K⁺ Absorption in Loop of Henle. We evaluated whether minoxidil stimulates NaCl and K⁺ transport in the TAL as a result of decreased urinary Na⁺ excretion by a microperfusion technique in vivo. Data obtained during perfusion of the loop of Henle with solutions containing minoxidil (100 μM) are summarized in Fig. 4. The data indicate a significant increase of Na⁺, K⁺, Cl⁻, and fluid absorption. As shown in Fig. 4, the rate of Na⁺ absorption ($J_{Na}$) increased significantly by 13.9% (from 1.96 ± 0.03 to 2.24 ± 0.05 nmol/min); chloride absorption ($J_{Cl}$) increased 21% (from 1.72 ± 0.05 to 2.08 ± 0.08 nmol/min). Potassium absorption was also sharply enhanced from 35.78 ± 1.85 pmol/min to 56.4 ± 3.87 nmol/min. $J_v$ also increased from 8.32 ± 0.12 to 11.95 ± 0.48 nl/min. These results demonstrated that minoxidil increases absorption of Na⁺, Cl⁻, and K⁺ in the loop of Henle.

Fig. 3. Effects of minoxidil on fractional excretion of Mg (FEMg; top) and K⁺ (FECa; bottom). Minoxidil was given by bolus i.v. at a concentration of 1.5 mg/kg. A similar amount of vehicle was given in the control group. *, significantly different from control values ($P < 0.05$).
Discussion

Minoxidil is a potent opener of ATP-sensitive K+ channels and acts on many cells including those of vascular smooth muscle and kidney (Meisheri et al., 1993). The mechanism of its strong antihypertensive effect is related to its ability to open K+ channels, which leads to cell hyperpolarization and inhibition of voltage-dependent Ca2+ channels (Andersson, 1992). This sequence of events produces strong vascular dilatation and lowers blood pressure (Kosman, 1980). The membrane effects of minoxidil on smooth muscle can be relieved by inhibitors of K+ channel conductance, such as glyburide and U-37883A (Meisheri et al., 1993). Although minoxidil is an effective antihypertensive agent, its use has been limited by significant side effects, such as sodium retention and edema (Ram and Reichgott, 1978; Kosman, 1980).

Our studies demonstrate that minoxidil decreases urine volume, sodium, chloride, and magnesium excretion and that these significant antidiuretic and antinatriuretic effects occur without causing major changes in urinary potassium and calcium excretion. Since minoxidil directly modulates transport in the loop of Henle, it is reasonable to suggest that the observed increase in sodium and chloride as well as fluid absorption at this nephron site contributes to the observed decline in sodium and chloride excretion in the urine. Moreover, the perfusion studies of the loop of Henle in which the hemodynamic effects and changes in flow rate of minoxidil are absent suggest that this K+ channel opener acts directly on tubule transport.

The loop of Henle consists of several distinct segments characterized by cell heterogeneity. Included in the loop of
Henle are the straight portion of the proximal tubule (S3) and the thin descending limb and TAL. It is most likely that minoxidil alters electrolyte transport in the TAL because ATP-sensitive K channels have been identified in the apical membrane of cells lining this nephron segment (Wang, 1994).

The action of minoxidil in the thick ascending limb may involve alterations in two transport operations. First, apical K+ channels are expected to stimulate absorption of sodium, potassium, and chloride through the electroneutral Na/2Cl/K-cotransporter by augmenting the rate of K+ recycling across the apical membrane. As a consequence of accelerated entry of K+ into the lumen, the activity of the cotransporter is expected to rise and thereby enhance the reabsorption of the ions translocated by the cotransporter. Several K+ channels have been identified at this site, including a low- and medium-conductance ATP-sensitive channels (Giebisch et al., 1993; Wang, 1994). Ca2+-activated K+ channel activity has also been reported in cultures of medullary thick ascending limb cells (Guggino et al., 1987). Moreover, recent studies has also been reported in cultures of medullary thick ascending limb cells other than the TAL. Our data showed that minoxidil was limited to the tubule perfusion fluid, making it possible that K+ channels alter fluid absorption in segments other than the TAL on is presently not known.

Our studies strongly support the view that the TAL is an important site of action of minoxidil, but it is likely that other nephron segments are also involved. First, our clearance studies demonstrate a larger reduction in sodium excretion than expected from the perfusion studies of the loop of Henle. One possibility is that K channel openers hyperpolarize the apical membrane of principal cells in the initial and cortical collecting tubule. Such an action would increase the driving force into principal tubule cells for sodium and thereby stimulate its reabsorption.

Our perfusion studies show that the effect of minoxidil includes both increased reabsorption of Na+, K+, and Cl– and enhancement of fluid absorption along the loop of Henle. Since the effects of K channel openers involve actions on the cells of the TAL, a tubule segment with very low water permeability (Kokko and Tisher, 1976), it is likely that minoxidil alters fluid movement in the S3 or thin ascending limb of Henle’s loop. It should be noted that administration of minoxidil was limited to the tubule perfusion fluid, making it unlikely that changes in the medullary interstitial environment affected fluid transport. The mechanism by which K+ channels alter fluid absorption in segments other than the TAL on is presently not known.

Our observations on the transport of potassium following administration of minoxidil suggest that this agent may affect nephron segments other than the TAL. Our data showed that minoxidil increased the K+ absorption in the loop of Henle but did not change urinary K+ excretion. We propose that K+ transport is modulated by the combined action of minoxidil in the TAL and initial and cortical collecting ducts. In the TAL, stimulation of recycling of potassium across the apical membrane of the cells increases reabsorption of sodium and chloride, whereas activation of potassium channels in the apical membrane of principal cells of the cortical collecting tubule could increase potassium secretion. As a consequence of these opposing mechanisms, the combined action of K+ channel openers could result in unchanged K+ excretion. We have previously shown that the KATP blockers glybenclamide and U-37883A produce diuretic and natriuretic effects but did not change the K+ excretion (Wang et al., 1995b). This supports the view that inhibition or stimulation of the K channels in both TAL and cortical collecting tubule changes urinary Na+ but not K+ excretion (Clark et al., 1993; Wang et al., 1995a).

Our clearance studies show that Mg2+ excretion was decreased by minoxidil, whereas that excretion of Ca2+ remained unchanged. The mechanisms by which K+ channel openers modulate transport of these divalent cations along the nephron have not been explored and need further study.

In conclusion, the present microperfusion study provides evidence that minoxidil increases sodium, chloride, and potassium absorption in the loop of Henle, and clearance experiments show that this K+ channel opener effects significant sodium and fluid retention without change in potassium excretion. It is proposed that stimulation of potassium recycling in the thick ascending limb and increased activity of the Na/2Cl/K-cotransporter contribute to these effects.

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References


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