Renal Effects of Glibenclamide: A Micropuncture Study

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ABSTRACT

The renal effects of glibenclamide were investigated using free flow micropuncture techniques in anesthetized Sprague-Dawley rats. Intravenous infusion of the drug (3 mg/hr) evoked a natriuresis and diuresis; potassium excretion remained unchanged. Fractional reabsorption in the proximal convoluted tubule in glibenclamide-infused rats did not differ significantly from that in control animals, although the late proximal tubular fluid to plasma concentration ratio for potassium was reduced. Fractional sodium delivery to the early distal tubule was elevated, while the fractional deliveries of water and potassium to this nephron site were unaffected. We conclude that glibenclamide impairs sodium reabsorption in one or more of the nephron segments that comprise the loop of Henle. These results are consistent with the hypothesis that the natriuresis resulting from glibenclamide administration is a consequence of blockade of potassium channels in the apical membrane of the thick ascending limb of Henle’s loop. The data suggest that glibenclamide may additionally inhibit a small secretory potassium flux in the proximal tubule.

The sulphonylurea glibenclamide is one of a series of structurally related compounds commonly used in the treatment of noninsulin dependent diabetes mellitus. It is now well established that glibenclamide’s extrapancreatic effects include a natriuretic and diuretic action on the kidney (Clark et al., 1993). Preliminary clearance measurements in this laboratory indicated a tubular action, because glomerular filtration rate was unaffected (Bailey and Walter, 1995a) and in a recent in vivo micropерfusion study Wang et al. (1995a) reported inhibition of sodium and potassium reabsorption in the loop of Henle when 250 μM glibenclamide was included in the perfusate. However, this intraluminal concentration is much higher than would be predicted in rats infused i.v. with glibenclamide at rates known to result in a natriuresis and diuresis, particularly when the extensive protein binding of the drug (Crooks and Brown, 1976) is taken into account.

Glibenclamide has been shown to inhibit the movement of potassium ions through ATP-sensitive channels in several tissues (Ashcroft and Ashcroft, 1990). Patch-clamp studies have demonstrated the presence of a glibenclamide-sensitive potassium channel in the basolateral membrane of the rabbit proximal tubule (Tsuchiya et al., 1992) that appears to play a central role in the efficient coupling of apical sodium entry to basolateral exit (Beck et al., 1993). Although no analogous channel has yet been identified in the proximal tubule of the rat, blockade by glibenclamide could, in principle, impair reabsorption in the proximal tubule and thus contribute to the natriuretic effect of the drug. Alternatively, inhibition of potassium movement through ATP-sensitive channels that are known to be present on the apical membrane of cells in the thick ascending limb of Henle (Wang, 1994) could be implicated in the natriuresis, because it has been shown, both in vitro and in vivo, that barium, a generic inhibitor of potassium channels, can inhibit sodium reabsorption in perfused loops of Henle (Greger and Schlatter, 1981; Walter et al., 1997). Our aim was to use free-flow micropuncture to investigate these two possible sites of action within the kidney. Some of the results have appeared in a preliminary communication (Bailey and Walter, 1995b).

Methods

Male Sprague-Dawley rats (weight range 230–270 g) were anesthetized with Trapanal (110 mg/kg body weight, i.p.; Byk Gulden, Constance, Germany) and prepared surgically for micropuncture experiments as described in previous studies from this laboratory (Walter et al., 1979; Shirley et al., 1990).

Infusion Protocol

Initially, the rats were infused i.v. with isotonic saline at a rate of 2 ml/hr. During the final hour of surgery an extra volume of saline, approximately equivalent to 0.5% body weight, was given to replace surgical losses. After the completion of surgery, rats were infused i.v. with both a 5% glucose solution (1 ml/hr) and isotonic saline (1.5

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ABBREVIATIONS: ATP, adenosine triphosphate; GFR, glomerular filtration rate; MABP, mean arterial blood pressure; PCV, packed cell volume; PNa, P K, P In, plasma concentration of sodium, potassium and inulin, respectively; SNGFR, single nephron glomerular filtration rate; TAL, thick ascending limb of Henle; TFNa, TFK, TFIn, tubular fluid concentration of sodium, potassium and inulin, respectively; Uosm, urine osmolality; V, UNaV, UKV, UPV, excretion rates of fluid, sodium and potassium, respectively; VTf, tubular fluid flow rate.
ml/hr). After a further hour had been allowed for equilibration, fluid and electrolyte excretion rates were measured over a 30-min period. This protocol was designed to establish baseline values for renal function before the administration of glibenclamide. Thereafter, \( ^{3} \text{H} \text{Hinulin} \) (60 \( \mu \text{Ci} \) primer; 40 \( \mu \text{Ci} / \text{ml} \)) was included in the saline infusate. The animals were split into two groups (\( n = 8 \) in each group). The glucose infusion for the first group was replaced by glibenclamide (Sigma Chemical Co., Poole, UK; 3 mg/ml) infused at 1 ml/hr in a 4:1 mixture of 5% glucose and 0.1 M NaOH; the second group received the vehicle alone. These infusions were maintained for the subsequent 5 hr. This protocol is summarised in figure 1. The rate of i.v. glibenclamide infusion would be expected to represent a submaximal dose, because even when the drug was administered as a bolus, 25 mg of glibenclamide per kg body weight were required for a maximal diuretic response.

**Micropuncture**

During the final 4 hr, free-flow micropuncture collections of tubular fluid were made from the final loop of superficial proximal tubules and from early segments of accessible distal tubules, the latter having been identified by intravenous injection of lissamine green (40 \( \mu \)l of a 5% solution). Typically three to four collections were obtained from each nephron segment per experiment. After each collection, a silicone rubber solution was injected into the nephron to allow subsequent confirmation of the puncture site by microdissection (Cortell, 1969). Proximal collections were accepted as “late” if the final or penultimate surface convolution had been punctured; distal collections were taken as “early” if the puncture site lay in the first third of the distal tubule. Samples of arterial blood (\( 40 \mu \)l) were taken at regular intervals throughout the period of micropuncture for the measurement of plasma \( ^{3} \text{H} \text{Hinulin} \) activity.

A 2-ml sample of arterial blood was taken at the end of each experiment for the measurement of PCV and plasma electrolyte concentrations.

**Analyses**

**Urine and plasma samples.** Sodium and potassium concentrations in urine and plasma were measured by flame photometry and urine osmolality by freezing point depression. PCV was measured using Hawksley microhematocrit tubes. \( ^{3} \text{H} \text{Hinulin} \) activity in 5-\( \mu \)l samples of urine and plasma, dispersed in Aquasol 2 scintillation cocktail (Dupont, Stevenage, UK), was measured by \( \beta \)-emission spectroscopy.

**Tubular fluid samples.** Micropuncture collections were deposited under water-saturated oil. Sample volume was assessed using calibrated constriction pipettes and duplicate samples taken for the measurement of \( ^{3} \text{H} \text{Hinulin} \), sodium and potassium. \( ^{3} \text{H} \text{Hinulin} \) activity was measured as above. Sodium and potassium concentrations were measured by helium glow photometry (Aminco, Silver Spring, MD) after diluting a 5-nl sample in 100 nl of deionised water.

**Intravenous infusions:**

- All rats: 0.9% saline (1.5 ml/h) → 0.9% saline + 40 \( \mu \text{Ci} \cdot \text{ml} \) \( ^{3} \text{H} \text{Hinulin} \) (1.5 ml/h) → 5% glucose soln (1 ml/h)

- **Glibenclamide group:** 3 mg/ml glibenclamide in a 4:1 mixture of 5% glucose and 0.1 M NaOH (1 ml/h)

- **Vehicle group:** Vehicle alone (1 ml/h)

**Calculations and statistics.** Appropriate plasma \( ^{3} \text{H} \text{Hinulin} \) activities for clearance measurements and for tubular fluid-to-plasma concentration ratios were interpolated from measured values. The corresponding ratios for sodium and potassium were calculated on the basis of plasma obtained from the terminal blood sample. GFR was calculated as the renal clearance of \( ^{3} \text{H} \text{Hinulin} \) and SNGFR determined, using distal samples only, as \( (\text{TFIN/PIN}) \). 

\[ V_{TFP} \text{, where } \text{TFIN and PIN are the activities of } ^{3} \text{H} \text{Hinulin in the tubular fluid and plasma samples, respectively, and } V_{TFP} \text{ is the rate at which fluid was collected. The fractions of filtered water, sodium and potassium delivered to each puncture site were calculated as } P_{IN}/\text{TFIN}, (\text{TFIN}/\text{PIN}), \text{ and } (\text{TFN}/\text{PIN}), \text{ respectively.} \]

Data are presented as means ± S.E. Values from the late proximal and early distal collections were pooled to give an average value for each site in each rat. These averages values were used to give a mean value for each site per group. Statistical comparisons were made using Student's \( t \) test for unpaired samples. A difference was taken as statistically significant if \( P < .05 \).

**Results**

PCV and plasma sodium and potassium concentrations measured on the final blood sample, together with body weight, kidney weight and mean arterial blood pressure, are shown in table 1. There were no significant differences between the two groups of rats.

**Whole kidney data.** During the control period there were no significant differences between the excretion rates of water, sodium or potassium measured in rats later given glibenclamide and the corresponding values in animals that received vehicle alone (data not shown). Excretion rates for the micropuncture kidney during the experimental period are shown in table 2, together with GFR and urine osmolality. Urine flow rate and sodium excretion were significantly higher and urine osmolality lower in rats receiving glibenclamide. There were no differences between measurements in the micropuncture and contralateral kidneys (data not shown) in either group of rats.

**Micropuncture data.** SNGFR (calculated from distal tubular collections) was similar in the two groups of rats (vehicle and glibenclamide) and the corresponding values in animals that received vehicle alone (data not shown). Excretion rates for the micropuncture kidney during the experimental period are shown in table 2, together with GFR and urine osmolality. Urine flow rate and sodium excretion were significantly higher and urine osmolality lower in rats receiving glibenclamide. There were no differences between measurements in the micropuncture and contralateral kidneys (data not shown) in either group of rats.
TABLE 1
Body weight, kidney weight, mean arterial blood pressure, packed cell volume and plasma sodium and potassium concentrations in rats receiving glibenclamide or vehicle alone

<table>
<thead>
<tr>
<th></th>
<th>Vehicle</th>
<th>Glibenclamide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>247 ± 4</td>
<td>244 ± 4</td>
</tr>
<tr>
<td>Left kidney weight (g)</td>
<td>0.99 ± 0.05</td>
<td>1.01 ± 0.04</td>
</tr>
<tr>
<td>MABP (mm Hg)</td>
<td>101 ± 4</td>
<td>101 ± 3</td>
</tr>
<tr>
<td>PCV</td>
<td>0.46 ± 0.01</td>
<td>0.45 ± 0.01</td>
</tr>
<tr>
<td>PNa (mM)</td>
<td>139 ± 2</td>
<td>136 ± 1</td>
</tr>
<tr>
<td>PK (mM)</td>
<td>4.2 ± 0.1</td>
<td>4.3 ± 0.2</td>
</tr>
</tbody>
</table>

Values are means ± S.E. n = 8 in each group. Packed cell volume and plasma data were measured on the final blood sample. MABP, Mean arterial blood pressure; PCV, packed cell volume; PNa,PK, plasma sodium and potassium concentration respectively.

TABLE 2
Whole-kidney data for the micropuncture kidney during the experimental period measured in rats receiving either vehicle or glibenclamide solutions

<table>
<thead>
<tr>
<th></th>
<th>Vehicle</th>
<th>P</th>
<th>Glibenclamide</th>
</tr>
</thead>
<tbody>
<tr>
<td>GFR (ml/min)</td>
<td>1.22 ± 0.09</td>
<td>NS</td>
<td>1.33 ± 0.09</td>
</tr>
<tr>
<td>V (μl/min)</td>
<td>3.72 ± 0.57</td>
<td>&lt;.05</td>
<td>6.62 ± 1.09</td>
</tr>
<tr>
<td>UNa V (μmol/min)</td>
<td>0.49 ± 0.17</td>
<td>&lt;.05</td>
<td>1.08 ± 0.19</td>
</tr>
<tr>
<td>UKV (μmol/min)</td>
<td>0.80 ± 0.01</td>
<td>&lt;.05</td>
<td>0.82 ± 0.14</td>
</tr>
<tr>
<td>Uosm (mosm/kg.H2O)</td>
<td>1870 ± 133</td>
<td>&lt;.05</td>
<td>1388 ± 188</td>
</tr>
</tbody>
</table>

Values are means ± S.E. n = 8 in each group. GFR, Glomerular filtration rate; V, UNa V, UKV, excretion rates of water, sodium and potassium, respectively; Uosm, urine osmolality.

univ. 0.98 ± 0.03; P < .05). Glibenclamide infusion resulted in a significant increase in fractional sodium delivery to the early distal tubule, associated with a raised TFNa/PNa (0.45 ± 0.02 univ. 0.35 ± 0.03; P < .05).

Discussion

The difference in sodium excretion and urine flow rate between the two groups of rats during the experimental period in our study provides confirmation of the natriuretic and diuretic effects of i.v. infused glibenclamide, because no such difference was apparent during the control period. As in previous studies, GFR was unaffected, indicating a tubular effect of the drug. Our aim was to use free-flow micropuncture to clarify the renal sites of action of glibenclamide. The main conclusions relating to sodium transport are clear-cut: glibenclamide had no effect on fractional sodium reabsorption in the proximal convoluted tubule, an observation that is consistent with lithium clearance data reported earlier (Bailey and Walter, 1995a) and with the findings of a recent in vivo microperfusion study using U37883A, an ATP-sensitive potassium channel blocker structurally dissimilar to glibenclamide (Wang et al., 1995b). The second finding of our study was that glibenclamide significantly increased the fraction of the filtered load of sodium delivered to the early distal tubule. Taken together, these observations indicate a reduction in sodium reabsorption in the loop of Henle.

The data permit only limited speculation as to the precise site within the loop at which glibenclamide acts. The loop of Henle of superficial nephrons comprises several distinct segments: the pars recta of the proximal tubule, the thin descending limb and the TAL. It seems inherently unlikely that glibenclamide would have no discernible effect in the pars convoluta and yet inhibit sodium transport in the pars recta, although it is possible that the drug may be present at a higher concentration in the latter nephron segment, because it may gain access to the lumen via a secretory pathway (Ullrich et al., 1994). However, if sodium transport were inhibited in the pars recta and if, as is generally assumed, reabsorption in this segment of the nephron is isosmolar, then an increase in fluid delivery to the early distal tubule would be anticipated. No such change was observed. Sodium transport in the thin descending limb of superficial nephrons is generally considered to be small in magnitude and passive in nature (Grantham et al., 1992). Consequently, the most plausible site of glibenclamide’s action within the loop is the TAL. The rise in TFNa/PNa at the early distal nephron is consistent with this hypothesis. Because reabsorption of sodium chloride in the TAL provides the driving force for the countercurrent multiplier, an inhibition of sodium reabsorption would lead to a fall in medullary hyperosmolality, which could explain the diuresis and reduction in urine osmolality observed during glibenclamide infusion. Although the mechanism underlying glibenclamide’s action in the loop remains uncertain, inhibition of sodium reabsorption secondary to blockade of apical potassium channels is an attractive possibility. The main conundrum associated with this putative mechanism concerns the delivery of an effective concentration of glibenclamide, administered i.v., to the luminal membrane. The total amount of glibenclamide infused into each rat (over a 5-hr period) in our study was 15 mg (30 μmol). Because the drug is known to be extensively protein bound in the plasma, it seems unlikely that its concentration in glo-
merular filtrate would ever have exceeded 10 μmol/liter. An additional potential route of entry to the lumen involves carrier-mediated secretion of the drug into the proximal tubule (Ullrich et al., 1994). However, analysis of both late proximal and early distal tubular fluid (collected during i.v. infusion of the drug) using micellar electrokinetic chromatography indicated a glibenclamide concentration below 50 μmol/liter (Bailey MA, Moss I and Walter SJ, unpublished observations). An alternative explanation for the renal effects of i.v. glibenclamide is that inhibition of potassium channels in the TAL may be dependent on its presence within the cells as opposed to the tubular fluid. Finally, other actions of the drug may mediate the inhibition of reabsorption in the TAL. For example, glibenclamide has been reported to inhibit a small-conductance chloride channel in the basolateral membrane of murine TAL cells (Guinamard et al., 1995).

The tubular fluid-to-plasma concentration ratio for potassium, measured in late proximal samples from the glibenclamide group was lower than in control animals, suggesting that the drug may inhibit a small secretory potassium flux in the proximal tubule. The result is interesting in that it implies that glibenclamide may gain access to the apical membrane of the proximal tubule, and, moreover, that glibenclamide-sensitive potassium channels mediate a small degree of secretion in this nephron segment. This is consistent with the results from a recent study in which it was reported that barium significantly reduced the potassium flux in perfused segments of the proximal tubule (>2 mm in the proximal tubule). However, when the potassium concentration of the perfusate was lower than that of an ultrafiltrate of plasma (Kibble et al., 1995).

The fractional delivery of potassium out of the loop of Henle was not significantly affected by glibenclamide. At first glance this may seem paradoxical if it is postulated that inhibition of sodium reabsorption in the loop is secondary to blockade of ATP-sensitive potassium channels in the apical membrane. Reducing the magnitude of potassium backflux into the lumen (via inhibition of recycling across the apical membrane) would be expected to increase net potassium reabsorption. However, inhibition of sodium chloride reabsorption would result in a transepithelial potential difference and thus decrease the driving force for paracellular potassium reabsorption.

In conclusion, free-flow micropuncture experiments indicate that the natriuretic and diuretic effects of glibenclamide, infused i.v., are, at least in part, the consequence of a drug-induced inhibition of sodium reabsorption in the loop of Henle.

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References


