Renal Effects of Glibenclamide in Cystic Fibrosis Mice

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Abstract. In vitro studies have shown that glibenclamide sensitivity is conferred upon Kir 1.1 K⁺ channels when they are co-expressed with the cystic fibrosis transmembrane conductance regulator (CFTR). In rats, glibenclamide acts as a K⁺-sparing diuretic by a mechanism that involves blockade of Kir 1.1 channels in the distal nephron. To test whether interaction between Kir 1.1 and CFTR is required to mediate the renal effects of glibenclamide (15 mg/kg), clearance experiments were performed comparing wild type (WT) and Cfr⁻/⁻ mice. Glibenclamide treatment was associated with an equivalent diuresis in both WT and CF mice. Glibenclamide was K⁺-sparing in both genotypes with no significant change in urinary K⁺ excretion observed. That glibenclamide was an effective K⁺-sparing diuretic in CF animals suggests that CFTR expression is not a requirement to mediate its renal actions in mice.

Glibenclamide is thought to act as a diuretic by inhibiting K⁺ channels in the thick ascending limb (TAL) (1). This prevents the K⁺ recycling required to load the Na⁺-K⁺-2Cl⁻ cotransporter, thereby reducing NaCl reabsorption. Glibenclamide is nonkaliuretic due to simultaneous inhibition of secretory K⁺ channels in the cortical collecting duct (CCD) (1). The K⁺ channels that mediate K⁺ secretion in the TAL and CCD belong to the Kir 1.1 (ROMK) family (2,3). Although glibenclamide blocks native K⁺ channels in renal cells (1), it is ineffective as a blocker of recombinant Kir 1.1 channels (4). However, coexpression with the cystic fibrosis (CF) transmembrane conductance regulator (CFTR) confers glibenclamide sensitivity on Kir 1.1 channels (4,5). The aim of this study was to test the physiologic significance of the Kir 1.1/CFTR interaction by studying the renal effects of glibenclamide in a CF mouse model. The hypothesis was that glibenclamide would not be able to act as a K⁺-sparing diuretic in animals with CF, which should not form Kir 1.1/CFTR complexes.

Materials and Methods
Renal Clearance Measurement
Adult mice were used throughout and were genotyped as described previously (6) (Figure 1). Animals were anesthetized with a combination of Na⁺ thiopentone (100 mg/kg IP) (Thiovet, C-Vet Veterinary Products, Leyland, UK), ketamine (10 mg/kg IP) and xylazine (1.5 mg/kg IP) (Research Biochemicals International, Natick, MA). Animals were placed on a thermostatically controlled heated blanket that was set to maintain body temperature at 38°C (Harvard Apparatus, Kent, UK). Cannulae were placed in a jugular vein for intravenous infusion, a carotid artery for BP monitoring and blood sampling, and in the bladder for urine collection. A tracheostomy was performed, and pure oxygen was blown over the neck area throughout.

After implantation of the venous cannula and until the end of surgery, all animals received intravenous infusion of 2.25% bovine serum albumin in 0.9% NaCl at 0.3 ml/hr. After surgery, 45-min equilibration was allowed and then urine was collected over a 60-min experimental period. Animals received 0.6 ml of the infusate over the first 15 min of the equilibration period. Animals then received a maintenance infusion at 0.6 ml/hr. ³H-inulin (Amersham Pharmacia Biotech, Piscataway, NJ) was infused to allow estimation of the GFR. Animals received a 1.5-µCi priming dose during the first 15 min of equilibration and then a 4.5 µCi/hr maintenance dose thereafter. Glibenclamide was prepared in polyethylene glycol 200 vehicle at a concentration of 25 mg/ml. At the end of the equilibration period, animals were given either 15 mg/kg glibenclamide or an equivalent dose of vehicle as a bolus injected directly into the carotid cannula. ³H-inulin was assayed by liquid scintillation counting, Na⁺ and K⁺ by flame photometry (Sherwood, Model 410, Scientific Laboratory Supplies, Nottingham, UK), CI⁻ by electrometric titration (Jenway PCLM3, Essex, UK), and osmolality by freezing point depression (Roebling osmometer, Camlab, Cambridge, UK). All experimentation was conducted in accord with the UK Animals (Scientific Procedures) Act 1986.

Statistical Analyses
Renal clearance, fractional excretion, and free water clearance were calculated with standard formulae. Data are expressed throughout as mean ± SEM for eight animals in each group. Statistical analysis was by ANOVA plus Tukey’s post hoc test, with a 5% significance level.

Results
Table 1 shows that hematocrit, plasma electrolytes, and osmolality were unchanged by glibenclamide treatment. A significant increase in mean arterial BP was observed in CF mice but not in WT animals. No significant differences in GFR were observed. Table 2 shows that glibenclamide caused a significant increase in mean arterial BP was observed in CF mice but not in WT animals. No significant differences in GFR were observed.
significant diuresis, which was of equivalent size in both genotypes. The diuresis was accompanied by a significant and equivalent increase in osmolar clearance in both genotypes. Negative free water clearance was not affected. Basal excretion rates of Na$^+$, Cl$^-$, and K$^+$ in vehicle-injected animals were not significantly different. Glibenclamide-induced natriuresis was significant in WT mice but just failed to reach statistical significance in CF animals ($P = 0.04$, t test). A significant chloruresis was observed in both WT and CF mice (Figure 2). No change in K$^+$ excretion was observed in either genotype (Figure 2).

Discussion

The key findings of this study were that glibenclamide was an equally effective K$^+$-sparing diuretic in WT and CF mice. These data indicate that CFTR is not necessary for the renal actions of glibenclamide, assuming that no CFTR/Kir interaction can occur in ΔF508-CF mice. This assumption is based on the observation that ΔF508-CFTR does not typically reach the plasma membrane (7). Consistent with this idea, we have recently provided evidence that regulation by CFTR of the epithelial Na$^+$ channel ENaC, is lost in the kidney of these ΔF508-CF mice (6). That glibenclamide was an effective K$^+$-sparing diuretic in these mice was surprising, particularly in view of recent findings by Lu and Giebisch (8), which described poor inhibition of the small conductance K$^+$ channel in mouse TAL by glibenclamide in a CF knockout mouse. If inhibition of NaCl reabsorption secondary to blockade of this K$^+$ channel is the basis of the diuresis, then glibenclamide should not have caused a diuresis in CF mice. It should be noted, however, that the origin of the diuresis caused by glibenclamide is unknown in mice. No change in free water clearance was observed, which suggests that ADH levels were probably unaltered. Comparison of the size of total osmolar clearance with that of Na$^+$, K$^+$, and Cl$^-$ (Table 2) indicates significant shifts in the excretion of other solutes, possibly
### Table 2. Renal excretion variables

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>( V ) (ml/min)</th>
<th>( C_{\text{osm}} ) (ml/min)</th>
<th>( F_{\text{Cl}} ) (%)</th>
<th>( C_{\text{Cl}} ) (ml/min)</th>
<th>( F_{\text{Na}} ) (%)</th>
<th>( C_{\text{Na}} ) (ml/min)</th>
<th>( F_{\text{K}} ) (%)</th>
<th>( C_{\text{K}} ) (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT vehicle</td>
<td>8</td>
<td>8.7 ± 1.3</td>
<td>16.3 ± 2.2</td>
<td>7.6 ± 1.4</td>
<td>14.6 ± 1.8</td>
<td>7.3 ± 1.6</td>
<td>11.9 ± 1.5</td>
<td>11.9 ± 1.5</td>
<td>11.9 ± 1.5</td>
</tr>
<tr>
<td>CF vehicle</td>
<td>8</td>
<td>7.4 ± 1.0</td>
<td>18.5 ± 2.1</td>
<td>8.4 ± 1.5</td>
<td>11.2 ± 1.7</td>
<td>11.3 ± 1.5</td>
<td>11.3 ± 1.5</td>
<td>11.3 ± 1.5</td>
<td>11.3 ± 1.5</td>
</tr>
<tr>
<td>WT glib</td>
<td>8</td>
<td>8.8 ± 1.1</td>
<td>25.4 ± 2.2</td>
<td>9.4 ± 1.7</td>
<td>11.9 ± 1.5</td>
<td>11.9 ± 1.5</td>
<td>11.9 ± 1.5</td>
<td>11.9 ± 1.5</td>
<td>11.9 ± 1.5</td>
</tr>
<tr>
<td>CF glib</td>
<td>8</td>
<td>16.0 ± 1.4</td>
<td>24.4 ± 1.7</td>
<td>8.4 ± 1.7</td>
<td>11.9 ± 1.5</td>
<td>11.9 ± 1.5</td>
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V, urine flow rate; \( C_{\text{osm}} \), osmolar clearance rate; \( F_{\text{Cl}} \), fractional free water clearance rate; \( C_{\text{Cl}} \), chloride clearance; \( F_{\text{Na}} \), fractional sodium clearance; \( C_{\text{Na}} \), sodium clearance; \( F_{\text{K}} \), fractional potassium clearance; \( C_{\text{K}} \), potassium clearance.

Figure 2. Effect of glibenclamide on the urine outputs (UV) of Na\(^{+}\), Cl\(^{-}\) and K\(^{+}\) in wild-type (WT) and Cfrtm2cam ΔF508 (CF) mice. Open bars indicate treatment with polyethylene glycol vehicle, and solid bars represent treatment with a 15 mg/kg bolus of glibenclamide. *, significant difference between glibenclamide and vehicle treatment within each genotype (ANOVA plus Tukey’s post hoc test, \( P < 0.05 \)).
urea. Recent studies have furthermore suggested that $K_{ATP}$ channel blockers can also act in the proximal nephron (9). Therefore, inhibition of Kir 1.1 in the TAL may be only one component of the observed diuresis. Whatever the cause of the diuresis, $K^+$ excretion was strikingly similar in all groups. The secretory $K^+$ channels in collecting duct principal cells largely determine final $K^+$ excretion (10); therefore, the simplest explanation of the data is that CFTR/Kir 1.1 is not the physiologic configuration of these channels in vivo. One possible alternative $\beta$ subunit for renal Kir 1.1 channels is the sulfonamide receptor SUR2B, which can also confer glibenclamide sensitivity on Kir 1.1 channels coexpressed in Xenopus oocytes (11) and is expressed in mouse distal nephron (12). In conclusion, the data suggest that CFTR expression is not a requirement for the renal actions of glibenclamide to occur.

Acknowledgments
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References