In Vitro Uptake of Gentamicin by Rat Renal Cortical Tissue

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The mechanism of gentamicin uptake in vitro by renal cortical slices of rat kidney was investigated. The cortical-slice-uptake ratio of gentamicin concentration in 1.0 g of tissue water to that of 1.0 ml of incubation medium (SW/M) was 1.44 ± 0.04. The uptake of gentamicin was inhibited by 2 × 10⁻⁵ M dinitrophenol (SW/M = 1.03 ± 0.04) and by anoxia (SW/M = 1.01 ± 0.04). The results indicate that aerobic phosphorylation is required to transport gentamicin into the cells. The uptake of p-aminohippurate and tetraethylammonium chloride by renal cortical slices was not affected by gentamicin.

Studies indicate that gentamicin accumulates in renal cortical tissue at a higher concentration than in the plasma and persists there for a period of time even after disappearance of gentamicin from the plasma (4, 8). Gentamicin clearance data indicate that net renal tubular secretion of gentamicin occurs and accounts for 25 to 30% of the total gentamicin excreted when corrections are made for serum protein binding (5). The present study investigates the mechanisms of gentamicin uptake in vitro by renal cortical slices of rat kidney.

MATERIALS AND METHODS

Sprague-Dawley rats weighing 200 to 220 g were used. The animals were given Purina Rat Chow and water ad lib. Rats were killed under ether anesthesia, and one kidney from each animal was removed rapidly and placed in ice-cold saline. The following studies were performed within 20 min after removal of the kidneys.

In vitro uptake of gentamicin sulfate by rat kidney slices. Cortical slices, approximately 4 mm in thickness, were obtained from five rat kidneys using a Stadie-Riggs microtome. Two cortical slices were taken from each kidney, whereupon these slices were sectioned in half. One section was discarded, leaving a total of three cortical slices from each original kidney. One of the three slices from each kidney was incubated in 3 ml of medium containing 115 mM NaCl, 25 mM NaHCO₃, 10 mM sodium acetate, 5 mM KCl, 1.2 mM NaH₂PO₄, 1.2 mM MgSO₄, 1.0 mM CaCl₂, 5% (vol/vol) rat serum, and gentamicin sulfate and its ¹⁴C-labeled compound (kindly supplied by Schering Corp.), 2.75 μg/ml. The slices were incubated in a Dubnoff metabolic shaker at 25°C; shaking was at 100 cycles/min for 90 min with 95% O₂ and 5% CO₂ as the gas phase. The remaining two kidney slices from each rat were treated identically, except that one was incubated with 2.5 × 10⁻⁸ M dinitrophenol (DNP) added to the medium, whereas the other was incubated with a gas phase of 100% nitrogen. After the incubation, 1 ml of medium was removed and added to 2 ml of 5% trichloroacetic acid followed by Vortex mixing and centrifugation. The tissue was also removed immediately, blotted, weighed, and homogenized in 2 ml of 5% trichloroacetic acid. After tissue homogenization, 1 ml of incubation medium without gentamicin was added, followed by mixing and centrifugation. One milliliter of supernatant from the centrifuged incubation medium and 1 ml from the centrifuged tissue homogenate were placed in separate vials containing 20 ml of Aquasol scintillation liquid (New England Nuclear Corp.). Isotopic counting was performed in a Tri-Carb liquid scintillation spectrometer (model 3003, Packard Instrument Co.) with correction for quenching. The ratio of isotopic counts in 1 g of cortical tissue to that in 1 ml of incubation medium was calculated.

Renal cortical tissue water content was determined in nine additional normal rats. Cortical slices were dried at 120°C for 48 h. Tissue water content was calculated from the wet and dry weights of the slices.

Effect of gentamicin sulfate on p-aminohippurate and tetraethylammonium chloride uptake by rat kidney slices. (l) Group 1. One cortical slice was taken from each of seven rat kidneys as previously described. Each slice was halved, and the sections were incubated separately in 3 ml of Cross and Taggart solution (3), pH 7.40. One half slice served as a control, and its corresponding half served as the experimental slice. p-Aminohippurate (PAH) and its tritiated compound (Amersham/Searle Corp.) and tetraethylammonium chloride (TEA) and its ¹⁴C-labeled compound (New England Nuclear Corp.) were added to both experimental and control media at a final concentration of 2 × 10⁻⁴ M. Gentamicin sulfate powder not radioactively labeled (kindly supplied by Schering Corp.) was also added to the experimental medium at a final concentration of 70 μg/ml; it was not added to the control medium. Incubation was performed in a Dubnoff metabolic shaker at 25°C; shaking was at 100 cycles/min with 100% O₂ for 90 min. After incubation, 1 ml of medium was added to 2 ml of 5% trichloroacetic acid. The tissue was removed, weighed and homogenized...
in 2 ml of 5% trichloroacetic acid; 1 ml of Cross and Taggart solution was then added. One milliliter of the medium-trichloroacetic acid solution and the supernatant of the tissue homogenate-trichloroacetic acid solution were added to scintillation liquid as above. PAH and TEA contents of the slices (S) and medium (M) were determined by double isotopic counting using a Tri-Carb scintillation spectrometer (model 3003, Packard Instrument Co.) as previously described (6, 7). A slice-to-medium (S/M) ratio was calculated from the isotopic counts in 1.0 g of tissue versus those in 1.0 ml of medium. This ratio reflects the renal tubular transport activity of organic acid (PAH) and organic base (TEA) (3, 9).

(ii) Group II. Renal cortical slices of eight rats were prepared as described above. A cortical slice from each rat kidney was halved, and one half section served as a control and its corresponding half served as the experimental slice. The slices were preincubated separately at 37°C in 3 ml of Cross and Taggart solution with shaking at 100 cycles/min and gassed with 100% O₂ for 2 h. Gentamicin sulfate was added to the Cross and Taggart solution containing the experimental slice at a final concentration of 1,100 μg/ml, but was not added to the medium with the corresponding control slice. After 2 h, the slices were removed for PAH and TEA uptake studies, which were performed by further incubation of the slices in Cross and Taggart solution containing [³H]PAH and [¹⁴C]TEA as previously described (6, 7).

The paired t test was used for statistical analysis.

RESULTS

Water content of renal cortical tissue was 76.8 ± 0.8%. The gentamicin sulfate concentration in 1.0 g of tissue water was calculated by dividing the isotopic counts per gram of tissue by 0.768. The tissue slice uptake of gentamicin sulfate was expressed as the ratio of the gentamicin sulfate concentration in 1.0 g of tissue water to that of 1.0 ml of medium (SW/M). Table 1 summarizes the results of renal cortical slice uptake of gentamicin. The concentration of gentamicin sulfate in the tissue water was significantly higher than that in the incubation medium for slices incubated with a gas phase of 95% O₂-5% CO₂, as reflected by the SW/M ratios greater than 1.0. Tissues incubated in media containing DNP and gentamicin sulfate or with gentamicin sulfate and gassed with 100% nitrogen did not achieve higher gentamicin concentrations than in their media. (SW/M ratios were not significantly different from 1.0.) In no instance was the SW/M ratio of a DNP- or N₂-treated slice greater than that of the corresponding control slice incubated under 95% O₂ and 5% CO₂ without DNP.

The uptakes of PAH and TEA by renal cortical slices were not affected by the presence of gentamicin sulfate in the medium or by preincubating at 37°C in a high concentration of gentamicin sulfate (1,100 μg/ml) for 2 h (Table 2).

DISCUSSION

The accumulation of aminoglycoside antibiotics in renal tissues has been reported by several investigators (1, 2, 4, 8; H. Wahlig, Proc. 8th Int. Congr. Chemother., Athens, Greece, 1973, Abstr. no. A-24). In animal experiments, it has been demonstrated that a higher concentration of gentamicin antibiotic occurs in the kidney, especially the renal cortex, than in the serum (4, 8; H. Wahlig, Proc. 8th Int. Congr. Chemother., Athens, Greece, 1973, Abstr. no. A-24). However, the concentration of antibiotic measured in the tissue could be elevated by the high concentration of antibiotic in the renal tubular fluid. Furthermore, Luft and Kleit (8) and Fabre et al. (4) demonstrated that gentamicin enhanced the accumulation of PAH in renal tissue.

Table 1. Uptake of gentamicin sulfate by rat kidney slices

<table>
<thead>
<tr>
<th>Gentamicin (2.75 μg/ml)</th>
<th>Tissue water-to-medium concn ratio (n = 5)</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>95% O₂ + 5% CO₂</td>
<td>1.44 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>DNP (2 × 10⁻³ M); 95% O₂ + 5% CO₂</td>
<td>1.03 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>100% Nitrogen</td>
<td>1.01 ± 0.04</td>
</tr>
</tbody>
</table>

* Data are expressed as means ± standard error of the mean. Statistical analysis by paired t test.

Table 2. Effect of gentamicin on PAH and TEA uptake by rat kidney slices

<table>
<thead>
<tr>
<th>No. of rats</th>
<th>Control—without gentamicin</th>
<th>With gentamicin (70 μg/ml)</th>
<th>Preincubation with gentamicin (1,100 μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PAH</td>
<td>TEA</td>
<td>PAH</td>
</tr>
<tr>
<td>n = 6</td>
<td>11.0 ± 0.72±</td>
<td>13.3 ± 1.39</td>
<td>11.3 ± 0.83±</td>
</tr>
<tr>
<td>n = 7</td>
<td>7.8 ± 0.45</td>
<td>11.8 ± 0.78</td>
<td>7.9 ± 0.43±</td>
</tr>
</tbody>
</table>

* Values are expressed as means ± standard error of the mean.

* Asterisk indicates not significant.
strated that gentamicin sulfate persists in the renal cortex for several days to weeks following a single parenteral injection. Gentamicin sulfate probably accumulates in the cortex by binding to cell membranes, organelle membranes, or intracellular proteins (4, 8, 13).

The present in vitro study clearly indicates that gentamicin sulfate, albeit weakly, is actively transported by the renal cortical slices of the rat kidney. This uptake of gentamicin sulfate is blocked by DNP or lack of oxygen, as reflected by the SW/M ratios of 1.03 and 1.01, respectively. The latter ratios suggest that the accumulation of gentamicin sulfate in the tissue slices under these conditions is mainly due to passive diffusion. Thus, energy from aerobic phosphorylation (3) is required to transport gentamicin sulfate into the cells. Such an active transport has also been demonstrated for cephaloridine (10, 11).

The tissue uptakes of PAH and TEA are not affected by high concentrations of gentamicin sulfate in the medium. A recent preliminary study (13) indicates that quinine, a blocker of the proximal tubular organic base secretory system (12), significantly reduces renal cortical gentamicin accumulation in vivo. Failure of gentamicin sulfate to interfere with organic base (TEA) transport could be due to the fact that gentamicin, an organic base, is either very weakly transported through this system or is transported by a different pathway.

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LITERATURE CITED