Effect of diazoxide on renal handling of sodium in the rat

L. G. FINE AND H. WEBER
Division of Nephrology, Department of Medicine, Albert Einstein College of Medicine, New York, U.S.A.

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Summary

1. To assess whether diazoxide-induced sodium retention is due to a direct renal action of the drug, independent of its systemic haemodynamic effects, diazoxide was infused directly into one renal artery of hydropenic rats. During the infusion period there was a small fall in mean systemic arterial blood pressure. There were no significant changes in the clearances of inulin or p-aminohippurate by either kidney.

2. Absolute and fractional excretion of sodium were unchanged on the non-infused side. On the side of infusion, there was a significant increase in absolute and in fractional sodium excretion.

3. Thus natriuresis rather than sodium retention seems to be the direct renal effect of diazoxide and may be related to its vasodilator properties.

Key words: diazoxide, extracellular fluid volume control, natriuresis, renal artery, sodium excretion.

Introduction

Diazoxide, a member of the benzothiadiazine group of compounds, has been shown to be a potent anti-hypertensive agent, which causes sodium retention and weight gain when administered parenterally or orally. Retention of sodium may occur without change in blood pressure or glomerular filtration rate (Johnson, 1971; Pohl, Thurston & Swales, 1972; Bartorelli, Gargano, Leonetti & Zanchetti, 1963), has been demonstrated in experimental animals (Taylor & Rubin, 1964) and has been attributed to increased proximal tubular reabsorption of sodium as an indirect response to changes in peripheral circulatory resistance (Pohl et al., 1972).

Swales, Thurston & Pohl (1972) demonstrated that patients with chronic renal failure treated with oral diazoxide were able to reduce urinary sodium excretion to levels substantially lower than could be obtained after a period of salt deprivation. In an attempt to clarify whether the sodium retention is due to a direct renal action of diazoxide independent of its systemic haemodynamic effects, the present study was designed to examine the effect of small doses of the drug on renal handling of sodium in the rat by using the method of direct infusion into the renal artery (Fine, Lee, Goldsmith, Weber & Blaufox, 1974).

Methods

Animals

Studies were performed on female Sprague–Dawley rats weighing from 250 to 270 g. The animals were maintained on standard Purina laboratory rat chow with free access to drinking water.

On the day of study the rats were anaesthetized with ether. A longitudinal incision was made in the left flank area and the left renal artery exposed retroperitoneally. The connective tissue around the artery was cleared gently with cotton applicators and a piece of 4-0 silk was placed loosely around the aorta proximal to the origin of the left renal artery. The left renal artery then was catheterized with a 33
gauge needle-cannula bent at 90° and connected to PE 10 tubing which had been flushed first with heparin solution and then with sodium chloride solution (150 mmol/l). The technique employed for the cannulation was as follows. The aorta was lifted gently by means of the silk-tie around it in order to stabilize the origin of the left renal artery and the needle was inserted into the artery close to its origin. The length of needle lying within the artery was 3 mm. The tension on the silk-tie around the aorta was released immediately upon placement of the catheter, the time of ischaemia to the kidney being no more than 15 s. A drop of Eastman 910 adhesive (Eastman Chemical Products Inc., Kingsport, Tennessee, U.S.A.) was placed at the site of insertion of the needle to fix it in position. The PE 10 tubing was tied in two places to the fat overlying the muscles of the posterior abdominal wall and was carried subcutaneously via a large-bore needle to emerge at the base of the tail. The tubing was connected to a syringe containing sodium chloride (150 mmol/l) and an infusion was initiated at a rate of 0.02 ml/min with a constant infusion pump. To confirm that the cannula was properly placed, the infusion was interrupted and 0.2 ml of 1% Lissamine Green was injected into the catheter and flushed with 0.2 ml of sodium chloride (150 mmol/l). When the tip of the needle was properly placed there was a greenish discoloration of the kidney, which abated rapidly. The infusion into the left renal artery then was restarted at the same rate (0.02 ml/min). If the discoloration persisted in any area of the renal parenchyma for longer than 15 s the experiment was terminated.

The bladder was catheterized with a silastic catheter and the middle third of the left ureter was catheterized with PE 50 tubing through a midline abdominal incision.

The femoral artery was cannulated with PE 50 tubing and connected to a mercury manometer for blood pressure measurement. The left jugular vein was cannulated with PE 50 tubing and an infusion of sodium chloride (80 mmol/l) was started at a rate of 0.02 ml/min. All wounds were sprayed with Rezifilm (E. R. Squibb and Sons, N.Y., U.S.A.) to prevent oozing and the rat was placed in a Lucite restraining device. The time for completion of all the preparatory procedures was approximately 90 min.

About 30 min after recovery from anaesthesia, a priming dose of 3 μCi of [14C]inulin and 5 μCi of p-amino[3H]hippurate was administered in 1 ml of sodium chloride (150 mmol/l) via the jugular cannula and a sustaining infusion containing 1.5 μCi of [14C]inulin and 5 μCi of p-amino[3H]hippurate/ml of sodium chloride (80 mmol/l) was started at a rate of 0.02 ml/min. Urine volume typically rose gradually after completion of the surgical procedure and tended to stabilize within 90–180 min. The experiments were initiated only after such stability had occurred. Urine was collected from each kidney separately under mineral oil in preweighed tubes. The urine from the left kidney drained directly into the tube via the ureteral catheter. The urine from the right kidney was collected via the bladder catheter and the bladder was massaged for the final 30 s of each collection period to facilitate complete emptying. At the midpoint of each urine collection period, an arterial blood sample was collected from the femoral artery cannula in a heparinized micro-haematocrit tube. The haematocrit was determined on each tube and the plasma was separated.

**Experimental stages**

After the 90–180 min period of equilibration, three to four 15 min collections of urine were obtained simultaneously from each kidney to establish the base-line (i.e. control) values for glomerular filtration rate, p-aminohippurate clearance and sodium excretion. Blood pressure was monitored throughout and the value at the midpoint of each period was recorded. At the completion of the control periods the infusate delivered into the left renal artery was changed to sodium chloride (150 mmol/l) containing 8.7 x 10⁻⁶ mol/ml (2 mg/ml) of diazoxide and the rate was continued at 0.02 ml/min. The average dose of diazoxide administered was 6.5 x 10⁻⁷ mol min⁻¹ kg⁻¹ (0.15 mg min⁻¹ kg⁻¹).

The infusion was continued for 45 min, during which three 15 min collections of urine were obtained from both kidneys. Blood pressure was monitored and recorded as during the control periods. The diazoxide infusion was then terminated. The infusion was changed back to sodium chloride at 150 mmol/l and a final 30 min clearance period was obtained.

Since it was necessary to raise the pH of the infusate to 9.5 to maintain diazoxide in solution, four control experiments were performed in which sodium chloride (150 mmol/l) titrated to pH 9.5 with sodium hydroxide was used as the infusate into the left renal artery during the experimental periods. The experi-
mental procedure was identical with that employed in the diazoxide experiments.

In three additional studies, the rats were maintained under light ether anaesthesia and after the introduction of the left renal artery catheter a guage 26 needle attached to a 5 cm length of PE 50 tubing was inserted into the left renal vein. Three samples of renal venous blood were obtained during the control period and three samples during the infusion of diazoxide. The blood was allowed to flow freely into a heparinized capillary tube, the collection being made simultaneously with the arterial blood sampling. Plasma was separated and analysed for radioactive p-aminohippurate.

For the determination of radioactive inulin and p-aminohippurate, 15 μl samples of plasma and 50 μl samples of urine were pipetted into counting vials containing 10 ml of Aquasol (New England Nuclear Corp.). The samples were counted in a Packard Tricarb Liquid Scintillation Counter. A minimum of 10 000 radioactivity counts was obtained for each sample. Sodium concentrations in serum and urine were analysed in a flame photometer with a lithium standard (Instrument Lab. Inc., Mass., U.S.A.). Experimental versus control data were compared separately for each kidney by paired t analysis and significance expressed as the 2P value.

**Results**

The surgical procedure and placement of the cannula in the left renal artery had no deleterious effects on renal function and inulin and p-aminohippurate clearances (C_{inulin} and C_{PAH}) were comparable in the infused (left) and non-infused (right) kidney (Table 1). C_{inulin} and C_{PAH} were stable in both kidneys and did not change significantly despite a fall in mean blood pressure of 11 mmHg from a mean value of 100 mmHg (2P < 0.001) (Fig. 1).

The infusion of diazoxide caused a significant increase of 0.76 ± 0.13 μmol/min in absolute sodium excretion (U_NaV) and 0.42 ± 0.09% in fractional sodium excretion (FENa) (Table 1 and Fig. 2). These changes were highly significant (2P < 0.001) and occurred only on the infused (left) side. On the non-infused (right) side no significant change in sodium excretion occurred through the experiment.

In the control experiments in which sodium chloride (150 mmol/l) at pH 9.5 was infused for 45 min no significant change occurred in C_{inulin}, C_{PAH}, U_NaV and FENa, and the blood pressure remained stable (Table 1). Furthermore, the baseline values of these variables were comparable with those in the experimental group.

In three experiments extraction of p-amino-

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**Table 1. Effects of infusion of (a) diazoxide and (b) sodium chloride solution (pH 9.5) into the left renal artery of conscious rats, on inulin and p-aminohippurate clearances, absolute sodium excretion (U_NaV) and fractional sodium excretion (FENa)**

<table>
<thead>
<tr>
<th></th>
<th>C_{inulin} (ml/min)</th>
<th>C_{PAH} (ml/min)</th>
<th>U_NaV (μmol/min)</th>
<th>FENa (%)</th>
<th>Blood pressure (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Left</td>
<td>Right</td>
<td>Left</td>
<td>Right</td>
<td>Left</td>
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<tr>
<td>(a) Diazoxide infusion (n = 10)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean control</td>
<td>1.30</td>
<td>1.20</td>
<td>2.80</td>
<td>2.59</td>
<td>0.62</td>
</tr>
<tr>
<td>± SE</td>
<td>±0.09</td>
<td>±0.08</td>
<td>±0.30</td>
<td>±0.33</td>
<td>±0.24</td>
</tr>
<tr>
<td>Mean Δ</td>
<td>+0.05</td>
<td>−0.07</td>
<td>−0.17</td>
<td>−0.27</td>
<td>+0.76</td>
</tr>
<tr>
<td>± SE</td>
<td>±0.08</td>
<td>±0.07</td>
<td>±0.25</td>
<td>±0.18</td>
<td>±0.13</td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>&lt;0.001</td>
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<tr>
<td>(b) Sodium chloride (150 mmol/l) infusion, pH 9.5 (n = 4)</td>
<td></td>
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<tr>
<td>Mean control</td>
<td>1.21</td>
<td>1.13</td>
<td>2.60</td>
<td>2.07</td>
<td>0.83</td>
</tr>
<tr>
<td>± SE</td>
<td>±0.04</td>
<td>±0.02</td>
<td>±0.10</td>
<td>±0.33</td>
<td>±0.30</td>
</tr>
<tr>
<td>Mean Δ</td>
<td>−0.02</td>
<td>+0.30</td>
<td>−0.30</td>
<td>+0.03</td>
<td>+0.04</td>
</tr>
<tr>
<td>± SE</td>
<td>±0.10</td>
<td>±0.29</td>
<td>±0.19</td>
<td>±0.13</td>
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<tr>
<td>P</td>
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hippurate ($E_{PAH}$) was calculated from the concentration of $p$-amino[3H]hippurate in systemic arterial ($A$) and renal venous blood ($V$) by using the formula $A - V/A \times 100$. During the control period $E_{PAH}$ in the three experiments was 61%, 58% and 76%; during the infusion of diazoxide the respective values were 47%, 45% and 61%. Thus the mean $E_{PAH}$ value during diazoxide infusion was reduced to 79% of control values. The low control values probably reflect retrograde contamination of renal venous blood with $p$-aminohippurate from the inferior vena cava or left ovarian vein, no attempt having been made to tie off these vessels distal to the site of collection.

**Discussion**

These studies demonstrate that diazoxide, when administered in moderate doses directly into the renal artery, causes a small, but significant, increase in sodium excretion. In no experiment was there a diminution in sodium excretion on the side of infusion. In the only previous study of infusion into the renal artery Greene (1967) found that diazoxide caused a significant natriuresis on the side of infusion in the dog, but in all cases this was associated with an increase in glomerular filtration rate (mean increase of 20.1% in ten experiments), and an unchanged systemic blood pressure. The natriuresis observed by Greene (1967) could thus be accounted for entirely by an increase in filtered load of sodium and an effect on tubular reabsorption is difficult to evaluate.
In the present studies glomerular filtration rate actually decreased minimally on the infused side in 50% of the experiments and systemic blood pressure fell by a mean value of 11 mmHg. Despite these potential antinatriuretic influences, absolute and fractional sodium excretion increased on the side of infusion indicating an inhibitory effect of diazoxide on tubular reabsorption of sodium.

In the light of these results it is evident that the sodium-retaining effect of diazoxide as witnessed in clinical situations is probably an indirect one secondary to peripheral circulatory changes sensed by the volume control system responding as though to extracellular volume contraction.

The mechanism for the natriuresis observed in the present studies is not clear. It is possible that in common with other benzothiadiazine compounds the drug exerts its effects by direct inhibition of tubular sodium reabsorption. However, there are no studies available which would support this mechanism in the case of diazoxide. It seems equally possible that with direct infusion of the drug into the renal artery, the vasodilator action (Rubin, Roth, Taylor & Rosenkilde, 1962; Nayler, McInnes, Swann, Race, Carson & Lowe, 1968) was responsible for the natriuresis via an effect on the renal vasculature. With the slow infusion rates employed in these experiments the amount of diazoxide escaping into and recirculating in the systemic circulation was presumably minimal; furthermore, avid protein binding (Sellers & Koch-Weser, 1970) would further have reduced its pharmacological activity on the systemic circulation. The effects on total peripheral resistance were thus small (mean blood pressure fall of 11 mmHg) and did not counteract the direct natriuretic effect on the kidney.

It is possible therefore that diazoxide has an effect similar to other vasodilator agents such as acetylcholine, bradykinin, prostaglandin E₂ and dopamine, which have been shown to cause an increase in urine flow and electrolyte excretion without alterations in glomerular filtration rate (Baer, Navar & Guyton, 1970; Cohen & Carter, 1967; Harvey, 1966). The mechanism of natriuresis observed with these agents is not necessarily uniform and may be due to a direct tubular action (Stein, Congbalay, Karsh, Osgood & Ferris, 1972), to an intrarenal redistribution of blood flow (Stein, Ferris, Huprich, Smith & Osgood, 1971) or to an alteration in net Starling forces in the peritubular circulation (Carriere, Friborg & Guay, 1971).

A decrease in E_PAH was observed in three experiments in the present study; a similar finding has been documented by Rubin et al. (1962), who suggested that diazoxide competitively inhibits tubular transport of p-aminohippurate. This phenomenon has, however, been observed with other vasodilator agents (Carriere et al., 1971). C_PAH may have thus significantly underestimated a real increase in renal plasma flow during diazoxide infusion but since E_PAH was not measured in the contralateral kidney the relationship to renal blood flow is inconclusive and the mechanism of natriuresis must remain speculative.

The results of the present studies demonstrate that the sodium-retaining effect of oral or parenteral diazoxide administration observed in man and experimental animals is not related to a direct renal action of the drug and suggest that, in clinical situations, the peripheral haemodynamic effects over-ride the direct natriuretic action of diazoxide on the kidney.

Acknowledgment

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