DIURETIC ACTION OF METOLAZONE IN DOGS

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Accepted August 28, 1978

Abstract—Metolazone, the sulfonamide diuretic was investigated to determine the sites of action. We used a radioactive microsphere, clearance and stop-flow method in anesthetized dogs. Urine flow and urinary excretion of sodium and potassium were increased at 5-60 min when metolazone was given intravenously at doses of 0.2-5.0 mg/kg, while total renal blood flow, distribution of cortical renal blood flow and GFR did not change. The urinary excretion rate of sodium to potassium (Na/K) increased from 5.69±0.82 to 8.07±0.76 in a dose of 1.0 mg/kg, i.v. Osmolar clearance and free water reabsorption increased almost proportionally, indicating that metolazone has little effect on the medullary portion of the ascending limb of Henle and may have a proximal site of action. In stop-flow experiments, a significantly raised U/PNa/U/Pretatinine was observed at the dip situated distally to the ascending limb of Henle. These findings indicate that the diuretic action of metolazone may be due to the inhibition of sodium reabsorption in the distal nephron segments, in addition to the absence of modification of the cortical regional blood flow.

Metolazone, (2-methyl-3-(O-tolyl)-6-sulfamyl-7-chloro-1,2,3,4-tetrahydro-4-quinazolin) is a natriuretic agent structurally similar to benzothiadiazine (Fig. 1). In comparison with benzothiadiazine metolazone induces a relatively more potent natriuresis accompanied by a minimal kaliuresis. The diuretic mechanism of the drug has been widely studied in the rat, dog and humans (1-4). It is generally considered that the diuretic action of metolazone is the result of inhibition of sodium reabsorption along with chloride in the cortical diluting segment of the ascending limb of Henle. Such conclusions followed clearance and micropuncture studies (5-8).

As another aspect of diuretic mechanism, the redistribution of cortical blood flow is an important factor, because different types of nephrons, morphologically and functionally, are located in the cortex (9). Since their GFR and sodium reabsorption rate differ considerably, an alteration in the ratio of blood supply to these nephrons could result in a marked change in renal function (10, 11). The change of intrarenal distribution of blood flow may contribute to the diuresis induced by bumetanide (12) and furosemide (13).

The present study was undertaken to determine the sites of action of metolazone using stop-flow and clearance methods. Since it is unknown whether or not metolazone affects the cortical regional blood flow, this effect was measured by the radioactive

FIG. 1. Chemical structure of metolazone.
microsphere method.

MATERIALS AND METHODS

The experiments were performed on mongrel dogs of both sexes weighing 11-15 kg. The animals were kept on a standard diet with free access to water, and were anesthetized with pentobarbital sodium (30-35 mg/kg, i.v.). Experiments were grouped into three:

A) Clearance studies

The left kidney was exposed through a retroperitoneal flank incision and the renal nerves were cut surgically as previously described (14). Renal blood flow (RBF) was measured by an electromagnetic flowmeter (Nihon Kohden MF-25) and systemic blood pressure (BP) was measured by a pressure transducer (Nihon Kohden MP-4T) connected to a catheter inserted into the abdominal aorta via the right femoral artery. Arterial blood samples were collected from the left brachial artery. A polyethylene tube was inserted into the left ureter for timed urine collections. A priming dose of creatinine (100 mg/kg) was given i.v., followed by a maintenance dose (50 mg/kg, hr.). Saline was infused into the left brachial vein at a rate of 3.5 ml/min throughout the experiment. In 17 dogs, following 1 hr equilibration period, urine was collected at 3 consecutive 10-min periods as a control renal clearance. At the midpoint of each period, systemic arterial blood samples were obtained for determinations of creatinine and electrolytes. After the 3rd control period, metolazone dissolved with 0.2 N-NaOH was administered by slow i.v. infusion at doses of 0.2, 1.0 or 5.0 mg/kg. Urine was collected at four 5-min, one 10-min and two 15-min periods during 60 min after metolazone injection, and at the midpoint of each period, arterial blood samples were withdrawn.

B) Stop-flow studies

Experiments were performed in 6 dogs. After completion of the operation similar to the experiment A), a priming dose of sodium para-aminobipyrurate (PAH) (20 mg/kg, i.v.) was given and was followed by an infusion of maintenance solution in 6 dogs. This solution consisted of 9 g of NaCl, 150 g of mannitol, 1.7 g of creatinine and 0.5 g of PAH per 1 liter, and was infused into the left brachial vein at a rate of 6 ml/min to increase urine flow and to maintain the plasma concentrations of the above substances. When urine flow rate was stabilized, 3 free flow clearance periods were obtained. The ureteral catheter was then clamped for 6 min and inulin (10 mg/kg, i.v.) was administered just before releasing the clamp. Upon release of occlusion, 0.5 ml of urine, 35 specimens, were collected serially. In 3 dogs, 30 min after, metolazone (5.0 mg/kg) was administered i.v. and the same procedure was repeated to estimate influence of the drug on the stop-flow pattern. In 3 other dogs, the same procedure was repeated without metolazone 30 min after.

C) Intrarenal hemodynamics studies

Intrarenal blood distribution was studied by the radioactive microsphere method previously reported (14). Microsphere injections were performed at control and 15 min after the injection of metolazone (1.0 mg/kg, i.v.). Four cortex zones, equal thickness from the surface to the juxtamedural area, were analyzed for individual isotope counts, and
The perfusion rate of each zone was calculated (15). The cortex zones were numbered sequentially downward from the surface.

GFR was calculated by creatinine clearance. Creatinine and PAH were determined by colorimetry. Sodium and potassium were measured by flame photometry (Hitachi 205D) and chloride was analyzed with a digital chloridemeter (Buchler). Osmolarity was measured with an osmometer (Fiske).

All results are expressed as the mean ± standard error (S.E.). Statistical analysis was performed using Student’s paired t-test.

RESULTS

A) Clearance studies

Effects of metolazone given in doses of 0.2, 1.0 and 5.0 mg/kg, i.v. were studied. The volume of the vehicle varied according to doses, and a maximal volume, 5 ml, decreased BP and RBF transiently after rapid injection. However it had no significant effect on BP, RBF and urine flow when it was slowly infused (Fig. 2).

Intravenous administration of metolazone in doses of 0.2 and 1.0 mg/kg had no effect on BP, RBF and GFR (Fig. 3, Table 1). Even when the dose was increased to 5.0 mg/kg, no significant change was observed in those parameters (Tables 1, 2). A marked increase in urine flow was observed within a few minutes, the maximum effect being achieved within 5 min and this effect was well maintained over 60 min after the injection regardless of the doses. Metolazone in a dose of 1.0 mg/kg increased in urine flow from 15.7±3.7 to 38.4±4.3 μl/g·min at 5 min and 36.6±3.8 μl/g·min at 60 min (Fig. 3). A similar pattern in diuretic response was seen with a dose of 0.5 mg/kg, and approx. a 5-fold increase of urine flow was observed (Tables 1, 2).

Urinary excretion of sodium and chloride was in fairly good parallel with urine flow. At a dose of 1.0 mg/kg, urinary excretion of sodium and chloride increased from 3.52±0.88 and 3.59±1.90 to 8.82±0.87, 8.57±2.17 μEq/g·min at 5 min and 9.43±1.32, 8.60±1.22 μEq/g·min at 60 min (Fig. 3).
Fig. 3. (A) Effects of i.v. administration of metolazone (1.0 mg/kg) on systemic blood pressure, renal blood flow, glomerular filtration rate and urine flow. (B) Effects of i.v. administration of metolazone (1.0 mg/kg) on urinary excretion of Na, K and Cl and the abstraction of solute free water ($T_{\text{H,O}}$).

$\mu$Eq/g-min at 60 min, respectively, while no significant changes were observed in the concentrations of plasma sodium, potassium and chloride (Table 3). Potassium excretion was less than sodium, therefore the calculated ratio of sodium to potassium (Na/K) in urine was increased significantly with each dose of metolazone (Tables 1, 2. Fig. 3).

Along with the increase of urinary excretion of electrolytes, $C_{\text{osm}}$ increased from $38.9 \pm 6.9$ to $79.3 \pm 5.1 \mu$Eq/g-min at 5 min and to $72.6 \pm 4.3 \mu$Eq/g-min at 60 min, and $T_{\text{C_H,O}}$ also increased from $23.4 \pm 3.7$ to $40.8 \pm 1.9$ and $36.0 \pm 2.6 \mu$l/g-min, respectively. In Fig. 4, a relationship between $T_{\text{C_H,O}}$ and $C_{\text{osm}}$ was seen during control period and after metolazone. Positive correlations between $T_{\text{C_H,O}}$ and $C_{\text{osm}}$ were obtained both in control and experimental periods, and there was no significant difference.
### Table 1. Effects of metolazone on renal hemodynamics and urine formation (5 mg/kg, i.v.)

| Time (min) | BP (mmHg) | RBF (ml/min) | GFR (ml/min) | UV (μl/min) | U\(_{Na}V\) (μEq/min) | U\(_{K}V\) (μl/min) | Na/K | C\(_{osm}\) (μl/min) | T\(_{H\_2O}\) (μl/min) |
|------------|-----------|--------------|--------------|-------------|------------------------|-------------------|------|---------------------|---------------------|
| Control    |           |              |              |             |                        |                   |      |                     |                     |
| 134 ± 11   | 134 ± 11  | 3.49 ± 0.60  | 0.90 ± 0.15  | 5.7 ± 0.8   | 1.04 ± 0.34            | 0.27 ± 0.04       | 4.15 | 20.1 ± 3.2          | 14.3 ± 3.2          |
| 137 ± 12   | 3.62 ± 0.52| 0.93 ± 0.16  | 6.1 ± 1.1    | 1.13 ± 0.36 | 0.85 ± 0.04            | 0.29 ± 0.04       | 3.97 | 20.5 ± 3.3          | 14.4 ± 3.0          |
| Metolazone | 5 mg/kg i.v.| 135 ± 11    | 3.72 ± 0.50  | 1.01 ± 0.24  | 31.3 ± 2.4             | 6.83 ± 0.85       | 0.85 | 7.67 ± 0.91         | 65.3 ± 7.7          |
| 5          | 136 ± 12  | 3.77 ± 0.50  | 0.78 ± 0.09  | 31.3 ± 2.8   | 6.78 ± 0.67            | 0.88 ± 0.16       | 8.19 | 62.1 ± 5.3          | 30.9 ± 5.7          |
| 10         |            |              |              |             |                        |                   |      |                     |                     |
| 20         | 134 ± 12  | 3.68 ± 0.43  | 0.80 ± 0.11  | 31.0 ± 2.9   | 6.88 ± 0.85            | 0.83 ± 0.13       | 8.62 | 61.8 ± 6.1          | 30.8 ± 5.3          |
| 30         | 134 ± 11  | 3.63 ± 0.26  | 0.78 ± 0.09  | 28.7 ± 3.2   | 6.03 ± 0.86            | 0.70 ± 0.13       | 9.72 | 58.0 ± 5.9          | 39.9 ± 4.2          |
| 45         | 132 ± 12  | 3.75 ± 0.28  | 0.85 ± 0.13  | 29.0 ± 3.1   | 6.85 ± 0.82            | 0.81 ± 0.18       | 9.09 | 60.3 ± 5.9          | 34.4 ± 2.1          |
| 60         | 132 ± 12  | 3.62 ± 0.39  | 0.85 ± 0.12  | 26.3 ± 2.7   | 6.32 ± 0.71            | 0.75 ± 0.19       | 9.43 | 55.1 ± 5.7          | 38.6 ± 3.9          |

All data are mean ± S.E. (N = 5). Abbreviations: BP = Systemic arterial pressure; UV = Urine flow; U\(_{Na}V\), U\(_{K}V\) = Urinary excretion of sodium and potassium; Na/K = Ratio of urinary concentration of sodium to potassium.
| Time (min) | BP (mmHg) | RBF (ml/g min) | GFR ($\mu$g/min) | UV ($\gamma$/g min) | $U_{Na}V$ ($\mu$Eq/g min) | $U_{K}V$ ($\mu$Eq/g min) | Na/K | $U_{Cl}V$ ($\gamma$/g min) | $C_{Cl}$ ($\mu$g/min) | $T^{V}_{H_{2}O}$ (ml/g) |
|-----------|-----------|----------------|------------------|-------------------|-----------------------------|--------------------------|-------|-----------------------------|-------------------------|------------------------|
| Control   |           |                |                  |                   |                             |                          |       |                             |                         |                        |
| 0         | 129       | 2.66           | 0.92             | 13.6              | 2.95                       | 0.35                     | 8.63  | 2.30                        | 31.5                    | 17.5                   |
| ± 10      | ± 0.32    | ± 0.07         | ± 2.7            | ± 0.69            | ± 0.07                     | ± 1.20                   | ± 0.33 | ± 5.7                      | ± 4.4                   |                        |
| 131       | 13        | 2.78           | 0.98             | 14.8              | 3.27                       | 0.36                     | 8.94  | 2.64                        | 31.8                    | 18.7                   |
| ± 13      | ± 0.20    | ± 0.12         | ± 2.9            | ± 0.79            | ± 0.04                     | ± 1.38                   | ± 0.47 | ± 7.5                      | ± 4.7                   |                        |
| Metolazone| 5         | 129            | 2.89             | 0.89              | 34.3                       | 7.73                     | 0.68  | 11.37                       | 7.51                    | 67.9                   |
| ± 13      | ± 0.12    | ± 0.06         | 8.5              | ± 1.43            | ± 0.03                     | ± 1.78                   | ± 0.45 | ± 9.9                      | ± 6.4                   |                        |
| 10        | 129       | 2.92           | 0.88             | 34.1              | 7.85                       | 0.67                     | 11.79 | 7.59                        | 66.3                    | 32.2                   |
| ± 12      | ± 0.14    | ± 0.07         | ± 3.9            | ± 1.19            | ± 0.02                     | ± 2.20                   | ± 1.13 | ± 9.9                      | ± 6.4                   |                        |
| 20        | 129       | 3.02           | 0.84             | 33.8              | 7.92                       | 0.68                     | 11.89 | 7.78                        | 67.2                    | 33.4                   |
| ± 13      | ± 0.09    | ± 0.04         | ± 3.2            | ± 1.15            | ± 0.07                     | ± 1.95                   | ± 1.10 | ± 10.1                     | ± 7.2                   |                        |
| 30        | 127       | 3.12           | 0.88             | 34.6              | 8.11                       | 0.71                     | 11.86 | 8.09                        | 69.6                    | 35.0                   |
| ± 14      | ± 0.08    | ± 0.04         | ± 3.0            | ± 1.11            | ± 0.12                     | ± 2.38                   | ± 1.17 | ± 10.2                     | ± 7.4                   |                        |
| 45        | 126       | 3.10           | 0.88             | 33.5              | 7.87                       | 0.77                     | 11.29 | 7.85                        | 67.9                    | 34.4                   |
| ± 11      | ± 0.07    | ± 0.05         | ± 2.4            | ± 0.91            | ± 0.18                     | ± 2.60                   | ± 0.90 | ± 8.5                      | ± 6.5                   |                        |
| 60        | 124       | 2.91           | 0.89             | 32.0              | 7.57                       | 0.81                     | 10.54 | 7.52                        | 65.4                    | 33.4                   |
| ± 12      | ± 0.03    | ± 0.04         | ± 2.8            | ± 0.90            | ± 0.20                     | ± 2.65                   | ± 0.74 | ± 8.0                      | ± 6.1                   |                        |

All data are mean ± S.E. (N=3). Abbreviations: BP = Systemic arterial pressure, UV = Urine flow, $U_{Na}V$, $U_{K}V$, $U_{Cl}V$ = Urinary excretion of sodium, potassium and chloride; Na/K = Ratio of urinary concentration of sodium to potassium.
TABLE 3. Effect of metolazone (1.0 mg/kg, i.v.) on plasma electrolytes concentration

| Time (min) | Plasma concentration | mEq/L |
|------------|----------------------|-------|
|            | Na (n=9)             | K (n=9) | Cl (n=4) |
| Control    | 146±1.4              | 3.38±0.12 | 118±1.34 |
|            | 147±1.2              | 3.38±0.12 | 116±1.41 |
| Metolazone | 10 146±1.8           | 3.24±0.06 | 117±1.33 |
|            | 30 146±1.4           | 3.27±0.12 | 116±1.51 |
|            | 60 145±1.4           | 3.26±0.13 | 118±1.07 |

All data are mean ± S.E.

Fig. 4. Relationship between the abstraction of solute free water and the osmolar clearance during control (open circle; O) and metolazone administration.

B) Stop-flow studies

A typical stop-flow experiment of 3 dogs is illustrated in Fig. 5. Metolazone did not affect the excretion pattern of PAH. In an analysis of the stop-flow pattern, the portion showing the highest PAH concentration was used as an indication of the proximal tubule, and the lowest sodium concentration as that of the distal tubule. The first appearance of inulin indicates the entry of new glomerular filtrate from the short nephrons. The specimens of which U/PNa/U/Pcreatinine showed a minimal value in the control experiments, so-called 'distal dip', contained urine mainly from the distal nephron. The administration of metolazone in a dose of 5.0 mg/kg resulted in a marked raise of U/PNa/U/Pcreatinine in each specimen, especially at the distal dip. This result indicates that metolazone inhibits sodium transport at the distal nephron. Further distally, a marked elevation of U/PK/U/Pcreatinine indicated that the secretion of potassium was stimulated in this segment. In control experiments, sequential stop-flow patterns in any animal were found to be reproducible. (Data not shown)

C) Intrarenal hemodynamics studies

Total renal blood flow did not change during metolazone administration. The flow rate of each zone differed significantly from that of the other three at the control period,
FIG. 5. Effect of i.v. administration of metolazone (5.0 mg/kg) on the stop-flow patterns. Solid line: control, dotted line: metolazone.

FIG. 6. Effects of metolazone (1.0 mg/kg) on renal blood flow (left panel) and on the percent distribution of the cortical blood flow.

i.e., the zonal flow rates were 3.43±0.56, 4.69±0.62, 3.62±0.47 and 2.75±0.62 ml/g·min in cortex zones 1, 2, 3 and 4, respectively. The zonal distribution and flow rate in each zone were not significantly affected by metolazone (Fig. 6).

DISCUSSION

A single intravenous administration of metolazone produced a diuretic effect within 5 min in dogs, and this effect was prolonged over the experimental period of 60 min. Urinary excretion of sodium, potassium and chloride increased without any significant change in BP, RBF and GFR. Although there was an increase in potassium excretion, this increase was relatively smaller than that of sodium. The urinary excretion rate of sodium to potassium (Na/K) increased significantly after metolazone. Plasma sodium and potassium levels were not altered throughout the experiment (Table 3).

In general, loop diuretics such as furosemide, ethacrynic acid and bumetanide produce a diuretic effect which is accompanied by an increase in RBF and a redistribution of intrarenal blood flow, in addition to their tubular actions (13–15). In contrast to loop diuretics,
metolazone had no effect on the total RBF nor the intrarenal distribution of blood flow. This result suggests that the medullary blood flow, which was considered to be postglomerular flow of juxtamedullary nephron, may not be affected by metolazone. These data indicate that an inhibition of sodium reabsorption in the tubules is probably the main mechanism of diuretic action of metolazone. As indicated in Fig. 4, metolazone did not affect the linear relationship between TC,H,O and C-osm obtained in control experiments. This result suggested that the drug did not inhibit sodium transport at the medullary portion of the ascending limb of Henle, as reported by Goldberg et al. (16). It is possible that, had the drug inhibited sodium transport in this portion, the generation of solute free water might have been remarkably depressed by the diminished hyperosmolality of medullary interstitium. In fact, loop diuretics, furosemide and bumetanide were reported to show a decrease of TC,O,H and an increase of C-osm (15, 17, 18). After metolazone injection, TC,H,O increased proportionally to the urine flow and the urinary excretion of sodium, indicating that metolazone may act on the proximal tubules or the distal nephron segment, or both. A drug that acts on the proximal tubules would augment the generation of solute free water with an increasing delivery of NaCl to the ascending limb of Henle.

The reabsorption of most of the filtered phosphate occurs proximally (19, 20). Steinmuller and Puschett (7) reported that metolazone inhibited a sodium transport mechanism linked to phosphate transport in the proximal tubule since they observed that metolazone induced a phosphaturia in man. Fernandez and Puschett (6) also reported inhibition of the sodium reabsorption at the proximal tubule without any change in the reabsorption of phosphate. Metolazone reportedly exhibits little carbonic anhydrase inhibitory activity (2). It was suggested that TC,H,O depends upon the sodium reabsorption of the ascending limb of Henle and that the segment has a large capacity to reabsorb the increased delivery, if uninhibited by a diuretic drug (17, 18). Thus, the proportional increase in C-osm and TC,H,O observed in the present study indicates that the increased delivery is probably due to an inhibition of the more proximal sites of nephron by metolazone.

Despite certain limitations with the stop-flow method (21), transport processes in the distal nephron can be demonstrated fairly accurately. In the present study, the stop-flow method was used to evaluate the effect of metolazone on sodium reabsorption in the distal nephron and to study its effect on the transport of potassium in this tubular site. Inspection of the stop-flow patterns revealed that the minimum ratio of sodium U/P to Creatinine U/P achieved in the distal nephron segment was quite evident after the administration of metolazone. This change in the above ratio in the distal dip may be the result of a depression of the distal tubular reabsorptive capacity (22). Sullivan and Pirch reported the ability of the thiazide diuretics to increase potassium secretion and discussed the potassium stop-flow pattern (23). In the present experiment, the peak of potassium; that is the increment of U/PK/U/Pcreatining is observed in the distal nephron, and our results indicate that metolazone, at least, does not inhibit potassium exchange system in this portion.

In conclusion, metolazone is a moderate diuretic agent which has a rapid onset and long action after a single i.v. administration with no significant changes in BP and RBF.
This compound has no effect on the intrarenal blood distribution. Although these observations do not rule out proximal action of metolazone, the distal nephron segment is probably the main site of action.

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