Comparison of the effect of two groups of diuretics on renin secretion in the anaesthetized dog

J. L. IMBS, M. SCHMIDT, J. VELLY AND J. SCHWARTZ

Institut de Pharmacologie et de Médecine Expérimentale, Faculté de Médecine, Strasbourg, France

(Received 27 January 1976; accepted 21 September 1976)

Summary

1. The effects of two groups of diuretics on renin secretion have been compared in dogs anaesthetized with pentobarbital.

2. Furosemide, ethacrynic acid and bumetanide cause an immediate rise in renin secretion which is not inhibited either by DL-propranolol or by a bilateral ureterovenous anastomosis which prevents salt and water loss.

3. Clopamide, metolazone and indapamide do not cause an immediate rise in renin secretion. Renin hypersecretion is induced only 1 h after intravenous injection of these diuretics.

4. Renin secretion was studied for 6 h after frusemide injection: the immediate rise was followed by a later increase in renin secretion. This later rise was inhibited by propranolol and by ureterovenous anastomosis.

5. These results allow us to distinguish between a direct renal mechanism responsible for early renin hypersecretion, which appears to be connected with the action of the diuretic on the ascending limb of Henle's loop, and an indirect mechanism responsible for late renin hypersecretion, which appears to be connected with salt and water loss.

Key words: clopamide, indapamide, loop diuretics, metolazone, propranolol, renin secretion.

Abbreviations: PRA, plasma renin activity; AI, angiotensin I.

Résumé

1. Nous avons comparé chez le chien anesthésié (pentobarbital) les effets sur la sécrétion de rénine de deux groupes de diurétiques.

2. Dès la 5ème minute après leur injection i.v., le furosumide, l'acide éthacrylique et le buméتانide entraînent une hypersécrétion de rénine qui n'est inhibée ni par le DL-propranolol, ni par la suppression des pertes d'eau et de sels grâce à une anastomose uretéro-veineuse.

3. Le clopamide, la métolazone et l'indapamide sont incapables de provoquer une hypersécrétion de rénine immédiate. Ce n'est que 1 h après l'injection de ces diurétiques que nous observons une hypersécrétion de rénine.

4. La sécrétion de rénine a été étudiée pendant les 6 h suivant une injection i.v. de furosumide: l'hypersécrétion de rénine précoce est suivie d'une deuxième phase, tardive. Cette hypersécrétion tardive est inhibée par le DL-propranolol et n'apparaît pas en l'absence de pertes hydro-salines chez les chiens préparés par anastomose uretéro-veineuse.

5. Ces résultats permettent de distinguer deux mécanismes à l'hypersécrétion de rénine provoquée par les diurétiques: un mécanisme rénal direct, propre aux diurétiques de l'anse et responsable d'une hypersécrétion de rénine précoce, et un mécanisme indirect responsable d'une hypersécrétion de rénine plus tardive, inhibée par le DL-propranolol et secondaire aux pertes d'eau et de sels.

Introduction

The rise in renin secretion induced by diuretics
can be explained by different mechanisms. (a) Salt and water losses modify the volume and composition of the extracellular space and diminish the renal perfusion pressure, thus inducing renin hypersecretion (Vander, 1967; Davis, 1971). Obviously this mechanism is common to all diuretics, irrespective of the site of their action on the nephron. (b) Early renin hypersecretion induced by frusemide is connected with an intrarenal mechanism which is independent of variations in extracellular volume (Meyer, Menard, Papanicolaou, Alexandre, Devaux & Milliez, 1968; Vander & Carlson, 1969; Imbs, Velly, Spach & Schwartz, 1969; Hofbauer, Zhiedrich, Hackenthal & Gross, 1974). We have shown that clopamide is incapable of stimulating this intrarenal mechanism (Imbs, Desaulles, Velly, Bloch & Schwartz, 1972).

The aim of this work was to study these two mechanisms according to the mode of action of the diuretic used. Six diuretics were tested; three of them act essentially on the ascending limb of the loop of Henle. These eliminate the sodium corticopapillary gradient and suppress urinary concentration processes, whereas the other three have no effect on it. In a second series of experiments the time-sequence of these two mechanisms after frusemide injection was investigated.

Materials and methods

General procedure

Thirty-seven mongrel dogs were used after starvation for 18 h, during which time they had free access to water. They were anaesthetized with pentobarbital (mean induction dose 23 ± 5 mg/kg intravenously, followed by maintenance doses; mean total dose 36 ± 1.5 mg/kg). After tracheal intubation, the animals were placed on a heating table.

The left kidney, reached by an incision into the lumbar region, was isolated from the adjacent organs. The nerves, lymphatics, collateral blood vessels and perivascular sheath were coagulated and sectioned. The ureter was catheterized and sectioned. The kidney was thus denervated and its lymphatic drainage suppressed. The sole efferent vessel was the renal vein. An electromagnetic flowmeter probe was placed on the renal artery and polyethylene catheters used for taking blood samples were inserted in the left renal vein through the previously ligated ovarian or spermatic vein, and in the abdominal aorta through a femoral artery. At the end of the experiment the left kidney was removed and weighed (average weight 60 ± 3 g).

Drugs

The sodium salts of frusemide (Hoechst), ethacrynic acid (Merck Sharp and Dohme) and bumetanide (Leo) were dissolved in distilled water brought to pH 9 (final pH) by solution of NaOH (1 mol/l). Clopamide (Sandoz), indapamide (Servier) and metolazone (Pennwalt) were dissolved in 50 ml of aqueous polyethylene glycol 400 (300 g/l) buffered to pH 7.4. DL-Propranolol hydrochloride (ICI) was dissolved in aqueous NaCl solution (150 mmol/l). The diuretics were injected intravenously over a period of 1 min through a polyethylene catheter inserted into a saphenous vein.

Experimental protocol

Short protocol (1 h). Renin secretion was measured after injection of the diuretics at the following doses: frusemide 15.1 μmol/kg (5 mg/kg), ethacrynic acid 16.5 μmol/kg (5 mg/kg), bumetanide 0.7 μmol/kg (250 μg/kg), clopamide 14.4 μmol/kg (5 mg/kg), indapamide 13.8 μmol/kg (5 mg/kg) and metolazone 13.7 μmol/kg (5 mg/kg). Eighteen dogs were divided in six groups of three and one of the six diuretics was administered to each group. In addition, four dogs served as control animals. Two of these were given 10 ml of aqueous NaOH (10 μmol/l, pH 9), the third was given 50 ml of aqueous buffered polyethylene glycol 400 (300 g/l, pH 7.4) and the fourth 0.7 μmol (250 μg) of bumetanide/kg dissolved in 50 ml of aqueous buffered polyethylene glycol 400 (pH 7.4). The purpose of these control experiments was to make sure that none of the solvents used affected the changes in renin secretion caused by the diuretics.

One minute before, and 5, 15, 30 and 60 min after injection of the tested substances, 6 ml of renal venous blood and 6 ml of arterial blood were taken simultaneously to determine the plasma renin activity. A further 5 ml of arterial blood was taken at the above times to determine the packed cell volume.

Urine from the left kidney was collected
under paraffin oil in fractions corresponding to the following five periods: (1) from minute 30 before injection of the diuretic to 1.5 min after; (2) from 1-5 min after injection to minute 5; (3) from minute 6 to minute 15; (4) from minute 16 to minute 30; (5) from minute 31 to minute 60.

Long protocol (6 h). Renin was measured in fifteen dogs during the 6 h after frusemide injection.

After induction of the anaesthetic, the dogs were given 30 ml/kg of aqueous NaCl solution (0.05 mol/l) by the gastric route. The blood samples for the measurement of renin secretion were taken immediately before the injection of frusemide or its solvent, 15 min after injection and then every hour for the following 6 h. Plasma potassium concentration and arterial packed cell volume were determined at the same times. The urine was collected in seven fractions: the first fraction during the 30 min preceding the frusemide injection, and the remaining six at hourly intervals after the injection.

Four dogs were given 60.6 µmol of frusemide/kg (20 mg/kg).

Five dogs received the same dose of frusemide during a propranolol infusion into the left renal artery. A priming dose of 6.8 µmol of propranolol/kg (2 mg/kg) was infused in the course of 30 min; this dose suppressed the renal vasodilation induced by intrarenal injection of 101.2 µmol of isoprenaline/kg (0.25 µg/kg). The frusemide was then injected and the propranolol perfused at a maintenance dose of 9.5 µmol 6 h⁻¹ kg⁻¹ (2.8 mg 6 h⁻¹ kg⁻¹).

Four dogs were given 60-6 µmol of frusemide/kg (20 mg/kg) after the two ureters had been anastomosed to the right iliac vein. Urine from the left kidney was collected for 1 min only from the ureterovenous anastomosis, immediately after injection of the diuretic, and then each hour after this injection. Urinary salt and water losses were thus prevented. The renin secretion was measured according to the above time-schedule.

Two dogs, used as controls, were given an injection of 10 ml of the solvent used to administer the frusemide.

**Haemodynamic measurements and analytic procedures**

Instantaneous and mean renal blood flow were measured by electromagnetic flowmeter (Nycotron). Blood pressure was measured in the abdominal aorta (Statham P23 Db). These variables were continuously recorded (Cardiopan 6). Volume and concentration of Na⁺, K⁺ (flame photometry) and Cl⁻ (mercurimetry in the presence of diphenyl carbazone) and osmolality (Fiske 230 osmometer) were measured in each urine sample.

Plasma renin activity was determined by radioimmunoassay (Haber, Koerner, Page, Kliman & Purnode, 1969) and expressed as ng of angiotensin I released by 1 ml of plasma in 1 h of incubation. The renin secretion rate was calculated as (renal venous PRA—systemic arterial PRA) × renal plasma flow (ml min⁻¹ g⁻¹ of kidney) and expressed as ng of angiotensin I equivalent min⁻¹ g⁻¹ of kidney. We verified that each PRA determination was performed in the presence of excess of substrate. Standards (Asp¹-Ile⁴-angiotensin I; New England Nuclear) or samples were always run in duplicate. A standard curve was prepared for each experiment. Blood samples taken from the same animal were always processed in one series. Angiotensin generated before incubation was measured in each blood sample. Under our conditions, pre-formed angiotensin was 4.8±0.15% (n = 230) of the angiotensin measured at the end of the incubation period. Verification *in vitro* showed that the diuretics or their solvents, added to the incubation medium in concentrations similar to those found to exist *in vivo* after injection of the drugs, did not affect the formation of angiotensin I nor, consequently, the results of PRA measurements (E. Desaulles, unpublished observations).

**Statistical evaluation**

The numerical data were expressed as mean results ±SEM; P values ≥0.05 were considered non-significant. The correlation between two parameters was analysed by the Pearson correlation coefficient.

**Short protocol (1 h).** The differences between the six groups of three animals were tested by analysis of variance. The six diuretics were chosen *a priori* as the representatives of two groups of drugs. The following three partial hypotheses, two with two orthogonals, were thus tested: (a) equality of value obtained with
the three diuretics acting on the ascending limb of the loop of Henle; (b) equality of values obtained with the three diuretics which do not act on the ascending limb of the loop of Henle; (c) the existence of a difference between the values obtained for these two groups of three diuretics. This last partial hypothesis can be tested only if the two preceding hypotheses have not been rejected.

*Long protocol (6 h).* For each stage of the experiment the results were submitted to variance analysis; differences between the experimental groups were then localized with the Scheffé (1953) method.

### Results

**Short protocol (1 h)**

*Basal values* (Table 1). There is no significant difference between the mean values measured in each of the six groups of animals.

*Values measured 5 min after injection of diuretics* (Table 1). The diuretic effect became apparent 1.5 min after injection of each substance. It was more marked in the nine dogs which were given loop diuretics, but there was no significant difference between the values of urine flow, natriuresis and chloruresis, observed in the two groups during the first 5 min after injection of the six drugs (Fig. 2).

The injection of frusemide, ethacrynic acid or bumetanide causes an increase (identical for each of the three substances) of arterial and venous PRA and renin secretion. On the other hand, the injection of clopamide, indapamide or metolazone causes no change in renin secretion. There is thus a highly significant difference between the two groups of diuretics (Table 1): the loop diuretics cause early renin hypersecretion, whereas clopamide, indapamide and metolazone, with the same urinary salt and water losses, do not.

A more marked reduction in urinary osmolality was also noted after injection of the loop diuretics. An inverse correlation was noted between the value of renin secretion measured 5 min after injection of the six diuretics and the value of urinary osmolality \( r = -0.4571; P < 0.05, n = 18 \). There was no correlation between renin secretion and urinary excretion of sodium.

Injection of the six diuretics was followed by a significant decrease in renal blood flow of the same magnitude for each drug (Table 2). Five minutes after injection of the three loop diuretics

| Table 1. Effects of injection of the two groups of three diuretics |
|------------------|------------------|------------------|
| Mean values (±1 SEM) of observations before (control values, \( n = 18 \)) and 5 min (\( n = 3 \)) after injection of the diuretics are shown. \( P = \) levels of significance of differences between the values obtained for the two groups of three diuretics. NS = not significant. | Loop diuretics |  |
| Arterial PRA (ng of AI \( h^{-1} ml^{-1} \)) | Control | Frusemide | Ethacrynic acid | Bumetanide | \( P \) | Clopamide | Indapamide | Metolazone |
| 1.08 (0.24) | 4.11 (1.11) | 2.75 (1.48) | 2.83 (0.46) | < 0.01 | 0.61 (0.14) | 1.28 (0.51) | 0.76 (0.41) |
| Venous PRA (ng of AI \( h^{-1} ml^{-1} \)) | 1.39 (0.28) | 6.90 (1.84) | 4.82 (1.78) | 7.20 (1.08) | < 0.001 | 1.00 (0.12) | 1.50 (0.66) | 0.84 (0.37) |
| Urine osmolality (mosmol/kg) | 992 (96) | 342 (21) | 580 (92) | 492 (134) | < 0.01 | 934 (121) | 631 (135) | 600 (231) |
| Urine flow (ml/min) | 0.44 (0.09) | 3.88 (0.79) | 3.57 (1.33) | 2.69 (0.36) | NS | 1.97 (0.35) | 2.90 (0.56) | 2.55 (0.62) |
| Urine Na (\( \mu \)mol/min) | 50 (10) | 532 (88) | 537 (216) | 332 (100) | NS | 355 (36) | 483 (69) | 263 (97) |
| Renal blood flow (ml min\(^{-1}\) g\(^{-1}\)) | 3.4 (0.2) | 3.8 (0.3) | 3.3 (0.6) | 2.9 (0.2) | NS | 2.8 (0.5) | 2.8 (0.3) | 3.2 (0.3) |
| Mean arterial pressure (mmHg) | 123 (3) | 125 (5) | 122 (4) | 109 (5) | NS | 126 (6) | 124 (6) | 127 (7) |
Table 2. Renal blood flow 1 min before and 5, 15, 30 and 60 min after injection of the diuretics

Mean values (±1 SEM) are shown; n = 3 for results with each diuretic. *P<0.05; **P<0.01.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Frusemide</th>
<th>Ethacrynic acid</th>
<th>Bumetanide</th>
<th>Group mean value (n = 9)</th>
<th>Clopamide</th>
<th>Indapamide</th>
<th>Metolazone</th>
<th>Group mean value (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>−1</td>
<td>4.3 (0.7)</td>
<td>3.5 (0.6)</td>
<td>3.4 (0.1)</td>
<td>3.7 (0.5)</td>
<td>3.2 (0.8)</td>
<td>3.1 (0.8)</td>
<td>3.4 (0.5)</td>
<td>3.2 (0.8)</td>
</tr>
<tr>
<td>+5</td>
<td>3.8 (0.3)</td>
<td>3.3 (0.6)</td>
<td>2.9 (0.2)</td>
<td>3.3** (0.7)</td>
<td>2.8 (0.5)</td>
<td>2.8 (0.3)</td>
<td>3.2 (0.3)</td>
<td>2.9* (0.6)</td>
</tr>
<tr>
<td>+15</td>
<td>3.5 (0.6)</td>
<td>3.6 (0.3)</td>
<td>2.9 (0.3)</td>
<td>3.3* (0.7)</td>
<td>2.8 (0.5)</td>
<td>2.8 (0.5)</td>
<td>3.1 (0.5)</td>
<td>2.9* (0.5)</td>
</tr>
<tr>
<td>+30</td>
<td>3.3 (0.6)</td>
<td>3.5 (0.9)</td>
<td>2.8 (0.2)</td>
<td>3.2* (0.6)</td>
<td>2.9 (0.7)</td>
<td>3.1 (0.7)</td>
<td>3.2 (0.6)</td>
<td>3.1 (0.6)</td>
</tr>
<tr>
<td>+60</td>
<td>3.2 (0.5)</td>
<td>3.2 (0.9)</td>
<td>2.8 (0.2)</td>
<td>3.1* (0.7)</td>
<td>2.9 (0.8)</td>
<td>3.2 (0.8)</td>
<td>3.7 (0.3)</td>
<td>3.3 (0.7)</td>
</tr>
</tbody>
</table>

Table 3. Renin secretion 1 min before and 5, 15, 30 and 60 min after injection of the diuretics

Mean values (±1 SEM) are shown; n = 3. P = levels of significance of differences between the values obtained for the two groups of three diuretics. At minute 30, P could not be calculated because of a significant difference existing within the loop diuretics group between ethacrynic acid and the other two drugs.

AI = angiotensin I. NS = not significant.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Frusemide</th>
<th>Ethacrynic acid</th>
<th>Bumetanide</th>
<th>P</th>
<th>Clopamide</th>
<th>Indapamide</th>
<th>Metolazone</th>
</tr>
</thead>
<tbody>
<tr>
<td>−1</td>
<td>1.19 (0.63)</td>
<td>0.37 (0.21)</td>
<td>0.85 (0.39)</td>
<td>NS</td>
<td>0.39 (0.12)</td>
<td>0.88 (0.34)</td>
<td>0.26 (0.04)</td>
</tr>
<tr>
<td>+5</td>
<td>6.05 (2.54)</td>
<td>4.18 (1.37)</td>
<td>8.03 (2.87)</td>
<td>&lt;0.01</td>
<td>0.61 (0.06)</td>
<td>0.43 (0.39)</td>
<td>0.16 (0.14)</td>
</tr>
<tr>
<td>+15</td>
<td>3.16 (2.08)</td>
<td>6.81 (2.66)</td>
<td>5.62 (1.61)</td>
<td>&lt;0.01</td>
<td>0.32 (0.15)</td>
<td>0.48 (0.42)</td>
<td>0.10 (0.05)</td>
</tr>
<tr>
<td>+30</td>
<td>1.13 (0.58)</td>
<td>7.04 (2.79)</td>
<td>4.57 (0.91)</td>
<td>---</td>
<td>0.40 (0.15)</td>
<td>2.26 (1.02)</td>
<td>0.20 (0.11)</td>
</tr>
<tr>
<td>+60</td>
<td>2.48 (1.87)</td>
<td>4.12 (1.79)</td>
<td>3.45 (1.61)</td>
<td>&lt;0.05</td>
<td>0.61 (0.34)</td>
<td>1.43 (0.58)</td>
<td>0.75 (0.45)</td>
</tr>
</tbody>
</table>

no change was observed in the mean arterial pressure. After injection of clopamide, indapamide and metolazone we noted a short-term increase in blood pressure in five out of nine dogs (7 mmHg on average).

Effects in 1 h. During the hour after injection of frusemide, bumetanide and ethacrynic acid, the saluretic effect increased and became significantly greater than that after clopamide, indapamide and metolazone (Fig. 2). The characteristics of the effect of the loop diuretic became evident: a decrease in urinary osmolality to isotonic values in relation to plasma (Fig. 3) and high urinary output of chloride. Renin secretion (Table 3), like arterial and venous PRA (Fig. 1), continued to increase up to minute 30, after which it remained stable or decreased.

The diuretic effect of clopamide, indapamide and metolazone was characterized by a chlorur-
FIG. 1. Mean values (± SEM) of arterial plasma renin activity (stippled bars: \(aPRA\)) and venous plasma renin activity (open bars: \(vPRA\)) 1 min before and 5, 15, 30 and 60 min after injection of the diuretics (\(F =\) frusemide; \(E =\) ethacrynic acid; \(B =\) bumetanide; \(C =\) clopamide; \(I =\) indapamide; \(M =\) metolazone). \(AI =\) angiotensin I. The levels of significance given at the foot of the Figure refer to the comparison by analysis of variance of the PRA values obtained for the two groups of three diuretics. NS = not significant.

FIG. 2. Mean values (\(\mu\)mol/min) of urinary elimination of Na (continuous lines) and Cl (broken lines) after injection of diuretics. The periods of urine collection are indicated at the bottom of the Figure: from the time of injection to minute 5; from minute 6 to minute 15; from minute 16 to minute 30; from minute 31 to minute 60 after injection of the diuretics. For abbreviations see Fig. 1. The levels of significance (\(P\) values) given at the foot of the Figure refer to the comparison by analysis of variance of the values obtained for the two groups of three diuretics.
Diuretics and renin secretion

Osmolality

Flow

Time (min)

Fig. 3. Mean values of urine flow (broken lines) and urine osmolality (continuous lines) after injection of the diuretics. The periods of urine collection are indicated at the bottom of the Figure as explained in Fig. 2. For abbreviations see Fig. 1. The levels of significance (P values) given at the foot of the Figure refer to the comparison by analysis of variance of the values obtained for the two groups of three diuretics.

esis inferior to natriuresis (Fig. 2) and a urinary osmolality which remained hypertonic in relation to plasma (Fig. 3). Arterial or venous plasma renin activity developed homogeneously in the case of the three diuretics (Fig. 1). The renin secretion values remained low and the difference between these and the loop diuretics remained significant up to minute 60 (Table 3).

At no time was there any significant difference between the values of renal blood flow or arterial pressure measured in the six groups of three dogs.

Controls. No significant variation was noted in renin secretion or urinary elimination of water and electrolytes in the animals given diuretic solvents. In the dog given bumetanide dissolved in polyethylene glycol, the diuretic effect and the development of renin secretion were identical with those observed after injection of bumetanide dissolved in distilled water.

Long protocol (6 h)

Frusemide only. Renin secretion remained raised during the 6 h after the injection of 60·6 pmol of frusemide/kg (20 mg/kg) (Table 5). Its evolution was biphasic: the values of renin secretion and the PRA of venous renal blood showed a first peak in minute 15 and a second around the third hour after injection of the diuretic (Fig. 4). The evolution of arterial PRA was identical, but the second peak appeared with a greater delay.

Frusemide and propranolol. During infusion of propranolol, the increase in renin secretion was not maintained throughout the 6 h after injection of 60·6 μmol of frusemide (20 mg/kg). Early renin hypersecretion, identical with that observed after injection of frusemide only, was evident from minute 15 on, but propranolol suppressed the second rise of renin secretion of
the biphasic curve described above. During the infusion of propranolol, the values of arterial and venous PRA and of renin secretion (Fig. 4) observed from hour 3 to hour 5 after injection of frusemide, were significantly lower than those noted when propranolol was not used.

In minute 15 after injection of the diuretic, salt and water elimination tends to diminish in animals treated with propranolol (Table 4). On the other hand, propranolol prolongs the saline secretory effect of frusemide beyond the sixth hour (Table 5). Salt and water losses during 6 h are identical in both groups of animals: 925 ± 332 pmol of Na/g of kidney and 7.81 ± 2.42 ml of water/g of kidney with frusemide only; 932 ± 112 pmol of Na/g of kidney and 7.38 ± 0.49 ml of water/g of kidney with frusemide and propranolol. Animals treated with propranolol appear to be incapable of concentrating their urine in the 6 h after the injection of frusemide: urinary osmolality remains significantly lowered from the fourth to the sixth hours after injection of the diuretic in the group treated with propranolol (Fig. 4).

Association with propranolol leads to a fall in the renal blood flow. There is no significant difference between serum potassium values and arterial pressure values between the control animals, those treated with frusemide only and those treated with propranolol and frusemide.

Frusemide and ureterovenous anastomosis. In the animals whose two ureters were anasto-

![Graph](image-url)

**Fig. 4.** Evolution of renin secretion (upper Figure) and of urine osmolality (lower Figure) in the 6 h after the injection of 60.6 µmol (20 mg) of frusemide/kg (○) or of its solvent (control: △), or of frusemide associated with propranolol (●) or with ureterovenous anastomosis (▲). Mean values ± SEM are shown; *P < 0.05; **P < 0.025; ***P < 0.001. AI = angiotensin I equivalent.
Diuretics and renin secretion

**TABLE 4. Effects of frusemide and propranolol after 15 min**

Mean values (± 1 SEM) observed in minute 15 are shown for animals treated with frusemide, 60.6 μmol/kg (20 mg/kg), or its solvent (control) or given frusemide associated with propranolol or with ureterovenous anastomosis. *P < 0.05; **P < 0.01 (test of Scheffé).

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Frusemide</th>
<th>Frusemide + propranolol</th>
<th>Frusemide + anastomosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial PRA (ng of Al h⁻¹ ml⁻¹)</td>
<td>2.2* (0.01)</td>
<td>10.9 (2.9)</td>
<td>16.8 (3.4)</td>
<td>14.0 (3.4)</td>
</tr>
<tr>
<td>Venous PRA (ng of Al h⁻¹ ml⁻¹)</td>
<td>2.4* (0.01)</td>
<td>15.7 (3.4)</td>
<td>23.8 (5.2)</td>
<td>19.8 (4.7)</td>
</tr>
<tr>
<td>Renin secretion (ng of Al-equiv. min⁻¹ g⁻¹)</td>
<td>0.4* (0.1)</td>
<td>12.2 (4.2)</td>
<td>8.7 (2.4)</td>
<td>10.0 (1.8)</td>
</tr>
<tr>
<td>Urine osmolality (mosmol/kg)</td>
<td>1217** (136)</td>
<td>321 (15)</td>
<td>305 (13)</td>
<td>308 (6)</td>
</tr>
<tr>
<td>Urine flow (ml/min)</td>
<td>0.13* (0.09)</td>
<td>3.86 (1.24)</td>
<td>3.05 (0.50)</td>
<td>4.99 (2.06)</td>
</tr>
<tr>
<td>Urine Na (μmol/min)</td>
<td>16* (9)</td>
<td>308 (166)</td>
<td>403 (57)</td>
<td>591 (273)</td>
</tr>
<tr>
<td>Renal blood flow (ml min⁻¹ g⁻¹ of kidney)</td>
<td>3.4 (0.8)</td>
<td>3.0 (0.5)</td>
<td>2.5 (0.3)</td>
<td>2.9 (0.4)</td>
</tr>
<tr>
<td>Mean arterial pressure (mmHg)</td>
<td>135 (5)</td>
<td>121 (9)</td>
<td>127 (3)</td>
<td>124 (6)</td>
</tr>
<tr>
<td>Blood K (mmol/l)</td>
<td>4.5 (0.1)</td>
<td>4.3 (0.1)</td>
<td>4.0 (0.2)</td>
<td>4.3 (0.1)</td>
</tr>
</tbody>
</table>

Mosed to an iliac vein, the saline effect remained maximal for 6 h. Urinary osmolality remained low, identical with that of the animals treated with propranolol. The variations in PRA and renin secretion values were the same as those noted after injection of frusemide and propranolol. Renin secretion only increased during the first hour after administration of the drug, after which it tended to revert to the initial values. The absence of urinary elimination resulted in a significant increase in serum potassium in the sixth hour (Table 5).

Control. No variation in renin secretion was observed in the two control dogs.

Discussion

The two groups of diuretics studied are clearly distinguished by their immediate effects on renin secretion.

Bumetanide, like frusemide and ethacrynic acid, induces renin hypersecretion from minute 5 after injection. This early renin hypersecretion cannot be explained by changes in renal perfusion pressure or by changes in the extra-cellular water volume. During the interval between measurements of renin secretion 1 min before and 5 min after injection of the three loop diuretics, the loss of water was on average only 15 ± 1 ml, the loss of Na⁺ 10 ± 0.3 mmol and the loss of K⁺ 9 ± 0.3 mmol (n = 9). Plasma potassium concentration did not vary significantly. In the dogs with a ureterovenous anastomosis, the early rise in renin secretion also occurred after injection of frusemide, even though there were no water and salt losses. Moreover, with a water and sodium excretion of the same magnitude, clopamide, indapamide and metolazone caused no increase in renin secretion during the 5 min after injection.

The early rise in renin secretion must, in fact, be considered in relation to the mechanism of action of the diuretics. Clopamide and its structural analogues thus appear to be incapable of activating the mechanism responsible for the early rise in renin secretion. Clopamide inhibits the active reabsorption of sodium at the level of the proximal tubule (Jucker, Lindenmann, Schenker, Flückiger & Taeschler, 1963; Terry & Hook, 1967) and of the distal tubule (Weid-
TABLE 5. **Effects of frusemide and propranolol after 6 h**

Mean values (± 1 SEM) observed in the sixth hour are shown for animals treated with frusemide, 60.6 μmol/kg (20 mg/kg), or its solvent (control) or given frusemide associated with propranolol or with ureterovenous anastomosis. * P< 0.05; ** P< 0.01; *** P< 0.001 (test of Scheffé).

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Frusemide</th>
<th>Frusemide + propranolol</th>
<th>Frusemide + anastomosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n = 2)</td>
<td>(n = 4)</td>
<td>(n = 5)</td>
<td>(n = 4)</td>
<td></td>
</tr>
<tr>
<td>Arterial PRA (ng of A1 h⁻¹ ml⁻¹)</td>
<td>3.7 (0.6)</td>
<td>9.9* (0.8)</td>
<td>4.6 (0.3)</td>
<td>3.4 (1.4)</td>
</tr>
<tr>
<td>Venous PRA (ng of A1 h⁻¹ ml⁻¹)</td>
<td>4.2 (0.2)</td>
<td>14.0** (2.7)</td>
<td>5.4 (0.3)</td>
<td>4.3 (1.2)</td>
</tr>
<tr>
<td>Renin secretion (ng of Al-equiv. min⁻¹ g⁻¹)</td>
<td>2.1 (1.9)</td>
<td>6.1* (2.4)</td>
<td>0.6 (0.2)</td>
<td>1.5 (1.0)</td>
</tr>
<tr>
<td>Urine osmolality (mosmol/kg)</td>
<td>1424* (398)</td>
<td>474* (43)</td>
<td>315 (10)</td>
<td>314 (16)</td>
</tr>
<tr>
<td>Urine flow (ml/min)</td>
<td>0.13 (0.06)</td>
<td>0.28 (0.07)</td>
<td>1.11 (0.19)</td>
<td>4.25*** (0.60)</td>
</tr>
<tr>
<td>Urine Na (μmol/min)</td>
<td>15 (0.1)</td>
<td>13 (3)</td>
<td>134 (27)</td>
<td>489*** (79)</td>
</tr>
<tr>
<td>Renal blood flow (ml min⁻¹ g⁻¹ of kidney)</td>
<td>4.0* (0.3)</td>
<td>2.6 (0.6)</td>
<td>1.5 (0.2)</td>
<td>1.5 (0.2)</td>
</tr>
<tr>
<td>Mean arterial pressure (mmHg)</td>
<td>125 (20)</td>
<td>100 (11)</td>
<td>114 (4)</td>
<td>133 (5)</td>
</tr>
<tr>
<td>Blood K (mmol/l)</td>
<td>4.0 (0.1)</td>
<td>3.7 (0.2)</td>
<td>3.5 (0.1)</td>
<td>4.5* (0.3)</td>
</tr>
</tbody>
</table>

mann & Siegenthaler, 1966). The kidney remains capable of concentrating urine during clopamide action (Flückiger, Schalch & Taeschler, 1963; Traeger, Ferioli, Pozet & Collard, 1967). Indapamide (Campbell & Phillips, 1974) and metolazone (Belair, Kaiser, van Denburg, Borrelli, Lawlor, Panasевич & Yelnosky, 1969; Fernandez & Puschett, 1973) appear to act in the same way as clopamide. The urine remains hypertonic in relation to plasma during their action. On the other hand, frusemide, ethacrynic acid and bumetanide suppress the process of urinary concentration by their action on the ascending limb of the loop of Henle (Goldberg, McCurdy, Foltz & Bluemle, 1964; Suki, Rector & Seldin, 1955; Bourke, Asbury, O’Sullivan & Gatenby, 1973). The corticopapillary gradient of concentration disappears after injection of ethacrynic acid and frusemide (Hook & Williamson, 1965; Cannon, Dell & Winters, 1968). In the nine dogs studied here, the urine became isotonic in relation to plasma from the sixth or eighth minute after injection of the diuretic. In the eighteen dogs, each of which was given one of the six diuretics, we noted an inverse correlation between the values of renin secretion and urinary osmolality \((r = -0.4571, \ P<0.05)\). The early rise in renin secretion would thus appear to be linked to the action of the diuretic on the functions of the cells of the ascending limb of the loop of Henle.

It is not possible to define the stimulus which triggers the early rise in renin secretion, nor to exclude the role of a redistribution of blood within the kidney, but we would emphasize the following.

(a) In the two groups of diuretics used, the quantity of Na⁺ reaching the cells of the macula densa increased owing to inhibition of the reabsorption of sodium upstream. With an equal amount of sodium excreted by the kidney, more sodium reaches the macula densa after frusemide, ethacrynic acid and probably bumetanide, which have no effect on the distal tubule, than after clopamide, indapamide and metolazone, which do have an effect on that tubule. Unfortunately, nothing is yet known about the
effect of the direct action of the diuretics on the cells of the macula densa.

(b) The two groups of diuretics appear to be distinguished also by their effect on the active reabsorption of Cl⁻ by the ascending limb of the loop of Henle. Inhibition of the active reabsorption of Cl⁻ by the ascending limb of the loop of Henle was demonstrated with ethacrynic acid and frusemide (Burg, Stoner, Cardinal & Green, 1973; Burg & Green, 1973). In view of the similarity of its action and chemical structure to frusemide (Ostergaard, Magnussen & Nielsen, 1972), it can be assumed that bumetanide has an identical mode of action. Thurau (1974) demonstrated in the rat on single nephron units that the PRA of the juxtaglomerular apparatus rises only if there is an increase in the quantity of Na⁺ and of Cl⁻ moving across the luminal membrane into the macula densa cells. Our results are in agreement with this finding.

The late renin hypersecretion common to the two groups of diuretics is not due to the same mechanism as the early renin hypersecretion analysed above. The difference between these two mechanisms is confirmed by the following results.

(a) Only the early renin hypersecretion occurred after injection of frusemide in the dogs with a ureterovenous anastomosis. The suppression of sodium and water losses coincided with the suppression of late renin hypersecretion, which would thus be a function of sodium and water loss. In fact, in the eighteen dogs studied according to the short protocol, the values recorded 1 h after injection of the diuretics showed a correlation between renin secretion and natriuresis ($r = 0.5210, P < 0.05; n = 18$).

(b) Inhibition of the β-receptors of the renal vascular bed also resulted in the suppression of late renin hypersecretion after administration of frusemide. The early rise in renin secretion persisted. This might be the explanation for the contradictions to be found in accounts of the effect of β-receptor blockers on renin hypersecretion induced by frusemide or ethacrylic acid (Winer, Chokshi, Yoon & Freedman, 1969; Bravo, Tarazi & Dusart, 1972; Johns & Singer, 1973; Bonvalet & Menard, 1974). β-Receptor blockers do not affect the stimulus produced by the direct action of the diuretic on the nephron. They do, on the other hand, inhibit later renin hypersecretion connected with sodium and water depletion.

Acknowledgments

The technical assistance of Mr J. P. Krieger is gratefully acknowledged. We thank the following companies for generous gifts of drugs: Hoechst, ICI, Leo, Merck Sharp and Dohme, Pennwalt, Sandoz and Servier.

References


