Theoretical blockage of putative sodium channels in the vascular smooth muscle cell membrane should produce vasodilation. By decreasing the inward current of sodium ions, blockade of such channels would decrease the positive charge on the inside of the cell and thus hyperpolarize the membrane. As calcium influx is partly voltage dependent, calcium influx would decrease, which would result in vasodilation. We tested the hypothesis that sodium channel blockade produces vasodilation by injecting amiloride intra-arterially in the dog forelimb. Amiloride reportedly blocks specific sodium channels in the luminal membrane of cells in both the late distal convoluted tubule and cortical collecting duct of the kidney, thereby producing natriuresis, diuresis, and antikaliuresis. We found that amiloride is a vasodilator in the dog forelimb and that it promptly lowers systemic arterial blood pressure on intravenous injection. These effects, however, required relatively large doses.

The report of an analogue of amiloride that is 60-fold more potent than amiloride as a sodium influx inhibitor in human fibroblasts prompted us to test 11 modifications of the amiloride molecule, 3 loop diuretics, and a hypotensive compound for vasodilator activity in the dog forelimb. Although the analogue that prompted the study proved to be less active than amiloride, we found one, 6-iodo-amiloride, with more activity than amiloride. Intravenous administration of 6-iodo-amiloride in relatively small doses immediately lowered systemic arterial blood pressure but did not abolish its natriuretic and diuretic properties. We therefore examined its actions in detail in the normotensive dog and in the normotensive and hypertensive rat.
rat. For the latter study we selected the spontaneously hypertensive rat because there is evidence that its vascular smooth muscle cells are abnormally permeable to sodium.1-3

Methods

The effects of amiloride, the amiloride analogues, and the other compounds were studied in 19 mongrel dogs, 7 Wistar rats, 7 Wistar-Kyoto rats (WKY), and 8 spontaneously hypertensive rats (SHR) of the Okamoto strain (all of the rats were from Charles River Breeding Laboratories, Wilmington, MA). National Institutes of Health and American Physiological Society guidelines for the care and use of animals were followed in all experiments.

The dogs were used to study the effects of local administration of amiloride and 15 other compounds on forelimb vascular resistance and the effects of intravenous administration of amiloride and 6-iodo-amiloride on systemic arterial pressure, forelimb vascular resistance, urine flow, urinary sodium excretion, and urinary potassium excretion. They were of both sexes and ranged in weight from 18 to 22 kg. They were anesthetized with sodium pentobarbital, 30 mg/kg i.v., and ventilated artificially through a cuffed endotracheal tube. The brachial artery in the right forelimb was exposed surgically above the elbow and ligated. The distal portion of the brachial artery was perfused at constant flow (129 ml/min) with aortic blood obtained through the left femoral artery. Flow was held constant with a finger pump, which delivered blood independently of the inflow and outflow pressures encountered. All collateral flow except that in bone was eliminated with ligatures above the elbow, which included all soft tissues except nerves, cephalic vein, and brachial vein. Perfusion pressure was measured in the perfusion line downstream to the pump. Solutions were injected into the perfusion line upstream to the pump to ensure adequate mixing with blood. The proximal portion of the brachial artery was cannulated for measurement of arterial blood pressure, and the femoral vein was cannulated for infusion of test solutions. Both ureters were cannulated retroperitoneally with polyethylene tubing through small flank incisions for the measurement of urine flow. Perfusion and systemic arterial blood pressures were measured with pressure transducers coupled to an oscillograph recorder. Urine flow from both kidneys was measured over consecutive 10-minute periods with a graduated cylinder and stopwatch.

The rats were used to study the effects of 6-iodoamiloride on blood pressure, urine flow, urinary sodium excretion, and urinary potassium excretion. Only male rats were used, and all were 15 to 16 weeks of age at the time of study. The WKY and SHR were weight matched. They consumed normal rat chow until the day of use, when they were anesthetized with sodium pentobarbital, 75 mg/kg intraperitoneally. A carotid artery was cannulated for the measurement of arterial pressure with a pressure transducer coupled to an oscillograph recorder. The urinary bladder was cannulated through a small abdominal incision for the continuous collection of urine into microcentrifuge tubes of known weight. Urine was collected for 10-minute periods, then the tubes were reweighed and the volume was calculated.

Sodium and potassium concentrations in the urine obtained from the dogs and rats were measured with flame photometry. The data were converted to excretion per 10-minute periods.

Amiloride, 11 of its analogues,6,7 3 other diuretics,8-10 and a hypotensive imidazopyrazine11 were studied. Compounds were placed in solution on the day of the experiment at a concentration of 1 or 2 mg/ml; some required the addition of small amounts of isethionic acid, sodium bicarbonate, or hydrochloric acid. The solutions were made isosmotic with plasma by adding sodium chloride. Solutions containing all components except the compounds to be studied (blank solutions) were always prepared, and none produced a response on intra-arterial or intravenous administration.

Results

Bolus injection of amiloride intra-arterially in the dog forelimb perfused at constant flow (129 ml/min) with the animal's own arterial blood (n = 9) produced a prompt, transient, dose-dependent decrease in perfusion pressure over the dose range 100 to 400 μg (Figure 1). The average maximal decrease with 200 μg...
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was 30 mm Hg, and the average duration of the response was 6 minutes (n = 6). Bolus intravenous injection produced a prompt, dose-dependent decrease in systemic arterial pressure, but effects were small and transient except in doses exceeding 10 mg.

We then tested 11 analogues of amiloride, 3 loop diuretics, and a hypotensive imidazopyrazine for vasodilator activity in the dog forelimb. All compounds were bolus injected intra-arterially with blood flow constant at 129 ml/minute. Amiloride was always the reference compound. Seven of the analogues produced vasodilation similar to that seen following injection of amiloride; four were less active than amiloride, two were equally active, and one, 6-iodo-amiloride, was more active than amiloride (Table 1). One of the analogues of amiloride (MK-875) produced potent vasoconstriction (see Table 1), and three, including 6-fluoro-amiloride and phenamil, were essentially inactive (not shown). One of the loop diuretics produced less vasodilation than amiloride; the other two loop diuretics and the hypotensive imidazopyrazine were without effect.

Figure 2 shows the effect of 6-iodo-amiloride relative to amiloride. Intra-arterial injection of 6-iodo-amiloride produced a greater and more prolonged decrease in perfusion pressure than amiloride and also produced a greater and more prolonged decrease in systemic arterial pressure on recirculation. We therefore concentrated subsequent efforts on 6-iodo-amiloride.

On bolus intravenous injection of 3 mg of 6-iodo-amiloride, systemic arterial pressure and forelimb perfusion pressure at constant flow promptly decreased 65 and 47 mm Hg respectively; the decreases lasted 13 and 9 minutes (n = 4). The decreases with the same dose of amiloride were only 5 and 23 mm Hg (lasting 1 and 5 minutes).

Intravenous infusion of 17 (n = 1), 34 (n = 1), 45 (n = 1), and 77 (n = 2) mg of 6-iodo-amiloride over a 20-minute period produced diuresis, natriuresis, and antikaliuresis and, with the two higher doses, hypotension. The onset of the hypotension occurred promptly on starting the infusion (Figure 3) and was sustained for the duration of the infusion. Blood pressure then gradually returned to the control level in 25 to 110 minutes. The urinary effects (see Figure 3) continued for 45 to 160 minutes beyond the end of the infusion. They were delayed in onset by the hypotension with the higher dose (see Figure 3); in fact, the diuresis was preceded by transient antidiuresis in the other animal.
Table 1. Vasodilator Activity of Amiloride and Its Analogues

<table>
<thead>
<tr>
<th>Name or Code of Compound</th>
<th>Position in formula (above)</th>
<th>Vasodilator activity†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amiloride</td>
<td>Cl H2N—</td>
<td>—NH2</td>
</tr>
<tr>
<td>6-Iodo-amiloride</td>
<td>I H2N—</td>
<td>—NH2</td>
</tr>
<tr>
<td>6-Bromo-amilorde</td>
<td>Br H2N—</td>
<td>—NH2</td>
</tr>
<tr>
<td>Benzamil</td>
<td>Cl H2N—</td>
<td>—NHCH3</td>
</tr>
<tr>
<td>MK-685</td>
<td>Cl (CH3)2N—</td>
<td>—NH2</td>
</tr>
<tr>
<td>No name or code</td>
<td>Cl (CH3)2CNH—</td>
<td>—NH2</td>
</tr>
<tr>
<td>No name or code</td>
<td>Cl CH3CH2(CH3)2N—</td>
<td>—NH2</td>
</tr>
<tr>
<td>Bromobenzamil</td>
<td>Br H2N—</td>
<td>—NHCH3</td>
</tr>
<tr>
<td>MK-875</td>
<td>Cl H2N—</td>
<td>NH—H2</td>
</tr>
</tbody>
</table>

†Relative to amiloride: ↑, more than amiloride; =, same as amiloride; ↓, less than amiloride.

receiving 77 mg. As our supply of 6-iodo-amiloride was limited, we were compelled to conduct all subsequent experiments in rats.

Wistar rats responded to intravenous infusion in the same manner. In preliminary experiments we found that 1 mg administered intravenously over a 10-minute period produced a small, transient decrease in blood pressure, diuresis, natriuresis, and antikaliuresis. As 2 mg administered intravenously over a 10-minute period produced a large, prolonged decrease in blood pressure and a delayed increase in urine flow and sodium excretion, we selected an intermediate dose for detailed study. The results are shown in Figure 4; 1.5 mg (0.38 mg/100 g body weight) administered intravenously over a 10-minute period to the 400-g Wistar rat produced a prompt, sustained decrease in arterial blood pressure and prompt, sustained diuresis, natriuresis, and antikaliuresis.

The findings were somewhat different when the same dosage was administered to WKY and SHR. In the WKY, blood pressure was unaffected and the changes in the urinary parameters were less pronounced (Figure 5A). In the SHR, blood pressure was greatly affected for a prolonged period and changes in the urinary parameters were essentially absent (Figure 5B). A lower dose (0.25 mg/100 g body weight) in these rats (n = 4 per group; results not shown) produced essentially the same results.

Discussion

These studies show that amiloride and certain analogues of amiloride produce vasodilation in the dog forelimb and that 6-iodo-amiloride is more active than amiloride. They also show that amiloride and 6-iodo-amiloride are depressor in the dog and that the latter is much more potent. In the normal dog and Wistar rat, intravenous infusion of 6-iodo-amiloride decreases arterial blood pressure but still produces diuresis, natriuresis, and antikaliuresis. In the SHR, it promptly produces a large, sustained decrease in arterial pressure in the absence of large changes in the renal excretion of water and salt.
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These studies indicate that certain sodium channel blockers, 6-iodo-amiloride in particular, are vasodilator and depressor as well as diuretic and natriuretic and that the depressor activity of 6-iodo-amiloride is particularly pronounced in SHR. The findings suggest that 6-iodo-amiloride and other sodium channel blockers may be useful as vasodilatory antihypertensive agents, particularly in those types of hypertension characterized by increased permeability of the vascular smooth muscle cell membrane to sodium.

The marked influence on vasodilator activity achieved by introducing iodo and fluoro substituents in the 6 position of amiloride suggests a more extensive study of 6- substituted derivatives is needed. The 6-iodo introduction enhanced activity, while the 6-fluoro introduction abolished activity. The 6-bromo introduction had little effect. Such a study must also bear in mind the influence on diuretic, natriuretic, and anti-kaliuretic activities. In a standard rat bioassay for natriuretic and anti-kaliuretic activity, the 6-chloro and 6-bromo compounds were shown to have maximal dual activity; the 6-iodo and 6-fluoro compounds were somewhat less active.

Amiloride blocks sodium transport in epithelia with a relatively high electrical resistance ("tight" epithelia). Epithelia with a low electrical resistance ("leaky" epithelia) are insensitive to amiloride. Its effect on passive sodium influx in vascular smooth muscle is unknown. Although the vasodilation observed is compatible with blockade of putative sodium channels in vascular smooth muscle, especially with 6-iodo-amiloride, transport and electrophysiological studies are needed to evaluate this possibility.

**FIGURE 4.** Effects of intravenous infusion of 6-iodo-amiloride (0.38 mg/100 g body weight) over a 10-minute period on systemic arterial pressure, urine flow, urinary sodium excretion, and urinary potassium excretion in WKY (average of 5 experiments).

**FIGURE 5.** Effects of intravenous infusion of 6-iodo-amiloride (0.38 mg/100 g body weight) over an 11-minute period on systemic arterial pressure, urine flow, urinary sodium excretion, and urinary potassium excretion in WKY (A) and SHR (B).
There were marked differences in the responses of the three strains of rats to the same dosage (0.38 mg/100 gm body weight over 10–11 minutes) of 6-iodo-amiloride. The agent influenced both blood pressure and renal excretion in the Wistar rat, influenced only renal excretion in the WKY, and initially influenced only blood pressure in the Okamoto strain of SHR (almost as if the agent switched its action from kidney to blood vessels). The differences between its actions in the two strains of normotensive rats again raises questions concerning the proper control for the Okamoto SHR. The large and prolonged effect on blood pressure in the SHR supports the idea that the hypertension in this strain is related to increased permeability of the vascular smooth muscle cell membrane to sodium.  

Studies over the last decade support the notion that two basic types of sodium transport defects exist in the vascular smooth muscle cell in essential hypertension in humans and that under certain circumstances the two defects may coexist and amplify their individual effects on cell sodium concentration. These defects are increased cell membrane permeability to sodium and decreased active pumping of sodium at a given internal sodium concentration. The first seems to be characteristic of heritable essential hypertension, and the second appears to be characteristic of low renin hypertension subsequent to an inability to excrete salt normally. Decreased active pumping apparently results from the release of a sodium pump inhibitor from the hypothalamus. Coexistence of the defects occurs when a patient with heritable essential hypertension also has defective renal function, particularly when salt intake is increased. Coexistence would drive internal sodium concentration to even higher levels, further increasing internal calcium concentration and contractions. If this is true, the ideal antihypertensive agent might be one that corrects both defects. This might be accomplished by an agent that blocks sodium channels in both the arteriole and renal tubule.

The findings in this study suggest that it might be profitable to examine the value of 6-iodo-amiloride and other amiloride analogues as antihypertensive agents in various animal models of hypertension and in hypertensive humans. The Dahl salt-sensitive rat should be of particular interest as there is indirect evidence that these rats also have vascular muscle cells that are abnormally permeable to sodium.  

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