Antihypertensive Action of Medullipin I Given by Mouth

E. Eric Muirhead, Bennie Brooks, Lawrence W. Byers, Kenji Toba, and Leonard Share

Perfusion of normal rat kidneys with 5% human albumin in a balanced salt solution bubbled with oxygen yielded medullipin I (Med I) in the renal venous effluent. The presence of Med I in the renal venous effluent has been established by thin-layer chromatography, by the type of vasodepressor effect when injected intravenously as a bolus into the hypertensive rat, by inhibition of the vasodepressor effect of the renal venous effluent by Tween 20 and SKF 525A (proadifen, inhibitor of cytochrome P-450), and by removal of the liver from the circulation (a procedure that inhibits extracted Med I). Med I so derived lowered blood pressure of spontaneously hypertensive rats when injected into the stomach by an indwelling tube or when given by mouth. The lowering of blood pressure was attended by no change in cardiac output and no change in heart rate. Med I given by mouth to the spontaneously hypertensive rat is a vasodilator that suppresses sympathetic tone, acting in the same way as Med I extracted from renal papillae and given intravenously. Importantly, the antihypertensive action was demonstrated in the spontaneously hypertensive rat, a model of hypertension considered to mimic idiopathic or essential hypertension of humans. Med I is a promising therapeutic agent for hypertension. (Hypertension 1991;17:1092-1096)

Medullipin I (Med I) is a hormone of the renal papilla. It is present in small amounts in the renal venous effluent (RVE) of normal rats and in large amounts in the RVE after unclipping of Goldblatt hypertensive rats. Med I is transported to the liver where it is activated into medullipin II (Med II). Med II causes vasodilation, suppresses sympathetic tone, causes diuresis-natriuresis, and in the rat has a suppressive effect on the central nervous system.

Med I can be derived by two approaches: 1) by extraction from fresh renal papillae and concentration by chromatography and 2) by perfusion of normal kidneys under elevated blood pressure (BP). The vasodepressor agent in the perfusate of the latter previously was shown by extraction and thin-layer chromatography to have the same Rf on the chromatographic plate as Med I extracted from renal papillae. Moreover, the medullipin of renal perfusate and extracted medullipin have the same biological properties and apparently represent the same entity.

Because the medullipins offer promise as antihypertensive agents, it is of interest to determine if Med I has an antihypertensive action when given by mouth to the spontaneously hypertensive rat (SHR) of Okamoto and Aoki. We report herein that this is the case.

Methods

National Institutes of Health guidelines for animal research were followed precisely.

Med I was derived by perfusing normal rat kidneys at 180 mm Hg with 5% human albumin in a balanced salt solution bubbled with oxygen, as described previously. The procedure included treating the donor rat with captopril (10 mg/kg i.p.) and the Burroughs-Wellcome compound BW 755C (10 mg/kg i.p.) (kindly furnished by Wellcome Research Laboratories, Beckenham, Kent, UK) to inhibit the renin-angiotensin, cyclooxygenase, and lipoxygenase systems, as reported by Higgs et al. Oxygen was bubbled through the albumin because of the suggestion that Med I synthesis involves an oxidative step. The albumin solution of the RVE was centrifuged at 4,000 rpm for 10 minutes to remove a minor residue and was assayed for antihypertensive action by injecting a bolus dose (0.5 ml) intravenously into SHRs. BP was measured directly from the abdominal aorta by an indwelling catheter. Intravenous injections were made by an indwelling catheter in the inferior vena cava. The assay SHRs were purchased from Taconic Farms, Inc., Germantown, N.Y., and

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weighed 300–350 g when used. The rats donating the kidney for perfusion were Sprague-Dawley rats from Bio-Lab Corp., St. Paul, Minn., and weighed 400–475 g on the day of perfusion.

Cardiac output (CO) was measured by the thermodilution method.\textsuperscript{15,16} SHRs weighing at least 350 g were placed under pentobarbital anesthesia (30 mg/kg i.p.), and the abdominal aorta was cannulated as described previously.\textsuperscript{14} In addition, a precision fine wire thermocouple (0.125 mm in diameter, Omega Engineering, Inc., Stamford, Conn.) was implanted in the aortic arch via the left femoral artery. This was followed by installation of a microbore plastic tube (0.375 mm i.d.×0.75 mm o.d.) into the right atrium via the right external jugular vein. Through a midline incision, an intragastric catheter was placed in the stomach. This tube was constructed by fusing with a soldering iron a 15-mm length of PE-90 tube with a 9-mm length of PE-50 tube. An oval bulb (3x1.5 mm) was formed at the tip of the PE-50 segment. This bulb was implanted just inside the stomach through an 18-gauge needle opening and anchored to the outer wall by a purse-string suture. The four lines, respectively, were channeled subcutaneously through a 17-gauge tube to the back of the neck. The jugular and aortic tubes were filled with saline containing heparin (each 50 units, Upjohn Co., Kalamazoo, Mich.). Penicillin G (20,000 units i.m.) was injected after the incisions were closed. After at least 7 days of recovery, the rats were placed in a wire cage (7.5×7.5×25 cm) having a slot for travel of the lines and were allowed to acclimate for 45 minutes before the experiment.

For hemodynamic measurements, mean BP was recorded from the abdominal aorta via a Statham P 231D transducer (Oxford, Calif.) connected to a Brush 2600 recorder (Gould Instruments, Cleveland, Ohio). Heart rate (HR) was recorded from the arterial pulse with a Brush Biotach. CO was measured by the rapid injection of 200 μl 0.9% NaCl at 20–24°C into the right atrium and recorded by the thermocouple in the thoracic aorta and a preamplifier designed by the Biomedical Instrument Division of the University of Tennessee, Memphis, on a Brush recorder. Rectal temperature was measured by a Telethermometer (Yellow Springs Instruments Co., Yellow Springs, Ohio).

Each CO determination was based on an average of at least two consecutive dilution curves with a minimum of 2 minutes between them. In preliminary experiments in which CO was measured 10 times at 2-minute intervals, the coefficient of variation was 5.1%.

### Table 1. Effect on Blood Pressure of 5% Albumin Solution Before Renal Perfusion in Assay Rats

<table>
<thead>
<tr>
<th>HBP (mm Hg)</th>
<th>BP, minutes after injection (mm Hg)</th>
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<tbody>
<tr>
<td>174.5±11</td>
<td>179.5±6</td>
</tr>
<tr>
<td>176.5±6</td>
<td>177±7</td>
</tr>
<tr>
<td>178±9</td>
<td>179±5</td>
</tr>
<tr>
<td>176.5±7</td>
<td></td>
</tr>
<tr>
<td>p</td>
<td>0.7</td>
</tr>
<tr>
<td>0.7</td>
<td>0.9</td>
</tr>
<tr>
<td>0.8</td>
<td>0.8</td>
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<tr>
<td>0.8</td>
<td></td>
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</tbody>
</table>

Dose, 0.5 ml i.v.; n=10. HBP, hypertensive blood pressure; BP, blood pressure.

Baseline BP was taken continuously for 1 hour and was followed by three control HR and CO measurements. The rats were allowed to recover. When baseline measurements were again steady, a bolus injection of Med I (10–15 ml RVE at 37°C) was given by gastric tube over 30 seconds. A second and third HR and CO measurement was performed at the nadir of BP and after its recovery to prior hypertensive levels. Six SHRs were used in the hemodynamic measurements. Altogether, 11 SHRs received Med I by stomach tube.

The localization of the thermocouple in the aorta was determined at autopsy.

For Med I given by mouth, SHRs were offered Med I in albumin solution at 37°C to drink (10 ml in a glass tube). BP was determined for 30 minutes before this offer. Those rats drinking the entire 10 ml had their BP followed for at least 50 minutes (n=8).

There were two sets of controls for the 5% albumin solution: 1) 0.5 ml plain albumin solution (not perfused through kidneys) was injected intravenously as a bolus into SHRs, and BP was followed (Table 1) (n=10); and 2) plain albumin solution (10 ml) was injected by stomach tube into SHRs, and their BP was followed (Table 2) (n=4).

Statistical analyses were by Student's t test. Values of \( p < 0.05 \) were considered significant. Results are given as mean±SEM.

### Results

**Vasodepressor Action of Renal Venous Effluent Containing Medullipin I Derived by Perfusion of Normal Kidneys**

Figure 1 depicts the type of vasodepressor effect of the RVE obtained from normal kidneys perfused under high pressure. Of special note is the 2-minute delay between the intravenous bolus injection and the beginning decline of BP, a characteristic feature of extracted Med I.\textsuperscript{1} BP dropped from 200 to 100 mm Hg in approximately 4 minutes, remaining so depressed for 8 minutes. Recovery required approx-
imately 40 minutes. In the present study, 16 perfusates (RVEs) of this type were used.

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Figure 2 shows the lowering of BP by Med I when injected by stomach tube. The average results indicated that approximately 30 minutes was required for BP to reach its nadir. Recovery began after 50 minutes.

Figure 3 depicts the state of BP at the nadir (the maximal effect) of all 11 examples. The nadir amounted to 166±6 mm Hg from 194±5 mm Hg (an approximate 20% drop in BP) and occurred in 46±12 minutes. By 63±4 minutes, recovery of BP had occurred.

Figure 4 shows the lowering of BP when Med I was given by mouth. As by stomach tube, the average drop of BP required approximately 30 minutes but remained depressed for at least 70 minutes.

Figure 5 gives the state of BP at the nadir (maximal effect) of all eight examples, which occurred at 49±10 minutes and amounted to 152.5 mm Hg from

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**FIGURE 1.** Representative recording of vasodepressor effect of renal venous effluent (RVE) (0.5 ml i.v.) injected into a spontaneously hypertensive rat. 1, Blood pressure (BP) 200 mm Hg; 2, 0.5 ml RVE injected i.v.; 3, BP 100 mm Hg; 4, 5, 6, BP 140, 180, 200 mm Hg, respectively. Response lasted 46 minutes.

**FIGURE 2.** Line graph showing lowering of blood pressure (BP) in spontaneously hypertensive rats after 10–15 ml renal venous effluent containing medullipin I was given by stomach tube (n=11). Continuous effect is shown. Values are for all 11 examples at each point on the time axis. HBP, hypertensive BP.

**FIGURE 3.** Plot showing lowering of blood pressure (BP) in spontaneously hypertensive rats when medullipin I in renal venous effluent was given by stomach tube (n=11). Maximal effect (at nadir) and recovery are shown. HBP, hypertensive BP.
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![Graph showing lowering of blood pressure](image)

**FIGURE 4.** Line graph showing lowering of blood pressure (BP) in spontaneously hypertensive rats after 10 ml renal venous effluent containing medullipin I was given by mouth (n=8). Continuous effect is shown. Values are for all eight examples at each point on the time axis. HBP, hypertensive BP.

![Graph showing maximal effect on blood pressure](image)

**FIGURE 5.** Plot showing maximal effect on blood pressure (BP) in spontaneously hypertensive rats after 10 ml of renal venous effluent containing medullipin I was given by mouth. HBP, hypertensive BP.

185±4 mm Hg (an approximate 17.5% drop in BP). By 76±8 minutes, recovery had occurred.

Table 1 demonstrates lack of change in BP when 0.5 ml plain 5% albumin was injected intravenously. Table 2 demonstrates lack of change in BP when 10 ml plain 5% albumin solution was injected by stomach tube.

**Hemodynamic Effect of Medullipin I in Renal Venous Effluent Given by Stomach Tube (n=6)**

Table 3 relates the hemodynamic findings during the antihypertensive action of Med I. The drop of BP from its hypertensive level to the nadir was significant. Recovery of BP was complete. There was no significant change in HR and CO as the BP lowered to the nadir nor as recovery of BP occurred. Thus, Med I acted as a vasodilator when given by stomach tube, and sympathetic tone was suppressed (no tachycardia).

**Discussion**

Using the principle elucidated by Karlstrom et al., namely, that Med I is secreted by an isolated kidney perfused with whole blood under elevated perfusion pressure, we derived Med I by perfusing isolated normal rat kidneys with 5% albumin solution bubbled with

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Nadir</th>
<th>p</th>
<th>Recovery</th>
<th>p vs. control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood pressure (mm Hg)</td>
<td>190±9</td>
<td>158±8</td>
<td>&lt;0.02</td>
<td>194±11</td>
<td>0.75</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>383±24</td>
<td>387±23</td>
<td>&gt;0.9</td>
<td>384±29</td>
<td>&gt;0.9</td>
</tr>
<tr>
<td>Cardiac output (ml/100 g wt)</td>
<td>19±1.1</td>
<td>18.6±2</td>
<td>&gt;0.9</td>
<td>18.2±1.6</td>
<td>&gt;0.7</td>
</tr>
</tbody>
</table>

Dose, 10 ml of renal venous effluent; n=6. Mean body wt of the six spontaneously hypertensive rats (SHRs) was 328±2 g. Cardiac output for these rats is similar to that reported by Albrecht et al. in SHRs with established hypertension using the dye dilution method.
oxygen at 180 mm Hg. Oxygen was used because prior observations suggested an oxidative step in the synthesis of Med I by the kidney. Med I so derived and Med I extracted from fresh renal papillae and concentrated by chromatography appear to be the same agent. Med I, therefore, has the characteristics of an antihypertensive hormone of the kidney and most likely its renomedullary interstitial cells.

In these experiments, we showed that Med I lowers BP of hypertensive rats when given by mouth or by stomach tube. This makes Med I a promising therapeutic agent for hypertension. It is of special interest that this per os antihypertensive effect occurred in the SHR, a rat model considered to resemble the most common type of human hypertension, idiopathic or essential hypertension.

The per os effect of kidney perfusion-derived Med I was the same as that previously described for intravenous Med I extracted from fresh renal papillae. In both cases, the agent obtained is a vasodilator that suppresses sympathetic tone. Moreover, extracted Med I causes diuresis-natriuresis. Med I must be converted to Med II by the liver to be active. This conversion appears to involve the cytochrome P-450-dependent enzyme system of the liver. Conversion of Med I injected intravenously requires 1–2 minutes (illustrated in Figure 1), whereas the ultimate drop in BP from Med I given by mouth required some 30 minutes (Figures 2 and 4). We consider the latter to result from the need for intestinal enzymes to digest the albumin to which Med I is attached before its absorption and conveyance to the liver.

The medullipin system and the renin-angiotensin system are feedback control systems. The former either lowers BP or keeps it lowered and is antihypertensive. The latter raises BP or keeps it elevated and is prohypertensive. These systems have a reciprocal behavior toward renal artery perfusion pressure. A drop in renal artery pressure stimulates the secretion of renin and the generation of angiotensin II and tends to shut off the secretion of Med I and the generation of Med II. An elevation of renal artery perfusion pressure stimulates the secretion of Med I and the generation of Med II while suppressing the secretion of renin and the generation of angiotensin II.

There are indications that an excess of one system can overpower the other system. An excess of circulating angiotensin II can overcome the antihypertensive action of an effective amount of Med II. By the same token, an excess of Med II can modulate the effectiveness of angiotensin II. Thus, the action of Med I taken by mouth likely will depend on the dosage available and the extent to which angiotensin II is operative in a given hypertensive state. In the SHR, Med I appears to be an effective antihypertensive agent by mouth.

Acknowledgment
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