The Role of Antihypertensive Polar Renomedullary Lipid in the Pathogenesis of Hypertension

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One-0-hexadecyl-2-0-acetyl-sn-glycero-3-phosphorylcholine (HAGPC), a component of semisynthetic antihypertensive polar renomedullary lipid (APRL) reported by Muirhead et al. in chemically treated rabbit renomedullary lipid had a strong hypotensive action, a cardiosuppressing action and an apparent anti-norepinephrine action. 3-(N-n-octadecy carbamoyloxy)-2-methoxypropyl-2-thiazolioethy] phosphate significantly inhibited the hypotensive action of HAGPC, whereas the hypotensive activities of prostaglandin I2, prostaglandin E3, bradykinin, histamine and acetylcholine were not affected by this drug. The blood pressure of rats with established hypertension produced by clipping one renal artery and contralateral nephrectomy normally decreases rapidly after unclipping the renal artery, but the initial rapid decrease was significantly inhibited by an intravenous infusion of 3-(N-n-octadecy carbamoyloxy)-2-methoxypropyl-2-thiazolioethyl] phosphate. This shows that endogenous 1-alkyl-2-acetyl-sn-glycero-3-phosphorylcholine participates in the rapid decrease of blood pressure after unclipping the renal artery in one-kidney, one clip hypertensive rats.

The renal medulla has been thought to contain hypotensive lipids, because the lipid extract of the renal medulla has potent hypotensive activity. In 1979, Blank et al. obtained antihypertensive polar renomedullary lipid (APRL) from a lipid extract of rabbit renal medulla with some chemical modifications such as hydrolysis of ester bond and reesterification with acetic anhydride. Its structure turned out to be 1-0-alkyl-2-0-acetyl-sn-glycero-3-phosphorylcholine with mainly C16:0 alkyl chain length at position 1. Rats with one renal artery clipped and contralateral nephrectomy become hypertensive and this high blood pressure is rapidly reduced after unclipping the renal artery In a normal rat perfusion of a declipp ed kidney of a two-kidney, one clip hypertensive rat also results in significant reduction of the blood pressure. These results strongly suggest that a humoral factor that lowers the blood pressure is released from the unclipped kidney. However, the pathophysiological mechanisms involved in the development of hypertension after clipping the renal artery and the reduction of the blood pressure after unclipping the artery are thought to be multifactorial. In this paper, the hypotensive mechanism of chemically synthesized 1-0-hexadecyl-2-0-acetyl-sn-glycero-3-phosphorylcholine and the role of this ether lipid in the rapid reduction of blood pressure in one-kidney, one clip hypertensive rats after unclipping were studied.

Key words: Antihypertensive polar renomedullary lipid
Blood pressure regulation
One-kidney
One clip Goldblatt rat

MATERIALS AND METHODS

Chemicals
1-0-Hexadecyl-2-0-acetyl-sn-glycero-3-phos-
Fig. 1. Comparative hypotensive activity of HAGPC, PGI₁, bradykinin and PGE₂. All drugs (1.1 μmol/kg body weight) were injected intravenously in male SHR. Hypotensive activity is defined as the net change in MBP before and after administration of each drug. Asterisks mean that value is significantly smaller (p < 0.001) than that of HAGPC. Data are shown as mean ± SEM (n = 7 for HAGPC and PGI₁, n = 5 for bradykinin and PGE₂).

Fig. 2. Change of the dose-response curve of norepinephrine by the infusion of HAGPC. Each point indicates mean ± SEM. The closed circle is saline infusion. The open circle and closed triangle are 2 and 20 n mol of HAGPC/kg body weight/ min, respectively.

Fig. 3. Effect of HAGPC on cardiac output in dogs. Values are mean ± SD for 6 experiments before (control) and 5 min after the injection of 50 n mol/kg body weight of HAGPC. Asterisks mean that the value is significantly smaller (p < 0.001) than that of control.

L Ltd., Osaka. Other reagents were obtained commercially.

Animals

Male SHR and normotensive Wistar Kyoto rats were purchased from Keari Co. Ltd., Osaka. One-kidney, one clip hypertensive rats were prepared as follows. Male Wistar rats (body weight 120–160g) were anesthetized intraperitoneally with 32.5 mg of pentobarbital/kg body weight. A ventral midline incision was used to expose the kidneys. A silver clip of 0.2 mm width was used to clip the left renal artery. The right kidney was removed. Four weeks later, rats with a mean arterial blood pressure (MBP) of over 150 mmHg were used for experiments.

Measurement of Mean Arterial Blood Pressure

MBP was measured through a polyethylene (PE50) tube inserted into the femoral artery using a Nihon Kohden polypreamplifier RMP-6008 equipped with a blood pressure stand MP-6S, blood pressure transducer and recorder WT-645G.

Measurement of Cardiac Output

Male or female dogs (body weight 8.5–12 Kg) were anesthetized with 13 mg/kg of pentobarbital (iv) following with 10 mg/kg of ketamine hydro-
TABLE I EFFECT OF CV-3988 INFUSION ON THE HYPOTENSIVE ACTIVITIES OF HAGPC, PGE$_2$, PGI$_2$, BRADYKININ, HISTAMINE AND ACETYLCHOLINE

<table>
<thead>
<tr>
<th>Hypotensive activity ($\Delta$mmHg)</th>
<th>Saline infusion</th>
<th>CV-3988 infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>HAGPC</td>
<td>52 ± 4</td>
<td>2 ± 2</td>
</tr>
<tr>
<td>PGI$_2$</td>
<td>32 ± 2</td>
<td>35 ± 3</td>
</tr>
<tr>
<td>PGE$_2$</td>
<td>12 ± 1</td>
<td>12 ± 1</td>
</tr>
<tr>
<td>Bradykinin</td>
<td>6 ± 1</td>
<td>6 ± 1</td>
</tr>
<tr>
<td>Histamine</td>
<td>8 ± 1</td>
<td>7 ± 1</td>
</tr>
<tr>
<td>Acetylcholine</td>
<td>12 ± 1</td>
<td>11 ± 1</td>
</tr>
</tbody>
</table>

All drugs were injected intravenously into femoral vein in 1K1C hypertensive rats under CV-3988 infusion (1 mg/kg body weight/min). Injection doses of HAGPC, PGE$_2$, PGI$_2$, bradykinin, histamine and acetylcholine were 3, 10, 1.25, 50, 100 and 50 nmol/kg, respectively. Hypotensive activity defined as the net change of MBP according to drug injection. Values are mean ± SEM for 10 experiments.

Fig. 4. Effect of CV-3988 infusion on the acute reduction of blood pressure in 1K1C hypertensive rats after unclipping.

chloride (im). Catheters were placed in the cubital vein for HAGPC injection and in the right femoral artery for measuring blood pressure. A Swan-Ganz catheter size 5F of Elecath Co. Ltd., USA, was inserted into the right femoral vein and the tip was placed in pulmonary artery for measuring pulmonary arterial pressure. The cardiac output was measured by a thermodilution method using a Nihon Kohden Thermo-dilution Amplifier AH-611V and a Cardiac Output Computer EQ-611V.

Administration of Drugs

CV-3988 was dissolved in a 0.9% NaCl solution and was infused continuously at a rate of 1 mg/kg body weight/min using a micro-injector KN-201 Model H, Natsume Seisakusho, Tokyo, Japan, through a PE50 polyethylene tube in the femoral vein. The infusion volume was 0.25 ml/kg body weight/min. This infusion volume had no influence on the MBP. All drugs except PGI$_2$ were dissolved in saline just before use and injected through the contralateral femoral vein cannulated with a PE50 tube. PGI$_2$ was dissolved in 0.1 M of glycine buffer (pH 10.4).

Unclipping in one-kidney, one clip hypertensive rats. One-kidney, one clip rats with an MBP of over 150 mmHg four weeks after clipping were anesthetized intraperitoneally with 32.5 mg of pentobarbital/kg body weight. A ventral midline re-incision was used to expose the silver clip. After removing the clip, the ventral wall was re-sutured. Four mg of CV-3988/ml of 0.9% NaCl solution were infused continuously at a rate of 1 mg/kg body weight/min into the femoral vein with a microinjector. The infusion was started 10 min before unclipping the artery and continued for 30 min. Sham unclipping and CV-3988 infused-sham unclipping were performed similarly without removing the silver clip. Unclipped- and sham unclipped-rats were infused with 0.9% NaCl solution without CV-3988.

Infusion of Norepinephrine

Norepinephrine in saline was injected at a graded dose of 0.5 to 10 $\mu$g/kg BW into the femoral vein in 5 normal Wistar rats and dose-response curves were obtained. Using the same rats the dose-response curves of norepinephrine were obtained again during the continuous infusion of HAGPC at a rate of 2 or 20 nmol/kg BW/min through the external jugular vein.

Statistical Analysis

The data were analyzed by the one-way or two-way analysis of variance and Fisher’s least significant difference method.

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RESULTS

**Hypotensive Activity of HAGPC in SHR**

When HAGPC was injected to the male SHR, acute drop of MBP was observed. A similar hypotensive action was also seen by PG12 and bradykinin administration, but a slight decrease of MBP was observed when PGE2 was administered under the same dose level. HAGPC showed the most potent activity for the lowering of MBP (Fig. 1), but the duration of hypotensive activity was the longest when PG12 was administered.

**Effect of HAGPC Infusion on Pressor Activity of Norepinephrine**

HAGPC inhibited the pressor activity of norepinephrine. The dose response curve of norepinephrine was significantly shifted to the right with the infusion of HAGPC at increasing rates (Fig. 2). On the other hand, HAGPC did not affect the pressor activity of angiotensin II.

**Effect of HAGPC on Cardiac Output in Dogs**

Cardiac output was suppressed significantly by the intravenous injection of 50 nmol/kg body weight of HAGPC with a drop in systemic blood pressure. Cardiac output decreased from 1.75 ± 0.23 L/min to 0.84 ± 0.16 L/min (mean ± SD, n = 6, p < 0.001) at 5 min after the injection of HAGPC (Fig. 3).

**Effect of CV-3988 on the Hypotensive Activities of HAGPC, PGE2, PG12, Bradykinin, Histamine and Acetylcholine**

The effect of CV-3988 on the hypotensive activities of several depressor substances generated endogenously was studied (Table I). The hypotensive activity of exogenous HAGPC (3 nmole/kg) in 1K1C hypertensive rats was inhibited about 95% (p < 0.001) by the infusion of 1 mg of CV-3988/kg body weight/min, whereas the hypotensive activities of PGE2, PG12, bradykinin, histamine and acetylcholine were not affected by CV-3988 infusion.

**Effect of CV-3988 on the Acute Reduction of Blood Pressure in 1K1C Hypertensive Rats After Unclipping**

MBP of 1K1C hypertensive rats decreased rapidly after unclipping the artery, but after an infusion of 1 mg of CV-3988/kg body weight/min, this decrease was significantly inhibited (Fig. 4). Sham unclipping with an infusion of saline or CV-3988 resulted in no change in blood pressure. The blood pressure in CV-3988-infused unclipped rats decreased, but statistically insignificantly through 30 min after unclipping as compared with that in the CV-3988-infused sham-unclipped rats.

**DISCUSSION**

APRL, which includes HAGPC as a major component was first obtained from the renal medulla with some chemical modifications, and termed APRL because of its strong hypotensive activity. Recently, APRL was obtained directly from renal perfusate. Renomedullary interstitial cells contain lipid granules. These granules are thought to contain precursors of prostaglandins since indomethacin treatment increases lipid granules. APRL was obtained after the chemical modification of extracts from renomedullary interstitial cells. The hypotensive activity of APRL is more potent than that of PG12. APRL, as well as prostaglandins may play an important role in the regulation of blood pressure.

The hypotensive mechanism of APRL has not yet been clarified. Microscopic observations of the response of cremaster muscle arterioles to APRL led Muirhead et al. to propose that APRL is a vasodilator. We also observed the vasodilating activity of HAGPC in the femoral artery and the decreasing activity of cardiac output in our dogs (Fig. 3). Continuous infusion of HAGPC partially blocks the pressor activity of norepinephrine. These results suggest that HAGPC has an ß-adrenergic blocking activity. In agreement with our observations, Smith et al. have reported that APRL is an ß-adrenergic antagonist. Unclipping of the renal artery in 1K1C hypertension decreases the elevated sympathetic tone with the release of a lipid from the kidney. Blood pressure in normotensive rats perfused with isolated-clipped kidney of two-kidney, one clip hypertensive rats decreases with a drop of cardiac output after unclipping. CV-3988, a specific inhibitor of HAGPC, inhibited the acute decrease of blood pressure in 1K1C hypertensive rats after unclipping the renal artery. These results indicate that endogenous 1-alkyl-2-acetyl-sn-glycero-3-phosphorylcholine participates in part in lowering the blood pressure of one-kidney, one clip hypertensive rats after unclipping the renal artery.

**REFERENCES**

1. MUIRHEAD EE, JONES F, STIRMAN JA: Anti-
hypertensive property in renoprival hypertension of extract from renal medulla. *J Lab Clin Med* **56**: 167, 1960


