Role of Acetyl Glyceryl Ether Phosphorylcholine in Blood Pressure Regulation in Rats

Fuminori Masugi, Toshio Ogihara, Shuichi Saeki, Atsuhiro Otsuka, and Yuichi Kumahara

SUMMARY The role of an endogenously occurring acetyl glyceryl ether phosphorylcholine (AGEPC) in blood pressure regulation was studied with an AGEPC antagonist in rats with hypertension of various etiologies. The hypotensive activity of an intravenously injected AGEPC was competitively suppressed by the intravenous infusion of 3-(N-n-octadecylcarbamoyloxy)-2-methoxypropyl-2-thiazolioethyolphosphate (CV-3988) and was dose-dependent. The CV-3988 was infused intravenously into one- and two-kidney, one clip hypertensive, deoxycorticosterone-salt hypertensive, adrenal regeneration hypertensive, spontaneously hypertensive, and normotensive control rats. The increase in blood pressure caused by CV-3988 infusion in spontaneously hypertensive and normotensive control rats was significant (p < 0.01 and p < 0.001, respectively, at 60 min) compared with that caused by vehicle infusion. The increase was not seen in rats with secondary hypertension. In rats with two-kidney, one clip hypertension, the initial rapid decrease in blood pressure seen after unclipping was significantly (p < 0.05) inhibited by CV-3988 infusion as compared with that by vehicle infusion. These results suggest that endogenous AGEPC may participate in the blood pressure regulation and pathophysiology of some forms of hypertension in rats. (Hypertension 7: 742-746, 1985)

Key Words • acetyl glyceryl ether phosphorylcholine inhibitor • two-kidney, one clip hypertensive rats • spontaneously hypertensive rat • unclipping

Acetyl glyceryl ether phosphorylcholine (AGEPC) has a strong hypotensive action after intravenous injection in experimental animals. In 1979, AGEPC was identified by Demopoulos et al. as a component of platelet activating factor. In the same year, Blank et al. reported that the AGEPC was an active component of antihypertensive polar renomedullary lipid derived from a lipid extract of rabbit renal medulla. Antihypertensive polar renomedullary lipid is a hypotensive lipid obtained semisynthetically by the acetylation of renomedullary lipid extract with an acetic anhydride following the reduction of the lipid extract with Vitride. The hypotensive mechanisms of exogenous antihypertensive renomedullary lipid and AGEPC have been reported to be 1) a short-lived depressor effect, 2) a prolonged depressor effect, 3) a vasodilative effect on the microcirculation, and 4) an apparent α-blocking effect against noradrenaline action. Recently, an AGEPC has been found in the renal venous effluent of the unclipped kidney, human urine, and cultured human endothelial cells. Caramello et al. reported that AGEPC is present in blood from human subjects and experimental animals but absent in blood from anephric patients and rats. The endogenous role of AGEPC in blood pressure regulation has not been investigated thoroughly.

In a preliminary paper we reported that the endogenous AGEPC participates in the short-term reduction of blood pressure in one-kidney, one clip (1K1C) hypertensive rats after unclipping. In this article we report on our extension of the former study, focusing on the possibility of a direct participation by endogenous AGEPC in blood pressure regulation with a competitive inhibitor of AGEPC in rats with experimental hypertension of various etiologies.

Methods

The AGEPC (1-hexadecyl-2-acetyl-sn-glycero-3-phosphorylcholine) was obtained from Eisai Co., Ltd., Tokyo, Japan. The 3-(N-n-octadecylcarbamoyloxy)-2-methoxypropyl-2-thiazolioethylphosphate
(CV-3988) was supplied by Takeda Chemical Industries, Ltd., Osaka, Japan. Angiotensin II was obtained from the Peptide Institute, Protein Research Foundation, Osaka, Japan, and norepinephrine hydrochloride was purchased from Wako Pure Chemical Industries, Ltd., Osaka, Japan.

Male Wistar rats, male spontaneously hypertensive rats, and male Wistar-Kyoto rats were obtained from Keari Co., Osaka, Japan. For the preparation of 1K1C and two-kidney, one clip (2K1C) hypertension, 5-week-old male Wistar rats (weighing 110–130 g) were used. For deoxycorticosterone-salt hypertension and adrenal regenerative hypertension, 4-week-old male Wistar rats (weighing 80–100 g) were used. After fasting overnight, rats were anesthetized with a single intraperitoneal injection of pentobarbital (37.5 mg/kg). Abdominal hair was removed with a blade, and a ventral midline incision was used to expose the kidneys. The 1K1C hypertension was produced by placing a 0.2-mm silver clip on the left renal artery and removing the right kidney. For 2K1C hypertensive rats, both kidneys were left intact and a 0.2-mm silver clip was placed on the left renal artery. For the preparation of deoxycorticosterone-salt hypertension, the left kidney was removed and 1.5 mg/kg of deoxycorticosterone acetate suspended in 4% gum arabic solution was injected subcutaneously every day for 6 weeks. The adrenal regenerative hypertensive rats were prepared by enucleating the left adrenal gland and removing the right kidney and right adrenal gland. The deoxycorticosterone-salt and adrenal regenerative hypertensive rats were given 1% NaCl solution ad libitum.

Mean arterial pressure (MAP) was measured through a polyethylene (PE-50) tube inserted into the right femoral artery with an RMP-6008 Nihon Kohden (Tokyo, Japan) polypreamplifier equipped with a blood pressure stand (MP-6s), blood pressure transducer, and recorder (WT-645G).

With the rats under pentobarbital anesthesia (37.5 mg/kg i.p.), PE-50 tubes were inserted into the right femoral artery and vein. The infusion was started after full recovery from the anesthesia. In preliminary experiments, no effect on blood pressure was observed at a dose of 0.1 mg/kg/min, blood pressure was increased at a dosage of 0.5 mg/kg/min for 60 minutes, and blood pressure was increased significantly at a dosage of 1 mg/kg/min for 60 minutes. Caramelo et al. reported that the range of AGEPC in blood of rats was 0.3 to 4.02 ng/ml of blood. From these data, the CV-3988 dose rate selected was 1 mg/kg/min.

The CV-3988, which was dissolved in 0.9% NaCl solution, was infused continuously with a microinjector (KN-201, Model H, Natsume Seisakusho, Tokyo, Japan) through a PE-50 tube in the right femoral vein. The infusion volume was 0.25 ml/kg/min. This infusion volume had no influence on the MAP in any hypertensive rat through 60 minutes of infusion time.

To determine the effect on AGEPC of infusing CV-3988, rats with experimental hypertension and their control rats were separated into two groups. Half of the animals received an infusion of vehicle for 60 minutes, followed by no infusion for 60 minutes, and finally by an infusion of CV-3988 for 60 minutes. To study the effect of CV-3988 on the action of pressor hormone, norepinephrine (20 nmol/kg) was injected into normotensive Wistar rats infused with CV-3988 or vehicle. Norepinephrine solution (40 nmol/ml saline) was injected into the left femoral vein through a PE-50 tube, and CV-3988 was infused at a rate of 1 mg/kg/min through a PE-50 tube in the right femoral vein of conscious rats. The effect of CV-3988 on angiotensin II (0.6 nmol/kg) was studied in the same manner. Blood pressure was monitored directly through a PE-50 tube inserted in the right femoral artery.

Hypertensive rats with a MAP greater than 150 mm Hg 4 to 6 weeks after clipping were injected intraperitoneally with pentobarbital (32.5 mg/kg). A ventral midline incision was used to expose the silver clip. After removing the clip, the ventral wall was resutured. Either CV-3988 or vehicle infusion was started 10 minutes before unclipping the artery and continued for 60 minutes.

The data were analyzed by one-way analysis of variance, two-way analysis of variance, and Fisher's method of least significant difference. The latter test was done to perform repeated t tests comparing 1) the blood pressure at different times after CV-3988 infusion with that immediately before starting the infusion and 2) the blood pressure at different times after unclipping with that immediately before unclipping.

Results

In male Wistar rats, AGEPC was a potent hypotensive agent acting in dose-dependent manner. The dose-effect curve was shifted to the right in a competitive way by the infusion of CV-3988 (Figure 1), and the hypotensive activity of AGEPC at a dose below 0.5 nmol/kg was completely suppressed. The dose of AGEPC for 50% of maximum response was increased from 0.36 to 13 nmol/kg (p < 0.001).

The possibility that CV-3988 can modulate the hypertensive activity of norepinephrine or angiotensin II was studied in normotensive male Wistar rats (Table 1). No effect was observed on the hypertensive action of norepinephrine or angiotensin II.

CV-3988 was infused into spontaneously hypertensive rats and normotensive Wistar-Kyoto rats to study the possible participation of endogenous AGEPC in blood pressure regulation. The MAP in CV-3988-infused rats was no different from that in vehicle-
infused rats (Figure 2A), although an increase in MAP was observed from 30 through 60 minutes after starting CV-3988 infusion. Thirty minutes after starting CV-3988 infusion the MAP was significantly increased \( p < 0.05 \) compared with that in vehicle-infused rats (Figure 2B, C). Compared with the effect on vehicle infusion on blood pressure, a 60-minute CV-3988 infusion had a significant effect on blood pressure only in normotensive Wistar rats \( p < 0.05 \); Table 2).

The blood pressure in vehicle-infused 2K1C hypertensive rats decreased rapidly after unclipping the artery. The MAP dropped from 171 mm Hg to 130 mm Hg by 20 minutes after unclipping \( p < 0.001 \). With CV-3988 infusion, the rapid decrease of blood pressure was also observed 10 minutes after unclipping. But after 10 minutes, the decrease in blood pressure was significantly inhibited by CV-3988 \( p < 0.05 \); Figure 3).

**Discussion**

In rat, dog, and baboon, AGEP has a potent hypotensive activity. It has been detected in human vascular endothelial cells, rabbit renomedullary interstitial cells, and human saliva, blood, and urine. The enzymes responsible for the biosynthesis of AGEP have also been reported and include acetyltransferase and choline phosphotransferase. The role of an endogenous AGEP in blood pressure regu-

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**Table 1. Effect of CV-3988 on the Actions of Angiotensin II and Norepinephrine in Normotensive Male Wistar Rats**

<table>
<thead>
<tr>
<th>Vasopressor</th>
<th>Dose (nmol/kg)</th>
<th>MAP change (mm Hg)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angiotensin II</td>
<td>4</td>
<td>CV-3988: +44±6</td>
<td>NS</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>20</td>
<td>Vehicle: +43±6</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data are presented as means ± SD. MAP = mean arterial pressure; NS = not significant.

**Table 2. Change in Mean Arterial Pressure (MAP) after 60 Minutes of Either Vehicle or CV-3988 Infusion in Rats with Hypertension of Various Etiologies**

<table>
<thead>
<tr>
<th>Group</th>
<th>CV-3988</th>
<th>Vehicle</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHR</td>
<td>105.24 ± 6.74*</td>
<td>98.51 ± 4.68</td>
</tr>
<tr>
<td>WKY</td>
<td>104.19 ± 2.95*</td>
<td>101.3 ± 2.3</td>
</tr>
<tr>
<td>1K1C</td>
<td>102.33 ± 1.68</td>
<td>101.3 ± 2.2</td>
</tr>
<tr>
<td>2K1C</td>
<td>103.27 ± 1.91</td>
<td>101.88 ± 3.39</td>
</tr>
<tr>
<td>DOC-salt</td>
<td>103.14 ± 2.48</td>
<td>101.62 ± 1.87</td>
</tr>
<tr>
<td>ARH</td>
<td>104.09 ± 4.05</td>
<td>102.48 ± 3.11</td>
</tr>
<tr>
<td>Wistar</td>
<td>104.12 ± 5.29*</td>
<td>98.71 ± 2.54</td>
</tr>
</tbody>
</table>

Values are means ± SD. The data shown are the immediate postinfusion values expressed as percent of blood pressure levels just before infusion \( *p < 0.01, \p  < 0.001, \p  < 0.05 \) (two-way analysis of variance between two groups). SHR = spontaneously hypertensive rats; WKY = Wistar-Kyoto rats; 1K1C = one-kidney, one clip; 2K1C = two-kidney, one clip; DOC = deoxycorticosterone; ARH = adrenal regenerative hypertensive.

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**Figure 1. Effect of 3-(N-n-octadecylcarbamoyloxy)-2-methoxypropyl-2-thiazolioethylphosphate (CV-3988) on dose-effect curve of acetyl glyceryl ether phosphorylcholine (AGEPC) in Wistar rats. The hypotensive activity of AGEPC was studied with CV-3988 (closed circles) or vehicle (open circles) infusion in male Wistar rats \( n = 3; \text{mean} ± \text{SEM} \).**

**Figure 2. A. Effect of 3-(N-n-octadecylcarbamoyloxy)-2-methoxypropyl-2-thiazolioethylphosphate (CV-3988) on mean arterial pressure (MAP) in spontaneously hypertensive (SHR) and Wistar-Kyoto (WKY) rats. B, C. The MAP change in SHR and WKY. The data were analyzed by two-way analysis of variance.**
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Figure 3. Effect of 3-(N-octadecylcarbamoyloxy)-2-methoxypropyl-2-thiazolioethylphosphate (CV-3988) on the reversal of blood pressure after unclipping in two-kidney, one clip hypertensive rats. *p < 0.05, **p < 0.01. blood pressure after unclipping was significantly lower than that before unclipping as determined by the one-way analysis of variance and Fisher's method of least significant difference. Analysis between the two groups was done by two-way analysis of variance.

Our results suggest that an endogenous AGEPC produced by kidney or vascular endothelial cells may participate in blood pressure regulation in spontaneously hypertensive and normotensive rats. A pathophysiological participation by AGEPC in secondary hypertension was not seen from our results (see Table 2). In addition, in rats with 2K1C hypertension, CV-3988 significantly inhibited the initial rapid decrease in blood pressure after unclipping (see Figure 3). Short-term hypotension in normotensive rats has been observed by Göthberg et al. after extracorporeal perfusion of the unclipped kidney of 2K1C hypertensive rats. These results strongly suggest that a humoral factor that lowers the blood pressure is released from the unclipped kidney and an endogenous AGEPC may be one of the factors that lower the blood pressure in the unclipping system in Goldblatt hypertensive rats.

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