ATTENUATION OF THE DEVELOPMENT OF HYPERTENSION IN SPONTANEOUSLY HYPERTENSIVE RATS BY CHRONIC ORAL ADMINISTRATION OF EICOSAPENTAENOIC ACID

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Chronic effects of highly purified eicosapentaenoic acid (EPA) on systolic blood pressure in spontaneously hypertensive rats (SHR) and normotensive rats were studied. Daily oral administration of 30 to 300 mg/kg EPA for eight weeks significantly decreased the development of hypertension in SHR dose-dependently. Eight weeks treatment of 30, 100, and 300 mg/kg EPA reduced mean systolic blood pressure by 23, 29, and 32 mmHg, respectively, compared to untreated rats. Hypotensive effect of EPA progressed slowly and was reversible after the termination of the treatment. However, daily administration of EPA to normotensive rats did not affect the systolic blood pressure. EPA may be useful as a hypotensive agent for treatment of hypertension.

Keywords — eicosapentaenoic acid; hypertension; spontaneously hypertensive rat; oral administration; systolic blood pressure

INTRODUCTION

Hypertension is believed to be initiated and maintained by multiple factors and the importance of the nutrition factor has been recognized. Numerous experimental and clinical studies show a strong positive correlation between salt intake and the development of hypertension. However, not many experimental studies have been focused on the role of nutrition factors other than salt intake for hypertension.

Eicosapentaenoic acid (EPA) is found in high concentration in fish such as sardine, mackerel and pike. Dyerberg and Bang suggested that a high dietary intake of this highly unsaturated fatty acid may be involved in the low incidence of cardiac infarction in Greenland Eskimos. Since Eskimos also have comparatively lower blood pressure, it is plausible that high dietary intake of EPA can reduce the blood pressure.

In the present study, the chronic effect of highly purified EPA on blood pressure in spontaneously hypertensive rats (SHR) as well as normotensive rats was investigated. Orally administered EPA showed a marked hypotensive effect in SHR but not in normotensive rats.

METHODS

As a preliminary experiment, the acute effect of EPA on blood pressure of SHRs was studied.

300 mg/kg EPA was given orally to three male 15-week-old SHR and the systolic blood pressure was monitored every one hour for 12 h, and for 1, 2, and 3 d after the treatment.

To study the chronic effect of EPA, male SHR were divided into 5 groups. Group 1 was the untreated control. Treatments were started when the rats were 10 weeks old and continued for 8 weeks. EPA was administered per os at a dose of 30, 100 or 300 mg/kg/d (group 3 to 5), and the chronic effect of the vehicle was also studied (group 2). EPA or the vehicle was administered 6 d a week, and the systolic blood pressure and heart rate were recorded every seven days before the daily treatment of EPA. Systolic blood pressures of group 1 and 4 were measured at the 11th week of the experiment, 3 weeks after the cessation of EPA treatment.

Nine week-old normotensive Wistar rats whose body weights were comparable to 10-week-old SHR were also used. EPA (100 mg/kg/d) was administered orally for 7 weeks.

The rats were fed standard animal chow for mice and rats (CE-2; Clea Japan), and tap water ad libitum. The systolic blood pressure and heart rates were measured with a tail-cuff plethysmograph (KN-0090; Natsume, Japan). EPA ethyl ester (purity 90%; Nissui Seiyaku, Japan) was dissolved in 0.5% carboxymethyl cellulose solu-
tion with one droplet/100 ml of polysorbate 80.

To compare means from more than two groups in the SHR experiment, an analysis of variance was first performed. Only if significant differences were detected, the Bonferroni t-test was applied to identify the source of difference. Since only one treatment was applied to normotensive rats, the data from these two groups were analyzed using unpaired Student's t-test.

RESULTS

Single administration of EPA did not reduce blood pressure in SHR. EPA (300 mg/kg p.o.) was given to three SHRs and the systolic blood pressure was monitored every one hour for 12 h, and every 24 h for 3 d. Any appreciable change in the blood pressure was detected after a single administration of EPA. We concluded that orally applied EPA did not affect blood pressure acutely. Therefore, EPA was administered for a longer period of time in the following study.

Daily treatment of EPA significantly diminished the hypertension dose-dependently in SHR. Figure 1 depicted the effect of orally administered EPA on systolic blood pressure during the course of the experiment. Untreated SHR developed hypertension and the systolic blood pressure exceeded 200 mmHg after the age of 15 weeks. Orally administered EPA attenuated the development of hypertension. Hypotensive effect of EPA was first detected after 3 weeks of treatment and the systolic blood pressure did not rise thereafter. Eight weeks treatment of 30 mg/kg EPA reduced systolic blood pressure by 23 mmHg and 300 mg/kg reduced by 32 mmHg.

The vehicle in itself did not affect the blood pressure. Although we did not examine systematically, neither treatment produced a toxic effect nor extraordinary behavior of the rats. Neither heart rates nor body weights were affected by any treatment. Data of pre-treatment control, the 4th week, and the 8th week are summarized in Table I.

Hypotensive effect of EPA was reversible. After 8 weeks of treatment, EPA administration was withdrawn for 3 weeks and blood pressure of group 1 and 4 were measured. After the cessation, the systolic blood pressure of group 4 increased to 208.3 ± 7.6 mmHg (mean ± S.D.), which was not significantly different from the value of group 1, 213.5 ± 5.6 mmHg.

In contrast to the result in SHR, no significant effect of EPA on the blood pressure of normotensive rats was detected. 100 mg/kg/d of EPA was administered to normotensive Wistar rats with the same procedure used for SHR. Although the systolic blood pressure showed a tendency to decrease with EPA treatment, values were not significantly different from the control (Fig. 2). Neither heart rate nor body weight was influenced.

DISCUSSION

Orally administered EPA produced a marked hypotensive effect in SHR. The effect was dose-dependent, reversible, and manifested very slowly. At least 3 weeks of treatment were necessary to detect a significant effect on the blood pressure. Hypotensive effect of EPA is reversible and disappeared completely after 3 weeks cessa-

![FIG. 1. Time Course of the Change in Systolic Blood Pressure of Five SHR Groups](image-url)

Untreated control (○), oral administration of vehicle (●), 30 mg/kg EPA (□), 100 mg/kg EPA (■), and 300 mg/kg EPA (■). Daily administration was started just after measuring 10th week blood pressure and continued for 8 weeks. 30 to 300 mg/kg/d EPA significantly retarded the development of hypertension after 3 weeks. The vehicle did not affect the blood pressure. Standard deviation of each point was between 2.0 and 11.4, however, only points to represent mean values from 6 to 8 rats are shown to avoid redundancy. Values of 10, 14, and 18 week-old rats are summarized in Table I.
TABLE I.  Chronic Effect of EPA on Body Weight, Heart Rate, and Systolic Blood Pressure in SHR

<table>
<thead>
<tr>
<th>No. Treatment</th>
<th>0 Week (^a/)</th>
<th>4 Week</th>
<th>8 Week</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Body weight (g)</td>
<td>Heart rate (/min)</td>
<td>Systolic blood pressure (mmHg)</td>
</tr>
<tr>
<td>1 Control</td>
<td>6 266.0 404</td>
<td>152.6</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>±16.8 ±78</td>
<td>±6.9</td>
<td>–</td>
</tr>
<tr>
<td>2 Vehicle</td>
<td>7 274.4 399</td>
<td>155.0</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>±23.3 ±104</td>
<td>±6.0</td>
<td>–</td>
</tr>
<tr>
<td>3 EPA 30 mg/kg</td>
<td>8 263.1 400</td>
<td>158.4</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>±15.3 ±91</td>
<td>±11.0</td>
<td>–</td>
</tr>
<tr>
<td>4 EPA 100 mg/kg</td>
<td>8 265.3 378</td>
<td>154.6</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>±17.2 ±63</td>
<td>±9.7</td>
<td>–</td>
</tr>
<tr>
<td>5 EPA 300 mg/kg</td>
<td>8 259.5 394</td>
<td>155.1</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>±8.7 ±101</td>
<td>±10.4</td>
<td>–</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.D.  
\(^a/\) Data from 0 week were recorded before the initiation of the treatment.  
\(^b/\) Systolic blood pressure was significantly different from the group(s) listed in this column (p < 0.05). See Methods for statistical analysis. No significant difference was detected from the values of body weight and heart rate.

![Systolic blood pressure vs. Age (week)](image)

FIG. 2.  The Effect of EPA on Systolic Blood Pressure in Normotensive Rats

Daily administration of EPA (100 mg/kg) was begun at the age of 9 weeks and continued for 7 weeks. Systolic blood pressure from the rats received EPA showed a tendency to decrease. However, statistical analysis failed to show the difference from the value of untreated control. Symbols and vertical bars represent mean ± S.D. from 7 (control) or 8 (EPA) rats. ○, control; ●, EPA 100 mg/kg.

The effect of dietary fatty acid on blood pressure is controversial. In SHR a high level of linoleic acid in the diet augments the hypertension.\(^5\) High fat diet exaggerates a salt-induced hypertension in rats.\(^6\) Nijkamp and de Jong\(^7\) showed that rats fed an essential fatty acid-deficient diet developed lower levels of renal hypertension and the supplement of linoleic acid eliminated the hypotensive effect. In contrast to these results, MacDonald \(et\ al.\)\(^8\) reported that the deficiency of linoleic acid results in an increased blood pressure in rats. SHR's fed high fat diet do not develop hypertension.\(^9\) Whether dietary linoleic acid shows hypotensive effect or hypertension may depend on the type of synthesized prostaglandins as suggested by Ahnfelt-Ronne and Arrigoni-Martelli.\(^10\) It is also important to examine what the active substance lost or supplemented is in a low fat or a high fat diet.

Scherhag \(et\ al.\)\(^11\) reported that 2.5 to 5 energy % dietary EPA with 5 energy % linoleic acid and 5 energy% oleic acid increase the systolic blood pressure in Sprague-Dawley rats. However, in the present experiment, 100 mg/kg/d of EPA, which corresponds about 0.4 to 0.5 energy %, assuming that the rat takes chow 20 g a day, did not affect the blood pressure in normotensive Wistar rats (Fig. 2). Since Scherhag \(et\ al.\) used cod liver oil as a source of EPA, ingredients other than EPA in cod liver oil may elevate the blood pressure. Lockette \(et\ al.\)\(^12\) reported the decreased response to norepinephrine and arachidonic acid of isolated aorta from rats supplemented with menhaden fish oil (containing 17% EPA) for 3 weeks. Low sensitivity to catecholamine in EPA-treated rats explains the hypoten-
sive effect, but these authors did not measure the blood pressure.

Morita et al. \textsuperscript{13} showed that the inhibitory effect of EPA supplementation on platelet aggregation is dependent on the species studied. EPA inhibited the platelet aggregation in humans but not in rats.\textsuperscript{14} Dyerberg et al. \textsuperscript{15} postulated that the formation of trienic prostaglandin (PG) I\textsubscript{3} and thromboxane (TX) A\textsubscript{3} from EPA, which inhibit platelet aggregation or have very weak aggregating activity, is the cause of the low rate of cardiovascular disease in Eskimos. In humans, EPA is easily converted to PGI\textsubscript{3} in cultured vascular smooth muscle cells and blocks TXA\textsubscript{2} synthesis in platelets.\textsuperscript{13} On the contrary, EPA is not converted to PGI\textsubscript{3} in vascular smooth muscle or TXA\textsubscript{3} in platelets of rat.\textsuperscript{13,14,16} Therefore, rats do not seem to respond to EPA. However, in the present study, EPA decreased systolic blood pressure in SHR. These results indicate either that the hypotensive effect of EPA is different from antiaggregative action or that the hypertensive animal is more sensitive to EPA. The present results that EPA did not affect the blood pressure of normotensive rats supports the latter possibility. The metabolic fate or the site of action of EPA may be different in SHR.

At present, the mechanism of how EPA reduces blood pressure in SHRs is not known. Single oral administration of EPA did not affect the blood pressure in SHR. The attenuation of hypertension by EPA was detected after 3 weeks of treatment. These results showed that EPA reduced blood pressure through a mechanism which required a long period. EPA inhibits the formation of PGI\textsubscript{2} in cultured rat vessel and in perfused rabbit ear possibly by reducing the content of arachidonic acid.\textsuperscript{13,14,17} Lukacsko et al. \textsuperscript{18} suggested decreased ability to utilize arachidonic acid for the synthesis of PGI\textsubscript{2} in SHR. However, the decrease in the production of PGI\textsubscript{2}, a potent vasodilator, will exaggerate hypertension. Blood pressure is known to be controlled by various factors. Studies of effects of EPA on renin-angiotensin, kallikrein–kinin, and sympathetic nervous systems as well as on arachidonic acid-cascade may be necessary to elucidate the hypotensive action of EPA.

Recently, the hypotensive effect of EPA was demonstrated in human subjects by two groups. Systolic blood pressure in an upright position of volunteers supplemented with EPA for 25 d was decreased.\textsuperscript{19} However, a hypotensive effect was not detected in a supine position and diastolic pressure was not altered. Two weeks of a diet supplemented with EPA decreased systolic but not diastolic blood pressure of patients with mild essential hypertension.\textsuperscript{20} Hypotensive effect of EPA was considerably stronger in normotensive volunteers. Although the effect is weak, chronic administration of EPA reduces the blood pressure of human subjects even in normotensive condition. There may be also a species difference in the hypotensive effect of EPA. However, since these studies on human subjects used cod liver oil\textsuperscript{19} or mackerel and herring fillets\textsuperscript{20} as the source of EPA, other ingredient(s) might have affected the blood pressure.

EPA may be useful to relieve hypertension as well as to prevent myocardial infarction. Further studies are necessary to investigate why dietary EPA relieve hypertension.

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REFERENCES
10) I. Ahnfelt-Ronne and E. Arrigoni-Martelli: Increased
Hypotensive Effect of EPA


