Release of Fatty Acids by Perfused Vascular Tissue in Normotensive and Hypertensive Rats

JACOB P. MTABAJI, MEHAR S. MANKU, AND DAVID F. HORROBIN

SUMMARY The release of fatty acids from perfused mesenteries of spontaneously hypertensive rats (SHR) and control Wistar-Kyoto rats (WKY) was studied. The release of the prostaglandin precursors dihomogammalinolenic acid, arachidonic acid, and eicosapentaenoic acid was reduced in SHR when compared with age-matched WKY. The release of all other fatty acids detected in the effluent was also reduced. The differences in fatty acid release were evident even when tissue levels of the fatty acids were similar or higher in SHR than in controls. The addition of evening primrose oil and fish oil into the diet partially corrected these defects. Evening primrose oil and fish oil both attenuated increases in blood pressure, but fish oil was more potent than primrose oil. Although both diets reduced vascular reactivity, primrose oil was more effective with lower doses of norepinephrine whereas fish oil blunted the effects of both low and high doses of norepinephrine. The possible mechanisms for the effects of primrose oil and fish oil on vascular reactivity are briefly discussed. (Hypertension 12: 39-45, 1988)

KEY WORDS • hypertension • fatty acids • arachidonic acid • dihomogammalinolenic acid • eicosapentaenoic acid

POLYUNSATURATED fatty acids (PUFAs) added to the diet attenuate the development of hypertension or lower blood pressure in both animals and humans.1-14 The mechanism of the hypotensive effect is not well understood, although it is commonly believed to result from alterations in arachidonate-derived cyclooxygenase metabolites. However, both n-6 and n-3 fatty acids lower blood pressure, although their effects on prostaglandin synthesis are dissimilar. The fatty acids themselves alter membrane fluidity and blood viscosity and may have effects on blood pressure that are independent of their conversion to prostaglandins.

The effect of diets rich in n-6 fatty acids in rats is generally an increase in tissue arachidonic acid (AA) and its conversion to cyclooxygenase metabolites.8-15 In contrast, diets rich in n-3 fatty acids are associated with a decrease in AA and a decrease in the synthesis of its metabolites.8, 15-17 These opposing observations cast doubt on the relevance of AA and its metabolites in the beneficial effects of PUFAs.

Dihomogammalinolenic acid (DGLA) is worthy of further consideration. It gives rise to metabolites that are hypotensive and antiaggregatory and that can inhibit conversion of AA to leukotrienes.18, 19 A low level of DGLA in adipose tissue in healthy men seems to be a strong predictor of future coronary heart disease.20 Whereas AA enhances vascular resistance in perfused vascular beds, DGLA reduces it.21-23 Diets rich in both n-3 and n-6 PUFAs, while having opposite effects on AA, raise DGLA, n-6 by increasing its rate of formation and n-3 by reducing its conversion to AA.24, 25

While considerable attention has been paid to production of prostaglandins in spontaneously hypertensive rats (SHR), little work has been done on the release of prostaglandin precursors such as DGLA, AA, and eicosapentaenoic acid (EPA) from vascular tissue. We have therefore compared release of fatty acids in SHR and in normotensive Wistar-Kyoto rats (WKY), paying particular attention to DGLA, EPA, and AA, the main prostaglandin precursors.

Materials and Methods

Male 4-week-old SHR and age-matched WKY were obtained from Charles River, Montreal, Que- bec, Canada. The animals were allowed a 1-week stabilizing period before experiments were started. They were kept in metabolic cages in groups of three to four, with free access to tap water and food. SHR were randomized into one of three groups (n = 8/group). One group was kept on a regular rat chow diet. The other two groups were given a
regular diet supplemented with 5 g of Efamol evening primrose oil (EPO) or 5 g of fish oil (both from Efamol, Guildford, Surrey, UK) per 100 g of diet. Fish oil was given in the form of Polepa, a partially concentrated fish body oil free of vitamins A and D. All WKY were kept on a regular diet (n = 8). The diets were continued for 12 weeks. The compositions of the diets are shown in Table 1. The EPO diet was particularly rich in n-6 fatty acids, mainly linoleic acid. The EPO diet was also unusual in that it contained γ-linolenic acid, the desaturated linoleic acid metabolite, important because of evidence of reduced desaturation in SHR. The fish oil diet was rich in n-3 fatty acids, mainly EPA and docosahexaenoic acid.

The systolic blood pressure of the animals was determined at the end of the feeding period in prewarmed, conscious animals using a tail pulse pickup device from Narco Bio-Systems (Houston, TX, USA).

Mesenteric vessels were prepared and perfused according to the method of McGregor as previously described. The superior mesenteric artery was cannulated with the rats under ether anesthesia, and the mesentery was dissected free at its border with the intestine. The preparations were suspended in organ baths and perfused with Krebs-Ringer bicarbonate buffer with a peristaltic flow inducer. The buffer contained 122 mM sodium, 4.3 mM potassium, 1.0 mM magnesium, 2.5 mM calcium, 1.7 mM phosphate, 25 mM bicarbonate (the remaining anion chloride), and glucose, 1 g/L. The buffer was bubbled continuously with 5% carbon dioxide in oxygen. Two types of experiments were then performed with the perfused vessels.

In one set of experiments, essential fatty acids-free bovine serum albumin was added to the perfusing medium in a concentration of 1 mg/ml to act as a trap for free fatty acids, as suggested by Isaksen et al. The effluent was then collected for 1 hour and analyzed for its free fatty acid content. A 20-minute washout period was allowed before collection of the effluent was started. During this washout period albumin-free buffer was used. In the other set of experiments, vascular reactivity was investigated with bolus injections of norepinephrine. Norepinephrine was injected and dissolved in a volume of 0.1 ml of normal saline.

Each effluent collection was extracted twice with 1.5 volumes of chloroform/methanol (2:1 vol/vol) containing 0.02% 2,6-di-tert-butyl-p-cresol to limit autoxidation. The solvent was then evaporated in vacuo, and a concentrate of the extract was applied on silica gel thin-layer chromatography plates. The plates were developed in a solvent mixture of hexane/ether/acetic acid in a ratio of 80:20:1. The fractions were then methylated with boron trifluoride in methanol (14% wt/vol) at 90 °C for 30 minutes. The resulting methyl esters were then taken up in hexane, and the fatty acid composition was analyzed using a Hewlett-Packard 5880 gas chromatograph (Palo Alto, CA, USA), as previously reported.

Before extraction, a known quantity of heptadecanoic acid (17:0) was added to the effluent as an internal standard. An aliquot of each 1-hour collection was used for direct radioimmunoassay without extraction for thromboxane B (the stable metabolite of thromboxane A2) and for 6-keto-prostaglandin F1α (6-keto-PGF1α; the stable metabolite of prostacyclin). The procedure has been described in detail previously.

Sensitivities of the assays, determined by the amount of standard per assay tube required to inhibit binding of the label to the antibody by 10%, were 8 pg for 6-keto-PGF1α and 5 pg for thromboxane B2. Cross-reactivity of the 6-keto-PGF1α antiserum was less than 1% for thromboxane and for E and F prostaglandins. Cross-reactivity of the thromboxane antiserum was less than 1% for 6-keto-PGF1α and for E and F prostaglandins.

After use, all mesenteries were weighed and homogenized and then extracted with 20 ml of chloroform/methanol. The extract was evaporated to a constant weight. An aliquot of the total lipid was then subjected to fatty acid analysis after methylation, as already described. The absolute amount of each fatty acid was calculated from the weight of the total lipid.

To determine the fatty acid composition of the phospholipid fraction, a preparatory separation of neutral and complex lipids was performed using the solvent partition procedure of Galanos and Kappoula. Briefly, the extract was dissolved in 15 ml of 87% ethanol and added to 45 ml of hexane in a separating funnel. After thorough shaking, the bottom layer was run into a second funnel containing 45 ml of hexane and the two phases were mixed. The bottom ethanolic phase was then run off. An additional 15 ml of fresh ethanol was added to the first funnel, and the procedure was repeated. Six further portions of fresh ethanolic phases were shaken with hexane in this way. The combined
Results

SHR on a regular diet had significantly higher systolic blood pressures (191 ± 4 mm Hg) than their normotensive controls (126 ± 5 mm Hg; p < 0.005). Pressure in the EPO-fed group (171 ± 5 mm Hg) was significantly lower than that in untreated SHR (p < 0.02), while pressure in the fish oil-fed group was significantly lower than that in the EPO-fed group (149 ± 9 mm Hg; p < 0.01).

Bolus injection of norepinephrine produced dose-dependent transient rises in perfusion pressure in both WKY and SHR (Figures 1 and 2). Mesenteric vascular beds in SHR were more reactive than those in WKY at all norepinephrine doses tested (p < 0.01). At low doses of norepinephrine, pressor responses were closer to those in WKY in the EPO group than in the fish oil group (see Figures 1 and 2). However, at high doses of norepinephrine, the EPO diet had little or no effect, whereas the fish oil diet was more effective than at lower norepinephrine doses.

A variety of fatty acids were released into the effluent. The major ones are shown in Table 2. For every single fatty acid, whether saturated, monounsaturated, or polyunsaturated, the quantity released was significantly less in SHR than in WKY.

The effects of fish oil and EPO diets in SHR are shown in Table 3. Fish oil lowered linoleic acid and AA release even further, but substantially increased the output of DGLA and, especially of EPA. EPO raised linoleic acid and DGLA output to values that were not significantly different from those in WKY, but it had no significant effects on either AA or
Table 3. Effluent Essential Fatty Acids Released from the Superior Mesenteric Vascular Bed of the SHR During 1 Hour of Perfusion

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Control SHR (μg)</th>
<th>SHR fed EPO (μg)</th>
<th>SHR fed FO (μg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>18:2 n-6</td>
<td>139±13</td>
<td>217±16*</td>
<td>104±11</td>
</tr>
<tr>
<td>20:3 n-6</td>
<td>0.9±0.1</td>
<td>3.9±0.3*</td>
<td>2.4±0.2*</td>
</tr>
<tr>
<td>20:4 n-6</td>
<td>23.7±1.4</td>
<td>27.0±1.6</td>
<td>13.8±0.9*</td>
</tr>
<tr>
<td>20:5 n-3</td>
<td>3.2±0.3</td>
<td>2.1±0.2</td>
<td>32.7±3.1*</td>
</tr>
</tbody>
</table>

Values are means ± SEM (n = 8/group). EPO = evening primrose oil; FO = fish oil.
*p < 0.05, compared with control values.

Table 4. Total Fatty Acid Content in the Mesenteric Vascular Beds

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>WKY (mg)</th>
<th>SHR (mg)</th>
<th>EPO (mg)</th>
<th>FO (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:0</td>
<td>31.3±1.8</td>
<td>35.2±1.8</td>
<td>25.9±3.4*</td>
<td>85.5±6.1</td>
</tr>
<tr>
<td>16:0</td>
<td>373.8±28.3</td>
<td>434.3±22.3</td>
<td>359.2±35.4</td>
<td>572.8±42.2</td>
</tr>
<tr>
<td>18:0</td>
<td>91.4±10.0</td>
<td>85.7±4.5</td>
<td>92.9±4.6</td>
<td>114.2±8.7*</td>
</tr>
<tr>
<td>18:1 n-9</td>
<td>608.6±32.2</td>
<td>562.0±44.5</td>
<td>437.3±24.0*</td>
<td>636.1±39.1</td>
</tr>
<tr>
<td>18:2 n-6</td>
<td>447.3±23.3</td>
<td>336.0±31.0*</td>
<td>857.8±56.9*</td>
<td>412.5±52.5*</td>
</tr>
<tr>
<td>18:3 n-6</td>
<td>1.1±0.6</td>
<td>1.7±0.14</td>
<td>44.5±4.4*</td>
<td>5.1±2.1</td>
</tr>
<tr>
<td>18:3 n-3</td>
<td>23.6±1.4</td>
<td>18.3±1.8</td>
<td>16.8±1.5</td>
<td>23.5±2.0</td>
</tr>
<tr>
<td>20:3 n-6</td>
<td>3.2±0.4</td>
<td>1.5±0.04</td>
<td>8.7±1.03*</td>
<td>3.4±0.36*</td>
</tr>
<tr>
<td>20:4 n-6</td>
<td>21.6±2.3</td>
<td>15.9±0.45</td>
<td>24.7±3.41*</td>
<td>16.7±1.6</td>
</tr>
<tr>
<td>20:5 n-3</td>
<td>2.9±0.14</td>
<td>2.1±0.1</td>
<td>1.7±0.19</td>
<td>37.9±3.8*</td>
</tr>
</tbody>
</table>

Values are means ± SEM (n = 8/group). EPO = evening primrose oil; FO = fish oil.
*p < 0.05, compared with values in control SHR.
†p < 0.05, compared with values in control WKY.

Table 5. Total Fatty Acids in the Phospholipid Fraction from the Mesenteric Beds

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>WKY (mg)</th>
<th>Control (mg)</th>
<th>EPO-fed (mg)</th>
<th>FO-fed (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:0</td>
<td>8.8±0.5</td>
<td>14.7±1.2*</td>
<td>15.5±1.9</td>
<td>17.3±1.1</td>
</tr>
<tr>
<td>18:0</td>
<td>3.4±0.5</td>
<td>4.3±0.4</td>
<td>5.1±0.4</td>
<td>5.0±0.4</td>
</tr>
<tr>
<td>18:1 n-9</td>
<td>10.7±1.0</td>
<td>17.8±2.0*</td>
<td>14.4±1.6</td>
<td>16.7±2.3</td>
</tr>
<tr>
<td>18:2 n-6</td>
<td>9.6±0.8</td>
<td>12.3±1.0*</td>
<td>29.3±3.6†</td>
<td>11.5±1.5</td>
</tr>
<tr>
<td>20:3 n-6</td>
<td>0.11±0.01</td>
<td>0.12±0.01</td>
<td>0.37±0.04†</td>
<td>0.27±0.05†</td>
</tr>
<tr>
<td>20:4 n-6</td>
<td>3.8±0.2</td>
<td>4.3±0.3</td>
<td>4.3±0.5</td>
<td>2.6±0.05†</td>
</tr>
<tr>
<td>20:5 n-3</td>
<td>0.27±0.02</td>
<td>0.28±0.08</td>
<td>0.12±0.01†</td>
<td>2.6±0.30†</td>
</tr>
</tbody>
</table>

Values are means ± SEM (n = 8/group). EPO = evening primrose oil; FO = fish oil.
*p < 0.05, compared with values in WKY.
†p < 0.05, compared with values in control WKY.
TABLE 6. Ratios of Effluent Fatty Acid Quantities to the Amounts of Fatty Acid in Total Lipid and in Phospholipid in the Mesenteric Bed

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Total lipid</th>
<th>Phospholipid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WKY (n = 8)</td>
<td>SHR (n = 8)</td>
</tr>
<tr>
<td></td>
<td>WKY (n = 8)</td>
<td>SHR (n = 8)</td>
</tr>
<tr>
<td>16:0</td>
<td>6.5</td>
<td>2.9</td>
</tr>
<tr>
<td>18:1 n-9</td>
<td>4.2</td>
<td>2.3</td>
</tr>
<tr>
<td>18:2 n-6</td>
<td>5.9</td>
<td>4.1</td>
</tr>
<tr>
<td>20:3 n-6</td>
<td>9.2</td>
<td>6.0</td>
</tr>
<tr>
<td>20:4 n-6</td>
<td>16.7</td>
<td>14.9</td>
</tr>
<tr>
<td>20:5 n-3</td>
<td>21.4</td>
<td>15.2</td>
</tr>
</tbody>
</table>

The total ratios have been multiplied by 10. Without exception the ratios in the WKY were greater than those in the SHR.

than levels in the WKY (p < 0.05). The dramatic changes were in the fish oil–fed SHR group, where levels of both eicosanoids were highly significantly reduced as compared with all other groups (p < 0.001). This reduction could not be accounted for on the basis of the reduced amount of free AA in the effluent, since the ratio of eicosanoid to free AA was dramatically reduced.

Discussion

The main observation in these experiments is the reduced release of all fatty acids in the SHR as compared with the WKY. This reduced release cannot be explained by smaller amounts of the fatty acids in either the total lipid or the phospholipid fraction of the whole vascular bed, including the adipocytes. The possibility that a phospholipid or other fraction present in blood vessel wall might contain smaller amounts of fatty acids in SHR than in WKY cannot be excluded by these experiments. Since the effect was apparent with all fatty acids, saturated, monounsaturated, and polyunsaturated, it is unlikely to be related to any of the specific mechanisms that have been proposed for the regulation of release of prostaglandin precursors.

The reduced release of prostaglandin precursors may help to explain the reduced rates of prostaglandin synthesis in perfused vascular beds from SHR reported in these and other experiments. These studies also provide direct proof of impaired DGLA release, as suggested earlier on the basis of indirect observations in SHR. Impaired substrate release would explain the reduced prostaglandin synthesis that has been reported in other forms of hypertension.

Our observations appear to contradict reports of increased conversion of exogenous AA to prostaglandins in various tissues from SHR. However, this contradiction is only apparent, and our experiments actually provide an explanation for the differing reports on whether prostaglandin synthesis is enhanced or reduced in SHR. Enhanced prostaglandin synthesis has been reported in situations in which exogenous AA has been used as the substrate. If, as our experiments show, free AA levels are reduced in SHR, then addition of labeled AA would meet with less competition from endogenous unlabeled AA and conversion of the exogenous material to prostaglandins would be enhanced. Enhanced microsomal prostaglandin synthetase activity in SHR may be a reflection of defective fatty acid release, an attempt to compensate for reduced substrate availability. Some evidence of this was seen in our study, in which a greater proportion of the available AA was converted to 6-keto-PGF1α in the SHR than in the WKY (see Table 7).

At present we have no knowledge as to the nature of the defect in fatty acid metabolism or the detailed pool of tissue fatty acids that gives rise to the effluent fatty acids. The pool concerned seems to be in equilibrium with the diet, since the reduced amounts of linoleic acid and DGLA in the effluent from SHR could be corrected by EPO, rich in linoleic acid and γ-linolenic acid, the immediate DGLA precursor. Further experiments are required to define whether the fatty acids are coming from the adipose tissue or the vascular wall itself in the mesenteric bed.

It seems unlikely that differences in AA release or formation of eicosanoids derived from AA can explain the hypotensive effects of both EPO and fish oil. The reduced AA release in the SHR as compared with WKY was slightly corrected by EPO but was exaggerated by fish oil. The reduced production of eicosanoids was made rather more pronounced by EPO and much more so by fish oil.

The only changes that were made in the same direction and toward WKY values by both EPO and fish oil were the rise in release of DGLA and the

TABLE 7. Concentrations of Thromboxane B2 and 6-keto-PGF1α (the Main Metabolite of Prostacyclin) in the 1-Hour Collection

<table>
<thead>
<tr>
<th>Group</th>
<th>TXB2 (pg/ml)</th>
<th>TXB2/AA (pg/ml)</th>
<th>6-keto-PGF1α (pg/ml)</th>
<th>6-keto-PGF1α/AA (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKY</td>
<td>381 ± 45</td>
<td>10.6</td>
<td>4580 ± 310</td>
<td>126.9</td>
</tr>
<tr>
<td>SHR</td>
<td>258 ± 57</td>
<td>10.9</td>
<td>3790 ± 620</td>
<td>159.9</td>
</tr>
<tr>
<td>Treated SHR</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EPO</td>
<td>219 ± 32</td>
<td>12.3</td>
<td>3100 ± 240</td>
<td>114.8</td>
</tr>
<tr>
<td>FO</td>
<td>28 ± 12*</td>
<td>0.49</td>
<td>450 ± 90*</td>
<td>32.6</td>
</tr>
</tbody>
</table>

Values, except ratios, are means ± SEM (n = 8/group). The eicosanoids were measured by direct radioimmunoassay without extraction as previously described. TXB2 = thromboxane B2; AA = arachidonic acid. EPO = evening primrose oil; FO = fish oil.

*p < 0.001, compared with values in all other groups.
reduced ratio of DGLA to AA. With EPO, the rise in DGLA was presumably due to increased provision of ω-6-linolenic acid coupled with the known low 5-5-desaturase and 5-6-desaturase activities in SHR. 24-27 With fish oil, EPA presumably inhibited 5-desaturase further, 28 leading to increased DGLA and reduced AA release.

Further investigation is required to define whether these relative changes in DGLA and AA are important in explaining the effects of EPO and fish oil. They may be relevant to the actions of the diets on vascular reactivity to norepinephrine. EPO was more effective than fish oil in attenuating responses to low doses of norepinephrine, whereas fish oil was much more effective than EPO at high norepinephrine doses. In the mesenteric bed, DGLA inhibits the pressor effects of agents that release intracellular calcium but not those that promote external calcium entry. 22-23-42 AA, in contrast, enhances the pressor effects of agents that release intracellular calcium. This defect appears to be more fundamental than the relative changes in DGLA and AA are important in explaining the effects of EPO and fish oil. These relative changes in DGLA and AA are important in explaining the effects of EPO and fish oil. These relative changes in DGLA and AA are important in explaining the effects of EPO and fish oil.

In conclusion, our results indicate that SHR have a defect in the release of all fatty acids from a tissue pool that appears to be in equilibrium with the diet. This defect appears to be more fundamental than previously reported differences between SHR and WKY in fatty acid and eicosanoid metabolism.

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