Fatty Acid Binding Protein in Kidney of Normotensive and Genetically Hypertensive Rats
SATOSHI FUJI, HIDEAKI KAWAGUCHI, AND HISAKAZU YASUDA

SUMMARY Fatty acid binding protein was purified from renal medulla, and its binding activity and fatty acid composition were determined in spontaneously hypertensive stroke-prone rats (SHRSP). Wistar-Kyoto rats (WKY) were used as controls. Fatty acid binding activity was higher in 5-week-old prehypertensive SHRSP than in control WKY (0.155 ± 0.006 vs 0.030 ± 0.001 mol palmitic acid/mol protein). However, in 40-week-old rats, the activity was decreased only in SHRSP with established hypertension (0.035 ± 0.002 vs 0.028 ± 0.003 mol palmitic acid/mol protein WKY). Fatty acid compositions were similar among 5-week-old and 40-week-old control WKY and 5-week-old SHRSP (palmitic acid, 24%; stearic acid, 14%; oleic acid, 30%; linoleic acid, 29%; arachidonic acid, 3%), although the total amount of bound long-chain fatty acids was decreased in 5-week-old SHRSP, explaining the high fatty acid binding activity in this preparation. Fatty acid binding protein from 40-week-old SHRSP had an elevated proportion of endogenous arachidonic acid, with other fatty acids being relatively reduced (palmitic acid, 8%; stearic acid, 2%; oleic acid, 4%; linoleic acid, 10%; arachidonic acid, 76%), indicating increased arachidonic acid transport in the cytosol. These results show that genetically hypertensive rats had an alteration in fatty acid transport mediated by fatty acid binding protein; this alteration may be involved in the pathogenesis of hypertension.

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KEY WORDS • fatty acid binding protein • genetic hypertension • fatty acid binding • kidney

PROSTAGLANDINS such as protacyclin and prostaglandin E₂ are thought to regulate blood pressure through their vasodilator and natriuretic properties. In spontaneously hypertensive rats (SHR), renal prostaglandin synthesis measured with medullary microsomes is increased compared with that of control Wistar-Kyoto rats (WKY). Arachidonic acid, the long-chain unsaturated fatty acid and major substrate for prostaglandin synthesis, is released by phospholipases A₂ and C from phospholipids in several tissues. We and others have observed increased phospholipase A₂ activity in the kidney of SHR, and we reported that changes in phospholipids and fatty acid with age are greater in the kidney of spontaneously hypertensive stroke-prone rats (SHRSP) than in WKY. These studies indicate that fatty acid metabolism is altered under pathological conditions of high blood pressure.

In cytosol, free fatty acids bind to a cytoplasmic protein that aids their distribution to various pools and pathways, affecting many enzyme reactions. This protein has been identified in several tissues (e.g., liver, small intestine, myocardium, and skeletal muscle). The best characterized is that of rat liver, the so-called Z protein. Although the precise nature and function have not been clearly defined, it seems to play an important role as a carrier protein in lipid metabolism. Some investigators have suggested that it acts as a substrate carrier in enzymatic reactions. Furthermore, it has been suggested that fatty acid binding protein (FABP) might participate in fatty acid movement through the cytosol from plasma membrane to endoplasmic reticulum. FABP has been detected in kidney, but its precise nature and physiological function remain undetermined. FABP, as a carrier protein, may play a role in hypertension through transport and metabolite regulation of fatty acids.

In the present study, we purified FABP from rat kidney cytosol and evaluated its role in hypertension. To examine fatty acid binding activity and the composition of endogenously bound fatty acids, FABP isolated from both WKY and SHRSP was used.

Materials and Methods
Male SHRSP and age-matched male control WKY were obtained from a large breeding colony at Hokkaido University School of Medicine (Sapporo, Japan). Original breeders for this colony were obtained from Okamoto-Aoki-derived SHR.
Fatty Acid Binding Protein Purification Procedure

The purification procedure was essentially similar to that reported by Takahashi et al. for Z protein. In brief, kidneys were excised from SHRSP and WKY anesthetized with α-chloralose (50 mg/kg) perfused immediately with cold 0.25 M sucrose, and sliced to 2 mm thickness. The medulla was separated carefully from the cortex, minced with scissors, and homogenized, first with a Polytron homogenizer (Model PT-10; Kinematica, Lucerne, Switzerland) at setting 7 for 60 seconds in 10 volumes (wt/vol) of 10 mM Tris HCl (pH 7.4), 10 mM KCl, 1 mM EDTA, 1 mM diothreitol (Buffer A), and then with a Potter-Elvehjem homogenizer (Model 7720, Iwaki Glass, Tokyo, Japan) filled with a Teflon pestle. EDTA was added to inhibit phospholipases A, and C, thus preventing fatty acid hydrolysis from phospholipids and triglycerides. The total homogenate was centrifuged at 30,000 g for 90 minutes at 4°C. The resultant supernatant was centrifuged at 105,000 g for 90 minutes at 4°C. The resultant supernatant was concentrated by ultrafiltration (Model UK-10; Toyo-roshi Co., Tokyo, Japan) and applied to a column (2.6 X 70 cm) of Sephadex G-75 (Pharmacia Chemical Co., Uppsala, Sweden) equilibrated with Buffer A. The fractions with high binding activity for palmitate, (Mr, 255) 0.3 M NaCl in the same buffer.

Electrophoresis and Other Methods

Polyacrylamide gel electrophoresis (PAGE) was performed according to the procedure of Maizel. Each lane was loaded with 10 to 20 μg of protein. Protein was determined by the method of Lowry et al. with bovine serum albumin as the standard. Blood pressure was measured directly through a femoral artery cannula connected to a Statham P23D pressure transducer (Oxnard, CA, USA) on the day before the animals were killed.

Statistics

Differences between the two groups of rats were tested for significance by means of the unpaired t test. Differences between groups were considered significant when the p value was less than 0.05. Results are expressed as means ± SEM.

Results

Blood Pressure

At 5 weeks of age, both SHRSP and WKY had blood pressures well within the normotensive range (100 ± 4/69 ± 3 vs 98 ± 3/69 ± 2 mm Hg), and the difference in blood pressure between strains was not significant (n = 6 per group). The 40-week-old SHRSP were distinctively hypertensive (231 ± 10/167 ± 9 mm Hg) and had significantly higher pressures than did age-matched WKY (142 ± 5/101 ± 4 mm Hg; p < 0.001 for both systolic and diastolic pressure; n = 8 SHRSP and 7 WKY).
Purification of Rat Kidney Fatty Acid Binding Protein

The FABP fractions obtained by Sephadex G-75 gel filtration were further purified by diethylaminoethyl cellulose chromatography. FABP-rich fractions were eluted by applying a linear NaCl gradient. The rat kidney FABP with or without delipidation was finally purified approximately ninefold with a 50% yield in all four FABP preparations. The amounts of purified FABP obtained per gram of tissue, wet weight, were 0.97 ± 0.09 mg for 5-week-old WKY, 0.93 ± 0.10 mg for 40-week-old WKY, 1.02 ± 0.08 mg for 5-week-old SHRSP, and 1.11 ± 0.12 mg for 40-week-old SHRSP (n = 5 per group). The 40-week-old SHRSP showed a significantly higher amount of FABP than did WKY of the same age (p < 0.05). Each preparation of FABP obtained from the two groups at different ages uniformly gave a single band with the same molecular weight, 15,500, on PAGE (Figure 1). FABP (20 μg) was labeled by a [14C]palmitate tracer, resolved in the sample buffer, and applied to the PAGE. Approximately 60% of the total radioactivity was found on the position corresponding to the band of molecular weight 15,500. The rest of the radioactivity comigrated with the tracking dye, suggesting that part of the [14C]palmitate was released from the protein during electrophoresis.

Binding Activity of Fatty Acid Binding Protein

The 5-week-old rats showed different binding activities (Table 1). The binding activities for palmitate, stearate, oleate, linoleate, and arachidonate from 5-week-old SHRSP were significantly higher than those of controls. However, strain differences were not apparent in 40-week-old animals. The binding activity of FABP in 40-week-old SHRSP was significantly lower than that in 5-week-old SHRSP. No significant difference was observed between the FABP binding activity of hypertensive 40-week-old SHRSP and that of normotensive 40-week-old WKY. There was no significant difference in binding activity between 5-week-old and 40-week-old WKY. Therefore, the effect of age on binding activity was considered negligible. When delipidated FABP samples were used, equal binding of fatty acids to all FABP samples was observed (Table 2; Figure 2). The apparent dissociation constant (Kd) values for palmitate, stearate, oleate, linoleate, and arachidonate found with SHRSP and WKY at 5 and 40 weeks of age were of the same order of magnitude. The differences in the Kd values were not clearcut in the various series of experiments. As a general tendency, FABP bound unsaturated fatty acids with higher affinity than saturated fatty acids. The values were of the same order of magnitude as observed with purified FABP from rat liver and heart.27 Although maximal binding capacity values were rather variable for all fatty acids, in delipidated samples the molar ratio of binding was estimated to be approximately 1 mol fatty acid/1 mol binding protein in all samples.

Fatty Acid Analysis of Fatty Acid Binding Protein

Table 3 summarizes the fatty acid compositions of FABP from four preparations. The molar ratios of binding (total moles of long-chain fatty acids endogenously bound to 1 mol of FABP) were 0.980 in 5-week-old WKY, 0.982 in 40-week-old WKY and 0.995 in 40-week-old SHRSP. However, in 5-week-old SHRSP this value was lower than that of the other three preparations and estimated to be 0.857. The higher binding activity of FABP in 5-week-old SHRSP can be postulated from these results. When exogenous fatty acid binding activity and endogenous fatty acid binding activity (expressed as endogenously bound fatty acids) were combined, the molar binding ratio was estimated as 1 mol of total long-chain fatty acids per mole of FABP in all four preparations, regardless of strain and age. Similar fatty acid compositions were shown in FABP of the 5-week-old and 40-week-old WKY and 5-week-old SHRSP. Proportions of long-chain fatty acids were similar in these three groups, and palmitic (23.1–24.0%), stearic (13.0–14.1%),

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**Figure 1.** Left panel: Electrophoresis of 10 μg of fatty acid binding protein (FABP) from 5-week-old WKY on 15% acrylamide gel was performed as described in Methods. Right panel: Determination of the molecular weight of the purified FABP. Proteins of known subunit molecular weight used to calibrate in the gel system included lysozyme (14,400), soybean trypsin inhibitor (21,500), carbonic anhydrase (31,000), ovalbumin (45,000), and bovine serum albumin (66,200). Rf = relative (electrophoretic) mobility.
Fatty Acid Binding of Renal Medulla Fatty Acid Binding Proteins from WKY and SHRSP

<table>
<thead>
<tr>
<th>Group</th>
<th>Palmitic acid</th>
<th>Stearic acid</th>
<th>Oleic acid</th>
<th>Linoleic acid</th>
<th>Arachidonic acid</th>
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</thead>
<tbody>
<tr>
<td>WKY</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>5 wk</td>
<td>0.030 ±0.001*</td>
<td>0.028 ±0.001*</td>
<td>0.025 ±0.001*</td>
<td>0.029 ±0.002*</td>
<td>0.030 ±0.003*</td>
</tr>
<tr>
<td>40 wk</td>
<td>0.028 ±0.003</td>
<td>0.027 ±0.002</td>
<td>0.026 ±0.002</td>
<td>0.024 ±0.003</td>
<td>0.024 ±0.002</td>
</tr>
<tr>
<td>SHRSP</td>
<td></td>
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</tr>
<tr>
<td>5 wk</td>
<td>0.155 ±0.006t</td>
<td>0.153 ±0.008t</td>
<td>0.149 ±0.009†</td>
<td>0.148 ±0.029‡</td>
<td>0.178 ±0.019§</td>
</tr>
<tr>
<td>40 wk</td>
<td>0.035 ±0.002</td>
<td>0.034 ±0.003</td>
<td>0.032 ±0.003</td>
<td>0.034 ±0.004</td>
<td>0.031 ±0.003</td>
</tr>
</tbody>
</table>

Values are means ± SEM of five rats per group. Binding is expressed as moles of fatty acid per mole of protein.

*p < 0.005, compared with values in age-matched SHRSP.

†p < 0.025, ‡p < 0.005, §p < 0.05, compared with values in 40-week-old SHRSP.

Fatty Acid Binding Parameters of Renal Medulla Fatty Acid Binding Proteins from WKY and SHRSP

<table>
<thead>
<tr>
<th>Group</th>
<th>Palmitic acid</th>
<th>Stearic acid</th>
<th>Oleic acid</th>
<th>Linoleic acid</th>
<th>Arachidonic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKY</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>5 wk</td>
<td>62 ±15 0.95 ±0.15</td>
<td>63 ±18 0.93 ±0.11</td>
<td>67 ±17 0.92 ±0.11</td>
<td>71 ±21 1.06 ±0.23</td>
<td>65 ±19 1.08 ±0.22</td>
</tr>
<tr>
<td>40 wk</td>
<td>65 ±16 0.98 ±0.15</td>
<td>66 ±12 1.03 ±0.03</td>
<td>73 ±12 1.04 ±0.07</td>
<td>68 ±23 1.17 ±0.33</td>
<td>69 ±23 1.20 ±0.30</td>
</tr>
<tr>
<td>SHRSP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 wk</td>
<td>59 ±10 0.90 ±0.10</td>
<td>59 ±18 1.02 ±0.10</td>
<td>69 ±15 1.07 ±0.20</td>
<td>69 ±15 1.37 ±0.53</td>
<td>74 ±25 1.37 ±0.25</td>
</tr>
<tr>
<td>40 wk</td>
<td>68 ±17 0.92 ±0.18</td>
<td>71 ±15 0.99 ±0.12</td>
<td>69 ±19 1.06 ±0.08</td>
<td>74 ±27 1.41 ±0.22</td>
<td>78 ±21 1.31 ±0.23</td>
</tr>
</tbody>
</table>

Values are means ± SEM of five rats per group. B_max = maximal binding capacity. Data are derived from Scatchard analysis as shown for palmitate in 5-week-old WKY in Figure 2.

In the present study, FABP was purified from genetically hypertensive rat renal medulla, and its function under pathological conditions was investigated. Alterations of fatty acid and prostaglandin metabolism have been observed mainly in medulla, and as expected, our preliminary study revealed the presence of only low concentrations of FABP in renal cortex and no appreciable changes in fatty acid binding activity and composition in the renal cortex of SHRSP compared with that of WKY. Thus, because cortical FABP did not seem to play an important role in the altered lipid metabolism observed in genetically hypertensive rats, medullary tissues were used in our experiments.

Interference of charcoal against protein recovery has been reported in the assay of fatty acid binding. However, our preliminary study showed that, when small but sufficient quantities of charcoal-dextran were used, interference was negligible and protein recovery was virtually unaffected and practically comparable to the values obtained in a hydroxylalkoxypropyl dextran-based assay. Although there were some variations among the K_d values after delipidation, all four FABP samples showed similar fatty acid binding activity, and these samples were not considered to reflect the natural state of FABP in the cytosol. Therefore, to investigate the lipid abnormalities in hypertension, we used FABP samples without a delipidation procedure. Fatty acid binding activity of renal medullary FABP in WKY was similar for palmitate, stearate, oleate, linoleate, and arachidonate, which is consistent with the assumption.

Discussion
that the fatty acid binding activity increases with chain length but decreases with the degree of fatty acid unsaturation.28-30

Altered renal eicosanoid metabolism in rats genetically predisposed to hypertension has been reported by several investigators. Armstrong et al.31 showed that spontaneously hypertensive New Zealand rats produced more renal prostaglandin E2 as a result of decreased metabolism through prostaglandin 15-hydroxydehydrogenase rather than increased synthesis, whereas Japanese spontaneously hypertensive rats did not exhibit decreased renal prostaglandin 15-hydroxydehydrogenase activity. Pace-Asciak32 demonstrated increased prostaglandin production and decreased metabolism in SHR when compared with WKY, and Grone et al.33 demonstrated that platelet and renal thromboxane increased in SHR compared with WKY. These results and the assumption that fatty acid metabolism changes during the development of hypertension2 led us to compare the fatty acid binding activity and composition of FABP between control and genetically hypertensive rats, after confirming that FABP exists in kidney and is a suitable fatty acid carrier protein. The results indicate striking and important alterations in fatty acid binding activity and composition of FABP in hypertensive animals. The binding activity was increased in 5-week-old prehypertensive SHRSP compared with that of control WKY. In 40-week-old SHRSP with established hypertension, this activity was reduced and was similar to that in control WKY. The importance of such changes is not entirely clear, but fatty acid composition data analysis suggests one possible explanation. The observed increased binding activity of FABP from 5-week-old SHRSP may be secondary to low levels of endogenously bound fatty acids. The results indicate that FABP from 5-week-old SHRSP contains relatively little endogenous fatty acids, which may allow easier binding of exogenous fatty acids. In 40-week-old SHRSP, the amount of endogenously bound arachidonic acid increased markedly and the binding activity for exogenous fatty acid was reduced and was similar to that of controls. This, together with the finding of an increased amount of FABP in 40-week-old SHRSP, may suggest that increased arachidonic acid transport occurs in the renal medullary cytosol of hypertensive animals. The altered composition of fatty acids associated with FABP has been observed by us as well as by Takahashi et al.14 who reported that compositions of endogenous fatty acids were strikingly different from one another, for instance, the DE-II fraction of liver FABP contained palmitic, stearic, oleic, linoleic, and arachidonic acids, whereas the DE-III fraction contained mainly arachidonic acid. They also reported that the molar ratio of endogenous long-chain fatty acids to DE-III was only 0.835. Since the presence of endogenous free fatty acids usually is associated with FABP,34 we did not analyze the possible variations of the fatty acid content of the renal medullary cytosol, which are expected to be closely correlated with the variations observed in FABP.

Our studies seem to support earlier reports revealing increased prostaglandin synthesis in genetically hypertensive rat kidney and to extend them by demonstrating that enhanced substrate delivery may occur in SHRSP when hypertension is maintained. The change in the fatty acid composition may reflect increased phospholipid breakdown by phospholipase A2, the activity of which is enhanced in hypertensive rats. Prostaglandin synthesis depends on substrate availability. Polypeptides, like angiotensin II and bradykinin, are thought to increase prostaglandin synthesis in isolated tissues by making more substrate available to the synthetase through intracellular phospholipase activation.34,35 Although the precise function of FABP in vivo is not known and hence the relevance of FABP to prostaglandin metabolism is not clearly established, our findings suggest that increased renal prostaglandin production in genetically hypertensive rats might be partly due to increased substrate delivery by the increased arachidonic acid found in FABP. Increased prostaglandin production might counteract the production and maintenance of hypertension until renal disturbances or other secondary alterations associated with established hypertension appear. These suggestions are speculative but worth pursuing.

We do not have a good explanation for the lower amount of FABP endogenous fatty acid found at one developmental age of SHRSP, the 5-week-old animals. This occurrence may not be secondary to the development of hypertension as it appears before the blood pressure increases and may be one of the primary genetic mechanisms altered in SHRSP. The possibility that this decrease is dependent on age difference

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### Table 3. Fatty Acid Composition of Renal Medulla Fatty Acid Binding Proteins from WKY and SHRSP

<table>
<thead>
<tr>
<th>Group</th>
<th>Palmitic acid</th>
<th>Stearic acid</th>
<th>Oleic acid</th>
<th>Linoleic acid</th>
<th>Arachidonic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKY</td>
<td>0.235 ± 0.013*</td>
<td>0.138 ± 0.005†</td>
<td>0.297 ± 0.006†</td>
<td>0.278 ± 0.015*</td>
<td>0.032 ± 0.002‡</td>
</tr>
<tr>
<td>5 wk</td>
<td>0.116 ± 0.003§</td>
<td>0.028 ± 0.001†</td>
<td>0.278 ± 0.015*</td>
<td>0.032 ± 0.002‡</td>
<td></td>
</tr>
<tr>
<td>40 wk</td>
<td>0.203 ± 0.006§</td>
<td>0.116 ± 0.003§</td>
<td>0.255 ± 0.004§</td>
<td>0.247 ± 0.018§</td>
<td>0.026 ± 0.001§</td>
</tr>
<tr>
<td>SHRSP</td>
<td>0.075 ± 0.002</td>
<td>0.020 ± 0.003</td>
<td>0.035 ± 0.007</td>
<td>0.102 ± 0.004</td>
<td>0.743 ± 0.018</td>
</tr>
</tbody>
</table>

Values are means ± SEM of five rats per group. Binding is expressed as moles of fatty acid per mole of protein.

* p < 0.05, † p < 0.005, ‡ p < 0.025, compared with values in age-matched SHRSP.

§ p < 0.005, compared with values in 40-week-old SHRSP.
is very small, since in WKY, FABP characteristics did not change significantly between 5-week-old and 40-week-old rats. Although Gray, has detected a significant, early increase in blood pressure in SHR, blood pressure differences did not become significant in our breeding colony until 9 weeks after birth and began to plateau around 12 to 13 weeks. Studies have not been conducted in neonatal SHRSP. Thus, firm conclusions cannot be drawn concerning the temporal relationship between low endogenous fatty acid content and hypertension in 5-week-old SHRSP; the possibility exists that it develops concurrently and very early in the developmental period. Finally, further studies are needed to clarify the mechanism that alters fatty acid binding activity and composition of FABP observed in hypertensive animals and to relate this mechanism to in vivo prostaglandin synthesis.

In summary, less endogenous fatty acid in FABP was found in young prehypertensive SHRSP than in normotensive controls. The difference does not appear to be secondary to hypertension. In aging SHRSP with established hypertension, endogenous arachidonic acid content in FABP was much greater than that in control WKY, which indicates the possible increase of substrate delivery for prostaglandin synthesis and an important role for FABP in the regulation of blood pressure.

Acknowledgments
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References
1. Vane JR, McGiff JC. Possible contributions of endogenous prostaglandins to control of blood pressure. Circ Res 1975;36(suppl 1):1-175
8. Ockner RK, Manning JA, Poppenhausen RB, Ho WKL. A binding protein for fatty acids in cytosol of intestinal mucosa, liver, myocardium, and other tissues. Science 1972;177:56-58
17. O'Doherty PJA, Kukas A. Stimulation of tricarboxylic acid synthesis by Z protein in rat liver and intestinal mucosa. FEBS Lett 1975;56:256-258
20. Glazc JFC, Veerkamp JH. A radiochemical procedure for the assay of fatty acid binding proteins. Anal Biochem 1983;132:89-95
26. Glitz GC, Janssen AM, Baerwald CCF, Veerkamp JH. Purification and characterization of fatty acid-binding proteins from rat heart and liver. Biochim Biophys Aeta 1985;837:57-66
27. Mishkin S, Stein L, Gatmaitan Z, Arias IM. The binding of fatty acids to cytoplasmic proteins: binding to Z-protein in liver and other tissues of the rat. Biochem Biophys Res Commun 1972;47:997-1003
32. Grene HJ, Grippos RS, Arendshorst WJ, Dunn MJ. Role of thromboxane in control of arterial pressure and renal function.
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