RESEARCH ARTICLE PHARMACOLOGIA

DOI: 10.5567/pharmacologia.2014.12.18

Comparison of Fatty Acid-binding Protein Expression in Vascular Smooth Muscle Cells from Stroke-prone Spontaneously Hypertensive and Wistar Kyoto Rats

Takuji Machida, Tomomi Hatanaka, Akina Onoguchi, Kenji Iizuka and Masahiko Hirafuji

Department of Pharmacological Sciences, School of Pharmaceutical Sciences, Health Sciences University of Hokkaido, Ishikari-Tobetsu, Hokkaido 061-0293, Japan

ABSTRACT

Background: Fatty acid-binding proteins (FABPs) modulate intracellular lipid metabolism by regulating fatty acid uptake and intracellular transport. **Aim:** This study was conducted to identify and compare FABP subtypes expressed in aorta and cultured Vascular Smooth Muscle Cells (VSMCs) derived from Wistar Kyoto rats (WKY) and Stroke-prone Spontaneously Hypertensive Rats (SHRSP). **Methods:** mRNA expressions of FABP subtypes were determined by Reverse Transcription-polymerase Chain Reaction (RT-PCR). The uptake of radioactive fatty acid by aorta was evaluated by a liquid scintillation counter. **Results:** RT-PCR analysis detected mRNA of heart FABP (H-FABP) and epidermal FABP (E-FABP) in the aorta and VSMCs. H-FABP mRNA expression was slightly higher in the VSMCs from SHRSP than from WKY and E-FABP mRNA expression was significantly higher in the VSMCs from SHRSP. The uptake of radioactive [^{3}H]-oleic acid was significantly higher in aorta isolated from SHRSP than in that isolated from WKY. Subsequently, the effect of peroxisome proliferator-activated receptor α (PPARα) activators on FABP expression in VSMCs was investigated. Fenofibrate, but not WY-14643, significantly reduced H-FABP mRNA expression in both WKY and SHRSP cells, whereas it increased E-FABP mRNA expression only in WKY cells. **Conclusion:** These results suggest that increased FABP expression in the VSMCs from SHRSP may be relevant to the initiation and establishment of vascular disorders and that fenofibrate may alter lipid metabolism in VSMCs by differentially modulating FABP expression via a mechanism that is independent of PPARα activation.

Key words: Fatty acid-binding protein, stroke-prone spontaneously hypertensive rats, vascular smooth muscle cells, fenofibrate

Pharmacologia 5 (1): 12-18, 2014

INTRODUCTION

Fatty acid-binding proteins (FABPs) are members of the superfamily of lipid-binding proteins. To date, nine tissue specific cytoplasmic FABPs have been identified. FABPs are believed to be involved in intracellular lipid metabolism because they are regulators of fatty acid uptake and intracellular transport (Chmurzynska, 2006). Although abnormal lipid metabolism is widely known to be associated with cardiovascular diseases such as hypertension and arteriosclerosis, the quantitative expression and the precise role of FABPs in cardiovascular function is still controversial. Vascular Smooth Muscle Cells (VSMCs) play important roles in the development of vascular diseases and during the repair of injury, by not only regulating their phenotypes and properties such as proliferation, migration, apoptosis, or production of extracellular matrix in response to local environmental changes, but also by inducing the production of potent vasoactive mediators such as prostaglandin I_2 or nitric oxide that may primarily compensate for the endothelial dysfunction. In view of the importance of VSMC function in vascular pathophysiology, the present study was conducted to compare the isotypes of FABP that are expressed in both aorta and cultured VSMCs derived from Stroke-prone Spontaneously Hypertensive Rats (SHRSP), which constitute an animal model of cardiovascular disease and hypertension (Okamoto *et al*., 1974; Aitman *et al*., 1997; Collison *et al*., 2000), with those derived from normotensive Wistar Kyoto rats (WKY).

There is a strict correlation between activation of Peroxisome Proliferator-activated Receptor (PPAR) and intracellular concentrations of FABP (Wolfrum *et al*., 1999). Expression of heart FABP (H-FABP) mRNA is regulated by long-chain fatty acids which activate PPARα in rat myoblasts and cardiomyocytes (Chang *et al*., 2001).

Corresponding Author: Takuji Machida, Department of Pharmacological Sciences, School of Pharmaceutical Sciences, Health Sciences University of Hokkaido, Ishikari-Tobetsu, Hokkaido 061-0293, Japan Tel and Fax: +81-133-23-3892

The aim of the present study was to clarify (1) if the amount of FABP expression in vascular walls relates the initiation and establishment of vascular disorders and (2) if the FABP expression in vascular walls is modulated by PPAR activation.

MATERIALS AND METHODS

Materials: Fetal calf serum and culture medium were obtained from Life Technologies (Grand Island, NY, USA). [9, 10-^{3}H]-oleic acid was purchased from PerkinElmer (Waltham, MA, USA). 2-[4-(4-chlorobenzoyl)phenoxy]-2-methylpropanoic acid isopropyl ester (fenofibrate), (4-chloro-6-(2,3-xylidino)-2-pyrimidinyl-thio)acetic acid (WY-14643) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Animals, organ harvesting and cell culture: This study was conducted in accordance with the Care and Use of Laboratory Animals of the Animal Research Committee of Health Sciences University of Hokkaido. Male WKY and SHRSP (6 to 7 weeks old) were sacrificed using ether anesthesia. The blood pressure but not heart rate, of the 6- to 7-week-old SHRSP was slightly but significantly higher than that of age-matched WKY (Table 1). The harvested organs were immediately used for analysis of fatty acid uptake or were frozen in liquid nitrogen and stored at -80°C until required for RNA analysis. VSMCs were enzymatically isolated from fresh aortic media using collagenase and elastase and cultured in Dulbecco's modified Eagle's medium containing 10% fetal calf serum, as previously described (Hirafuji *et al*., 2002). Primary cells were used throughout the experiments. After 5-6 days of culture, the cells were incubated for 1-3 days with PPARα activators.

RNA extraction and reverse transcription-polymerase chain reaction: FABP isotypes that are expressed in both aorta and cultured VSMCs were identified by qualitative reverse transcription-polymerase chain reaction (RT-PCR). Total RNA in isolated tissue and cultured VSMCs was extracted using TRI reagent® (Sigma-Aldrich) according to the manufacturer's instructions. cDNA was synthesized from 2 μg of each RNA sample using 0.5 mg oligo $(dT)_{12-18}$ (Life Technologies, Invitrogen, Carlsbad, CA, USA) and 100 U reverse transcriptase (Invitrogen) at 42°C for 60 min. For PCR, 0.42 U Taq polymerase (Roche Diagnostics GmbH, Mannheim, Germany) and 0.24 μL 10 μM primer were added to 12 μL 1×PCR buffer (Hirafuji *et al*., 2002). The thermo cycling program consisted of 3 min at 95°C, 40 cycles of 1 min at 94°C, 1 min at 54°C and 1 min at 72°C and a final extension for 7 min at 72°C in a thermal cycler (Takara PCR thermal cycler Dice, Takara Bio, Shiga, Japan). The specific primers for FABP and Glyceraldehyde 3-phosphate Dehydrogenase (GAPDH) were as described by Guthmann *et al*. (1998) and Hirafuji *et al*. (2002), respectively. The RT-PCR products were separated and visualized on an ethidium bromide stained 1.0% agarose gel. Semi-quantitative analysis of each product was performed by densitometry using Image J software and concentrations were calculated relative to GAPDH.

H-FABP and epidermal FABP (E-FABP) expression in VSMCs were quantified by real-time RT-PCR using a 7500 Fast Real-Time PCR system (Life Technologies, Applied Biosystems, Foster City, CA, USA) and SYBR Green Real-Time PCR Master Mix (Toyobo, Osaka, Japan). The sense primer for H-FABP was 5' ACGCCTTTGTCGGTACCTGGA 3' and the antisense primer was 5' GGTCATGCTAGCGACCTGTCT 3'. The sense primer for E-FABP was 5' AGTGGGAAGGGAAAGAAAG 3' and the antisense primer was 5' GAGTTAGCCAGTCCTCATTG 3'. The sense primer for GAPDH was 5' ATGACTCTACCCACGGCAAG 3' and the antisense primer was 5' TCCACGACATACTCAGCACC 3'. H-FABP and E-FABP products was calculated relative to GAPDH.

Uptake of [^{3}H]-oleic acid by aorta: After the aortic segments were weighed, they were incubated with 1 ml Hepes buffer containing 0.75 μCi [^{3}H]-oleic acid at 37°C, for 1 h in a shaking waterbath. After discarding the Hepes buffer, the segments were washed three times with phosphate-buffered saline followed by washing twice with 0.9% NaCl. [^{3}H]-oleic acid incorporation was estimated using a liquid scintillation counter (Hitachi Aloka Medical, Tokyo, Japan).

Statistical analysis: All values are given as Mean±SEM Statistical analysis of the results was performed using Student's t-test or the Mann-Whitney U test for unpaired data and analysis of variance, followed by a Fisher's test for multiple comparisons. Significance was established at the $p<0.05$ level.

Table 1: Basal parameters of the male 6-7 week old WKY and same aged SHRSP

Parameters	WKY (n = 10)	SHRSP (n = 8)
Body weight (g)	127.60±6.24 0	115.50±3.11
Heart rate (bpm)	451.41±32.75	440.84±6.29
Systolic blood pressure (mmHg)	125.13±2.450	127.68±4.32*
Mean blood pressure (mmHg)	90.17±3.370	105.00±3.48*
Diastolic blood pressure (mmHg)	72.75±4.020	88.80±4.07*

WKY: Wistar Kyoto rat, SHRSP: Stroke-prone spontaneously hypertensive rat. Values are Mean±SEM. Values are statistically significant at *$p<0.05$ vs. WKY

RESULTS

FABP expression profiles and fatty acid incorporation: The expression profiles of FABP mRNA in rat aorta and cultured VSMCs were examined

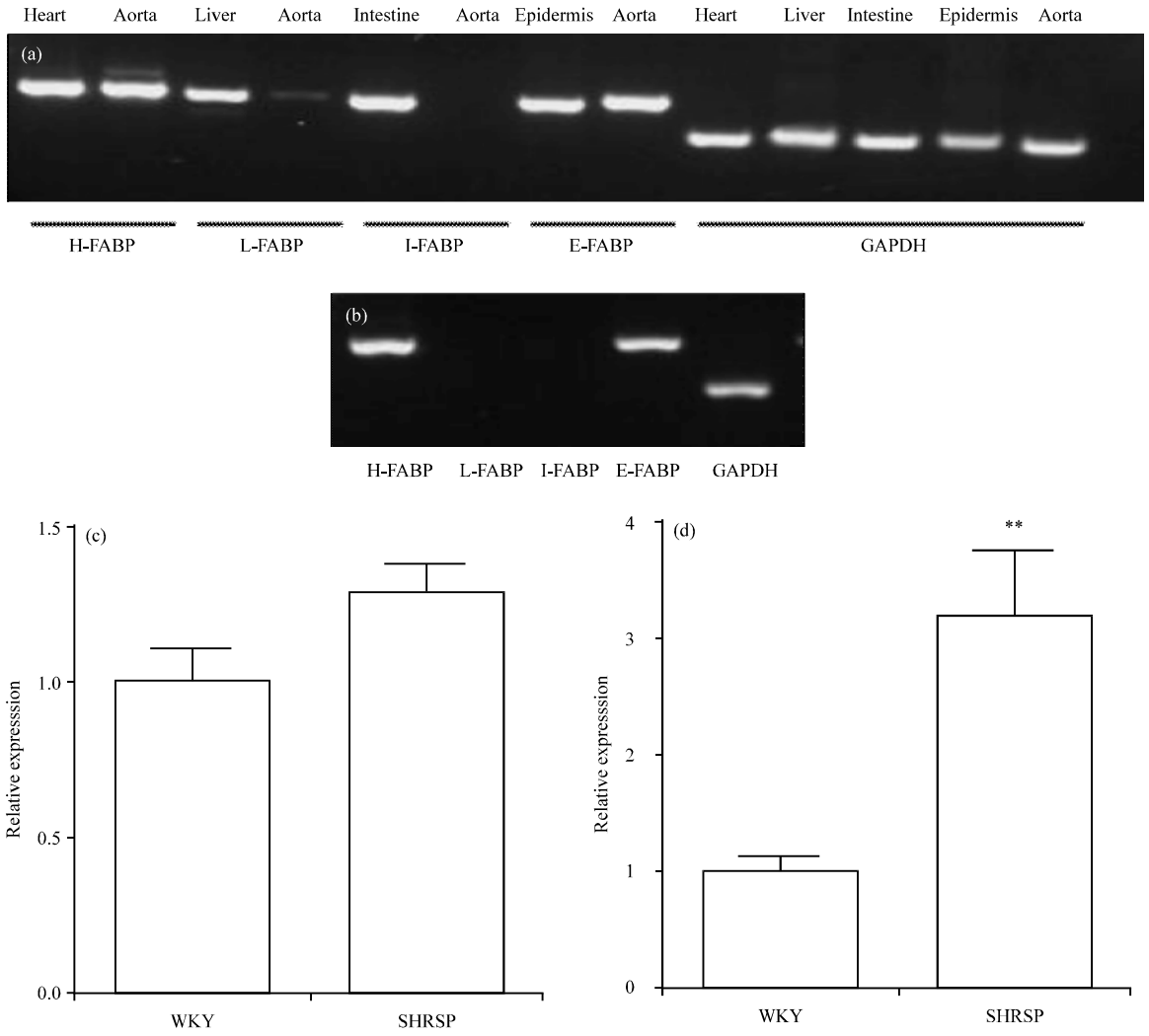

Fig. 1(a-d): FABP mRNA expression in aortic tissue and cultured VSMCs isolated from WKY and SHRSP. Representative fluorescence images of indicated (a) FABP expression in aortic tissue and (b) Cultured VSMCs isolated from WKY. FABP mRNA expression was determined by RT-PCR. mRNA extracted from heart, liver, intestine and epidermis was used as the positive control to identify the expression of FABP subtypes with primers for heart (H-FABP), liver (L-FABP), intestinal (I-FABP) and epidermal FABP (E-FABP), respectively. Comparison of (c) H-FABP and (d) E-FABP mRNA expression in cultured VSMCs from WKY and SHRSP. FABP mRNA expression was determined by real-time RT-PCR. Each column represents the Mean±SEM of 6 experiments. **$p<0.01$ vs. WKY

by RT-PCR. PCR amplification was performed for 40 cycles in order to reach saturation. mRNA of H-FABP and E-FABP were clearly detected in aorta isolated from WKY, whereas liver FABP (L-FABP) or intestinal FABP (I-FABP) mRNA were barely detectable (Fig. 1a). Similar results were found for the cultured VSMCs isolated from WKY (Fig. 1b) and for aorta and VSMCs isolated from SHRSP.

The mRNA expression levels of H-FABP and E-FABP in cultured VSMCs from WKY and SHRSP was then compared. As shown in Fig. 1c and d, mRNA expression of H-FABP was slightly higher and that of E-FABP was significantly higher in VSMCs from SHRSP compared with that from WKY (128.9±8.82 and 319.3±0.33%, versus WKYs).

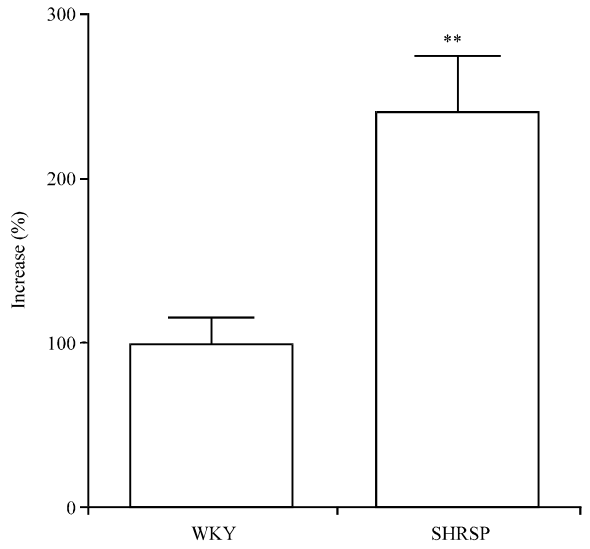

Fig. 2: Uptake of fatty acid by aorta isolated from WKY and SHRSP. Fatty acid incorporation was determined by radioactivity of [^{3}H]-oleic acid. Each column represents the Mean±SEM of 11 experiments. **p<0.01 vs. WKY

The aortic uptake of fatty acid was next compared between WKY and SHRSP. As shown in Fig. 2, [^{3}H]-oleic acid incorporation was significantly higher in aorta isolated from SHRSP than in that isolated from WKY.

These results suggest that the higher FABP expression in VSMCs could be one reason for the accelerated fatty acid incorporation by aorta in SHRSP.

Fenofibrate modulates FABP mRNA expression: There is a strict correlation between activation of PPAR and intracellular concentrations of FABP (Wolfrum *et al*., 1999). Several studies have demonstrated that the expression of FABP is regulated by PPAR activators, such as fibrates or fatty acids. Therefore, the effects of PPARα activators on FABP mRNA expression in VSMCs was next investigated. As shown in Fig. 3a, treatment with fenofibrate (30 μM), a PPARα-specific fibrate (Brown *et al*., 1999), for 1-3 days significantly reduced H-FABP mRNA expression in VSMCs from both WKY and SHRSP. By contrast, fenofibrate significantly increased E-FABP mRNA expression in VSMCS from WKY (Fig. 3b). However, fenofibrate had no effect on E-FABP mRNA expression in VSMCs from SHRSP (Fig. 3b). The synthetic PPARα activator, WY-14643 (30 μM; Fig. 3c and d) had no such significant effect.

These results suggest that fenofibrate treatment may affect intracellular lipid metabolism in VSMCs by differentially modulating FABP via a mechanism that is independent of PPARα activation.

DISCUSSION

The present study revealed that mRNA of H-FABP and E-FABP but not of L-FABP or I-FABP are co-expressed in rat aorta and VSMCs. However, Western blot analyses could not confirm these protein expressions, as they could barely be detected by the commercially available antibodies to H-FABP and E-FABP used in this study. Although several studies have shown the presence of H-FABP in VSMCs (Sakai *et al*., 1995; Antohe *et al*., 1998), the results of the present study indicate that VSMCs co-express H-FABP and E-FABP mRNA. In contrast with our results, Masouye *et al*. (1997) were not able to detect E-FABP expression in larger blood vessels with muscular walls, although they were able to detect it in endothelial cells of the human microvasculature using immunohistochemistry. At present, we have no clear explanation for this discrepancy, but it may be due to differences in the species or experimental methods used.

Sarzani *et al*. (1988) showed, for the first time, that a quantitative change in H-FABP is related to the pathogenesis of cardiovascular disease. They found reduced aortic H-FABP protein and mRNA expression in three experimental hypertensive rat models: deoxycorticosterone and salt-treated hypertensive rats, Goldblatt two kidney one clip renal hypertensive rats and angiotensin II-infused hypertensive rats. Reduced H-FABP mRNA expression has also been found in aorta isolated from streptozotocin-induced diabetic rats (Sakai *et al*., 1995). By contrast, Fujii *et al*. (1988) reported that FABP concentration is significantly higher in renal medulla of SHRSP more than 10 weeks old than in that of aged matched WKY and that antihypertensive treatment of 24 week old SHRSP caused a significant decrease in medullary FABP levels. They suggested that a higher FABP concentration might reflect accelerated fatty acid metabolism in renal medulla in SHRSP (Kawaguchi *et al*., 1987). In the present study, mRNA expression of H-FABP tended to increase in VSMCs from SHRSP compared to that in VSMCs from WKY and that E-FABP mRNA expression significantly increased in VSMCs from SHRSP compared to that in VSMCs from WKY. Furthermore, the uptake of fatty acid in aorta isolated from SHRSP was higher than that isolated from WKY. These results suggest the higher FABP expression in VSMCs could be one reason for the accelerated fatty acid incorporation by aorta in SHRSP. The VSMCs used in the present study were isolated from 6-7 week old SHRSP, which were at a very early stage of development of hypertension (Minami *et al*., 1997). In fact, the blood pressure but not heart rate, of the 6-7 week old SHRSP was slightly but significantly higher than that of

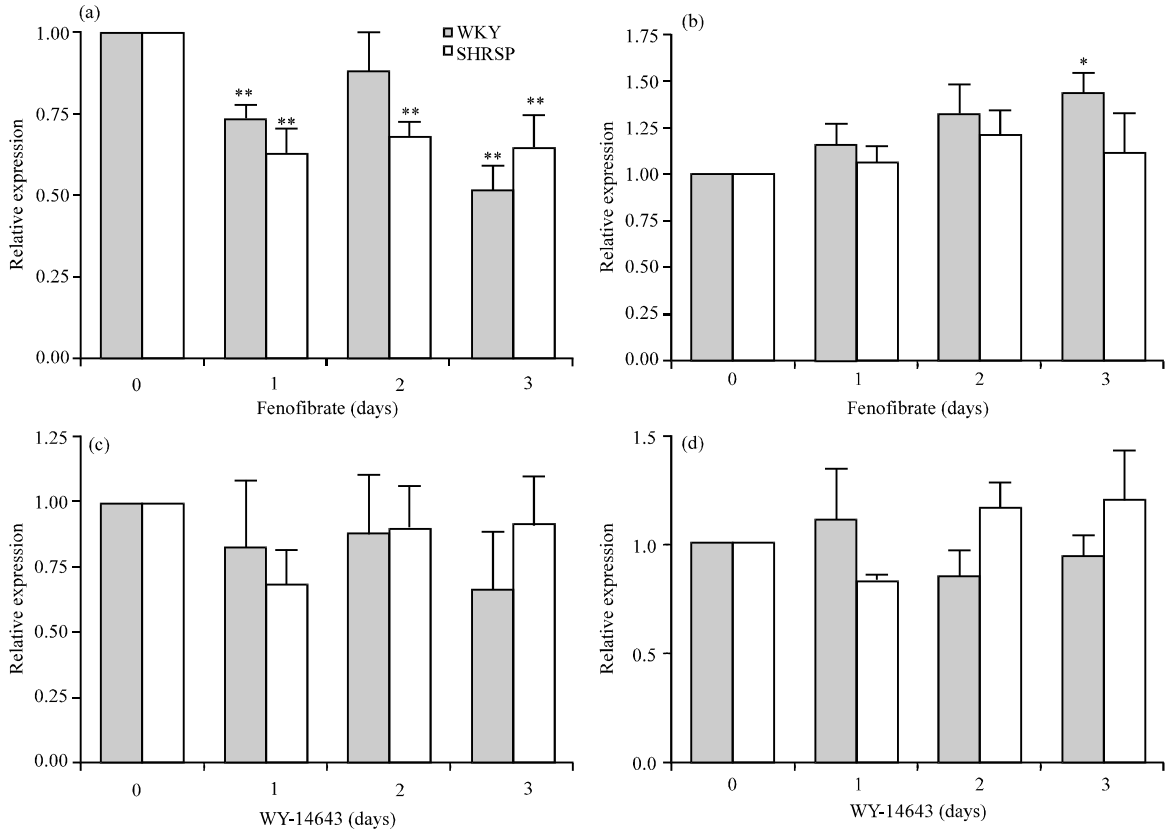

Fig. 3(a-d): Effect of fenofibrate and WY-14643 on mRNA expression of H-FABP and E-FABP in VSMCs isolated from WKY and SHRSP. FABP mRNA expression was determined by real-time RT-PCR. Cells were treated with either fenofibrate (30 μM) or WY-14643 (30 μM) for the indicated time periods. (a and c)H-FABP and (b and d) E-FABP mRNA expression was expressed relative to day 0. Each column represents the Mean± SEM of 6 experiments for WKY of a and b, 5 experiments for SHRSP of a and b and 3 experiments for c and d, respectively. *p<0.05, **p<0.01 vs. corresponding day 0

age matched WKY (Table 1). Thus, it is unlikely that high blood pressure and increased shear stress after the establishment of hypertension leads to changes in FABP mRNA expression. Although the main reason for the increased induction of FABP mRNA expression in the VSMCs of SHRSP is unclear, our results suggest that altered FABP expression may be relevant to the initiation and establishment of cardiovascular disorders in SHRSP.

PPARα plays an important role in the regulation of energy and lipid metabolism and thus in the pathophysiology of cardiovascular disease (Schiffrin *et al*., 2003). Furthermore, PPARα activators decrease the inflammatory response by negatively regulating nuclear factor-κB transcription activity in VSMCs (Staels *et al*., 1998). Several studies have demonstrated that the expression of FABP is regulated by PPAR activators, such as fibrates or fatty acids. Daoud *et al*. (2005) reported that the non-selective PPAR agonists bezafibrate and linoleic acid reduce H-FABP and L-FABP expression in human trophoblast cells. It has also been reported that WY-14643 induces L-FABP expression in rat hepatoma cells (Landrier *et al*., 2004). However, whether PPARα activators affect FABP expression in vascular cells remains unclear. As both PPARα and PPARα are expressed in the cardiovascular system (Bishop-Bailey, 2000), in endothelial cells (Inoue *et al*., 1998; Satoh *et al*., 1999) and in VSMCs (Staels *et al*., 1998), it is possible that PPARα activators influence FABP function by affecting the levels of FABP expression. In the present study, fenofibrate significantly reduced H-FABP mRNA expression in VSMCs derived from SHRSP and WKY. On the other hand, fenofibrate significantly increased E-FABP mRNA expression in VSMCs from WKY but not in those from SHRSP. These effects were observed at 30 μM fenofibrate, which is approximately the plasma concentration of its active metabolite fenofibric acid

found in humans following administration of the drug (Adkins and Faulds, 1997). Therefore, our results suggest that fenofibrate treatment may affect intracellular lipid metabolism in VSMCs by differentially modulating FABP expression. These effects were not observed with another selective PPARα activators WY-14643 even though it has been reported that WY-14643 induces H-FABP in liver and skeletal muscle in mice (Motojima, 2000). Thus, the effects of fenofibrate on FABP mRNA expression seem to be independent of PPARα activation. Further studies are required to determine the mechanism by which fenofibrate modulates E-FABP expression.

In conclusion, the results of the present study demonstrate that mRNA expression of H-FABP and E-FABP is increased in VSMCs isolated from SHRSP. This increase may be relevant to the initiation and establishment of vascular disorders in SHRSP. Furthermore, fenofibrate and an anti-hyperlipidemic agent may affect lipid metabolism by differentially modulating FABP expression in the vascular walls via a mechanism that is independent of PPARα activation. Further studies are required to clarify the precise roles of FABPs in the pathophysiology of vascular diseases and the mechanism by which fenofibrate regulates FABP expression.

ACKNOWLEDGEMENTS

This study was supported in part by a Grant-in-Aid for Young Scientists from the NOASTEC Foundation.

REFERENCES

Adkins, J.C. and D. Faulds, 1997. Micronised fenofibrate: A review of its pharmacodynamic properties and clinical efficacy in the management of dyslipidaemia. Drugs, 54: 615-633.

Aitman, T.J., T. Gotoda, A.L. Evans, H. Imrie and K.E. Heath *et al*., 1997. Quantitative trait loci for cellular defects in glucose and fatty acid metabolism in hypertensive rats. Nat. Genet., 16: 197-201.

Antohe, F., D. Popov, L. Radulescu, N. Simionescu, T. Borchers, F. Spener and M. Simionescu, 1998. Heart microvessels and aortic endothelial cells express the 15 kDa heart-type fatty acid-binding proteins. Eur. J. Cell Biol., 76: 102-109.

Bishop-Bailey, D., 2000. Peroxisome proliferator-activated receptors in the cardiovascular system. Br. J. Pharmacol., 129: 823-834.

Brown, P.J., D.A. Winegar, K.D. Plunket, L.B. Moore and M.C. Lewis *et al*., 1999. A ureido-thioisobutyric acid (GW9578) is a subtype-selective PPARα agonist with potent lipid-lowering activity. J. Med. Chem., 42: 3785-3788.

Chang, W., J. Rickers-Haunerland and N.H. Haunerland, 2001. Induction of cardiac FABP gene expression by long chain fatty acids in cultured rat muscle cells. Mol. Cell. Biochem., 221: 127-132.

Chmurzynska, A., 2006. The multigene family of Fatty Acid-Binding Proteins (FABPs): Function, structure and polymorphism. J. Applied Genet., 47: 39-48.

Collison, M., A.M. Glazier, D. Graham, J.J. Morton and M.H. Dominiczak *et al*., 2000. Cd36 and molecular mechanisms of insulin resistance in the stroke-prone spontaneously hypertensive rat. Diabetes, 49: 2222-2226.

Daoud, G., L. Simoneau, A. Masse, E. Rassart and J. Lafond, 2005. Expression of cFABP and PPAR in trophoblast cells: Effect of PPAR ligands on linoleic acid uptake and differentiation. Biochim. Biophys. Acta Mol. Cell Biol. Lipids, 1687: 181-197.

Fujii, S., H. Kawaguchi, H. Okamato, H. Togashi, H. Saito and H. Yasuda, 1988. Increased renal fatty acid binding protein in spontaneously hypertensive rats. J. Hypertens., 6: 671-675.

Guthmann, F., C. Hohoff, H. Fechner, P. Humbert, T. Borchers, F. Spener and B. Rustow, 1998. Expression of fatty-acid-binding proteins in cells involved in lung-specific lipid metabolism. Eur. J. Biochem., 253: 430-436.

Hirafuji, M., M. Tsunoda, T. Machida, N. Hamaue, T. Endo, A. Miyamoto and M. Minami, 2002. Reduced expressions of inducible nitric oxide synthase and cyclooxygenase-2 in vascular smooth muscle cells of stroke-prone spontaneously hypertensive rats. Life Sci., 70: 917-926.

Inoue, I., K. Shino, S. Noji, T. Awata and S. Katayama, 1998. Expression of peroxisome proliferator-activated receptor α(PPARα) in primary cultures of human vascular endothelial cells. Biochem. Biophys. Res. Commun., 246: 370-374.

Kawaguchi, H., H. Okamoto, H. Saito and H. Yasuda, 1987. Renal phospholipase C and diglyceride lipase activity in spontaneously hypertensive rats. J. Hypertens., 10: 100-106.

Landrier, J.F., C. Thomas, J. Grober, H. Duez and F. Percevault *et al*., 2004. Statin induction of liver fatty acid-binding protein (L-FABP) gene expression is peroxisome proliferator-activated receptor-α-dependent. J. Biol. Chem., 279: 45512-45518.

Masouye, I., G. Hagens, T.H. van Kuppevelt, P. Madsen and J.H. Saurat *et al*., 1997. Endothelial cells of the human microvasculature express epidermal fatty acid-binding protein. Circ. Res., 81: 297-303.

Minami, M., S. Kimura, T. Endo, N. Hamaue and M. Hirafuji *et al*., 1997. Dietary docosahexaenoic acid increases cerebral acetylcholine levels and improves passive avoidance performance in stroke-prone spontaneously hypertensive rats. Pharmacol. Biochem. Behav., 58: 1123-1129.

Motojima, K., 2000. Differential effects of PPARαactivators on induction of ectopic expression of tissue-specific fatty acid binding protein genes in the mouse liver. Int. J. Biochem. Cell Biol., 32: 1085-1092.

Okamoto, K., Y. Yamori and A. Nagaoka, 1974. Establishment of the stroke-prone spontaneously hypertensive rats (SHR). Circ. Res., 34-35: 143-153.

Sakai, K., H. Fujii, T. Yamamoto, J. Sakakibara, T. Izumi, A. Shibata and T. Ono, 1995. Tissue-specific suppression of aortic fatty-acid-binding protein in streptozotocin-induced diabetic rats. Eur. J. Biochem., 229: 201-206.

Sarzani, R., K.P. Claffey, A.V. Chobanian and P. Brecher, 1988. Hypertension induces tissue-specific gene suppression of a fatty acid binding protein in rat aorta. Proc. Natl. Acad. Sci. USA, 85: 7777-7781.

Satoh, H., K. Tsukamoto, Y. Hashimoto, N. Hashimoto and M. Togo *et al*., 1999. Thiazolidinediones suppress endothelin-1 secretion from bovine vascular endothelial cells: A new possible role of PPARã on vascular endothelial function. Biochem. Biophys. Res. Commun., 254: 757-763.

Schiffrin, E.L., F. Amiri, K. Benkirane, M. Iglarz and Q.N. Diep, 2003. Peroxisome proliferator-activated receptors: Vascular and cardiac effects in hypertension. Hypertension, 42: 664-668.

Staels, B., W. Koenig, A. Habib, R. Merval and M. Lebret *et al*., 1998. Activation of human aortic smooth-muscle cells is inhibited by PPARαbut not by PPARã activators. Nature, 393: 790-793.

Wolfrum, C., C. Buhlmann, B. Rolf, T. Borchers and F. Spener, 1999. Variation of liver-type fatty acid binding protein content in the human hepatoma cell line HepG2 by peroxisome proliferators and antisense RNA affects the rate of fatty acid uptake. Biochim. Biophys. Acta Mol. Cell Biol. Lipids, 1437: 194-201.