Original Article

The Role of Cytochrome P-450 in Salt-Sensitive Stroke in Stroke-Prone Spontaneously Hypertensive Rats

Chen-Jiang YING^{1,2)}, Takanori NOGUCHI³⁾, Hiroaki ASO¹⁾, Katsumi IKEDA⁴⁾,
Yukio YAMORI⁵⁾, and Yasuo NARA¹⁾

Stroke-prone spontaneously hypertensive rats (SHRSP/Izm) are salt sensitive: they develop severe hypertension and die of stroke within a short time after salt loading. We studied the role of cytochrome P-450 (CYP) isoforms in the brain and the effect of clofibrate to investigate the mechanism of salt sensitive stroke-proneness in SHRSP/Izm. Male SHRSP/Izm at 9 weeks of age were fed a regular diet with or without 0.25% clofibrate and given a 1% NaCl solution for drinking water for 10 d. The expression levels of CYP4A1, 2C11, and 2C23 were measured by Western blotting. Cerebral blood flow was measured with a laser Doppler method and blood vessel diameters were measured under microscopic observation. SHRSP/Izm died within 60 d after salt loading; however, clofibrate prolonged the survival (mean life span, 33 ± 7 vs. 215 ± 23 d, $p < 0.0001$) without significant attenuation of the severe hypertension. CYP4A1 and CYP2C11 expression levels were lower in SHRSP/Izm than those in age-matched male spontaneously hypertensive rats (SHR/Izm) in the cerebral cortex ($p < 0.05$). Salt loading down-regulated CYP2C11 expression in the cerebral cortex of SHRSP/Izm ($p < 0.05$). No obvious change in cerebral CYP4A1 was observed in either salt-loaded SHRSP/Izm or SHR/Izm. Clofibrate significantly up-regulated the expression of cerebral CYP2C11 and significantly attenuated its salt-induced suppression ($p < 0.05$). Additionally, clofibrate significantly increased blood vessel diameters ($p < 0.01$) and cerebral blood flow ($p < 0.0001$). CYP2C11 plays an important role in regulating cerebral blood flow and, as a result, in preventing stroke in the salt-sensitive stroke-prone SHRSP/Izm. (*Hypertens Res* 2008; 31: 1821–1827)

Key Words: stroke-prone spontaneously hypertensive rat, salt-sensitive, cerebral blood flow, cytochrome P-450, peroxisome proliferator-activated receptor- α

Introduction

Stroke-prone spontaneously hypertensive rats (SHRSP) are an inbred animal model for cerebrovascular disease resembling human stroke in many respects. Stroke occurs in the

course of the development of severe hypertension, and the development of severe hypertension and stroke are accelerated in response to high-salt dietary conditions. Rubattu *et al.* reported that SHRSP were genetically predisposed to stroke and had blood pressure-independent genetic factors for stroke based on their linkage analysis in salt-loaded F₂

From the ¹⁾Department of Pharmacy, School of Pharmacy, Shujitsu University, Okayama, Japan; ²⁾Department of Nutrition and Food Hygiene, School of Public Health, Huazhong University of Science and Technology, Wuhan, P.R. China; ³⁾Department of Nutrition and Health Science, Fukuoka Women's University, Fukuoka, Japan; and ⁴⁾Department of Pharmacy and ⁵⁾Institute for World Health Development, Mukogawa Women's University, Nishinomiya, Japan.

This work was supported in part by the Japan-China Sasagawa Medical Fellowship.

Address for Reprints: Yasuo Nara, Ph.D., Department of Pharmacy, School of Pharmacy, Shujitsu University, Okayama 703–8258, Japan. E-mail: nara@shujitsu.ac.jp

Received April 28, 2008; Accepted in revised form July 2, 2008.

hybrids bred from SHRSP and spontaneously hypertensive rats (SHR) (1). This result indicates that SHRSP possess genetic factors related to salt-sensitive stroke-proneness distinct from the genetic factors related to hypertension. To explore the genetic factors underlying the stroke proneness, the effect of blood pressure should be eliminated. However, this is very difficult, because if their blood pressure is controlled to be less than 200 mmHg, the prevalence of stroke is reduced in SHRSP. We have finally found suitable conditions, since in SHRSP/Izm fed a clofibrate-containing diet with a 1% NaCl solution for drinking water, the life span was significantly prolonged without attenuation of severe hypertension.

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors belonging to the nuclear receptor superfamily. Three isoforms of PPARs (α , β/δ , and γ) have been identified and display distinct physiological and pharmacological functions. The activation of PPAR- α by agonists such as lipid-lowering fibrates regulates lipid and lipoprotein metabolism. In addition to metabolic effects, PPAR- α agonists could also preventively protect the brain against noxious biological reactions induced by cerebral ischemia (2, 3).

Ishizuka *et al.* reported that a PPAR- α agonist induced cytochrome P-450 (CYP) 4A expression in the rat kidney (4). In the kidney, the CYP4 family metabolizes arachidonic acid (AA) to ω -terminal hydroxyeicosatetraenoic acids (HETEs, such as 20-HETE and 19-HETE) (5). HETEs have central roles in blood pressure regulation, and deficiency in the renal production of HETEs is associated with the development of hypertension in Dahl salt-sensitive rats (6). On the other hand, the CYP2C family, which is also induced in the kidney by the PPAR- α agonist fenofibrate, generates a series of epoxides (5,6-, 8,9-, 11,12-, and 14,15-epoxyeicoatrienoic acids, EETs) (7). EETs dilate cerebral arterioles and enhance the K^+ current in vascular smooth muscle cells from large cerebral arteries (8).

In this study, we focused on cerebral CYP isoform expression and the effect of clofibrate on their expression to explore the salt-sensitive stroke-proneness in SHRSP/Izm. The expression levels of cerebral CYP4A1, 2C11, and 2C23 were compared between SHRSP/Izm and SHR/Izm, and the effect of salt loading on their expression was examined. Furthermore, the effects of clofibrate on systolic blood pressure, cerebral arteriole diameter, cerebral blood flow, and CYP expression were examined in salt-loaded SHRSP/Izm.

Methods

Animals and Experimental Protocol

Male SHRSP/Izm and SHR/Izm at the age of 9 weeks were purchased from the Disease Model Cooperative Research Association (Kyoto, Japan) and housed in cages under controlled conditions (a 12 h light/dark cycle; 22–26°C; 40–50%

humidity). All experiments in the present study were carried out according to the Guiding Principles for Care and Use of Animals in the Field of Physiological Sciences, as drawn up by the Physiological Society of Japan (9).

Long-Term Experiment

To examine the effect of long-term administration of clofibrate on the survival rate of 1% salt solution-loaded SHRSP/Izm, 10 animals were randomly divided into two groups: the control group ($n=5$) was fed a regular diet (SP diet; Funabashi, Funabashi, Japan) with a 1% NaCl solution for drinking water, and the clofibrate treatment group ($n=5$) was fed the SP diet containing 0.25% clofibrate with a 1% NaCl solution for drinking water. Animals were carefully monitored and deaths were recorded every day. Clofibrate was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Systolic blood pressure was measured in the conscious state by the tail-cuff method (UR 5000; UEDA, Tokyo, Japan) (10). Survival rates were compared among the groups at the end of the experiment.

Short-Term Experiment

Twenty male SHRSP/Izm at the age of 9 weeks were randomly divided into four groups of five rats each. The rats were fed a SP diet supplemented with NaCl (1% NaCl solution for drinking water) clofibrate (0.25% in the SP diet), or NaCl plus clofibrate, or they were fed the SP diet without NaCl or clofibrate. Ten male SHR/Izm at the same age were divided into two groups and were fed the SP diet supplemented or not supplemented with NaCl. The experiments were continued for 10 d. At the end of the experiments, all rats were sacrificed under deep anesthesia with ether, and the cerebral cortex separated from the brain was rapidly removed, immediately frozen in liquid nitrogen, and then stored at -80°C until analysis.

Cellular Organelle Preparation

Sample preparation was carried out by Krapfenbauer's method (11). Briefly, the cerebral cortex tissue (about 500 mg) was suspended in 4.0 mL of solution containing 20 mmol/L HEPES-OH (pH 7.5), 320 mmol/L sucrose, 1 mmol/L EDTA, 5 mmol/L DTT, protease inhibitor cocktail (Sigma, St. Louis, USA), and 0.2 mmol/L sodium orthovanadate (Na_3VO_4). After the suspension was homogenized with a Teflon potter homogenizer, nuclei and undissolved material were removed by centrifugation at $800\times g$ for 10 min, and further centrifugation was performed at $10,000\times g$ for 20 min to separate the mitochondrial fraction. The supernatant was then further centrifuged for 1 h at $100,000\times g$ to obtain the microsomal fraction in the pellet. The fractionated organelles were used for gel electrophoresis.

Electrophoresis and Immunoblotting

The fractionated organelles were lysed in extraction buffer containing 50 mmol/L Tris (pH 8.0), 150 mmol/L NaCl, 0.1% SDS, 1% Nonidet P-40, 0.5% sodium deoxycholate, 1 µg/mL aprotinin, 100 µg/mL phenylmethylsulfonyl fluoride (PMSF), and 1% inhibitor cocktail (Sigma) and incubated on ice for 2 h. Electrophoresis and immunoblotting were performed as described previously (12). Namely, lysates were centrifuged at 12,000 rpm/min for 15 min and supernatants were collected. The protein concentration was quantified with BIO-RAD Dc protein assay reagent (Bio-Rad, Hercules, USA). Equal amounts of protein were mixed with SDS sample buffer and incubated for 5 min at 98°C before loading. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and immunological blotting were performed according to the methods recommended by Amersham Biosciences. The primary antibodies used were goat anti-rat CYP2C11 antiserum or goat anti-rat CYP4A1 antiserum (which reacts with CYP4A1, 4A2, and 4A3)(both from Dai-ichi Pure Chemicals Co., Ltd. Tokyo, Japan), rabbit anti-rat CYP2C23 antiserum (a gift from Dr. Jorge H. Capdevila, Vanderbilt University), rabbit anti-β-actin polyclonal antibody (Santa Cruz Biotechnology, Santa Cruz, USA), and mouse anti-β-actin monoclonal antibody (Sigma). Immunoreactive bands were detected using a Sigma ProteoQwest™ Colorimetric Western Blotting Kit or the ECL plus Western Blotting Detection System (Amersham Biosciences, Buckinghamshire, UK) according to the manufacturer's instructions and quantified with a lumino-image analyzer (LAS-1000 plus; Fujifilm, Tokyo, Japan).

Measurement of Cerebral Blood Flow and Vessel Diameter

After SHRSP/Izm ($n=5$) were given a 1% NaCl solution for drinking water with or without 0.25% clofibrate in the diet for 10 d, cranial windows were created as described by Morii *et al.* (13) and Noguchi *et al.* (14). Briefly, the experimental animals were anesthetized with sodium pentobarbital (60 mg/kg) and, after tracheotomy, artificially ventilated with 25% oxygen in air. The rate of respiration and the stroke volume of the respirator were adjusted to maintain constant blood gases and pH levels. Animals were immobilized in a stereotaxic frame, and a cranial window 5 mm in diameter was made in the center of the right parietal bone. A cover-slip 14 mm in diameter was placed over the window and secured with dental resin. Artificial cerebrospinal fluid (1.3 mmol/L $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 2.6 mmol/L KCl, 0.9 mmol/L $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 21.0 mmol/L NaHCO_3 , 125 mmol/L NaCl, 3.5 mmol/L dextrose, pH 7.2–7.4) was continuously infused within the cranial window and the intracranial pressure was adjusted to 3–5 mmHg to avoid brain herniation. The animal in the stereotaxic frame was placed on the stage of an Olympus SZX7 microscope equipped with a long working distance objective (Olympus

SZX-TR30 and U-TV0.5xC2; Olympus, Tokyo, Japan) and the cerebral vessels were monitored with a CCD camera (HDR-HC1; Sony, Tokyo, Japan).

The diameters of cerebral arterioles were determined from a montage of video images of the entire closed cranial window captured on a personal computer. The vessels were classified according to the scheme originally devised by Ley *et al.* (15). The branches of the middle cerebral arterioles were defined in order from A1 to A4. Cerebral blood flow (v) in the cerebral somatosensory area was measured with a laser-Doppler anemometer (PIM-II; Perimed, Stockholm, Sweden) (16, 17).

Statistical Analysis

Data are expressed as mean $\pm$ SEM. Protein expression experiments were reproduced three to five times, and representative experiments are shown. The data were analyzed by one-way ANOVA, Tukey-Kramer's HSD test, and Kaplan-Meier analysis. Differences were considered significant when p was <0.05 .

Results

Clofibrate Prolongs Life Span in Salt-Loaded SHRSP/Izm

To explore the effect of clofibrate on blood pressure and life span, SHRSP/Izm given a 1% salt solution for drinking water were fed a diet with or without clofibrate. Severe hypertension developed in both groups with or without clofibrate after salt loading, and there was no significant difference between the groups (Fig. 1A). All SHRSP/Izm with NaCl died of cerebral hemorrhage within 60 d, and the mean life span was 33 ± 7 d after salt loading. However, the mean life span after salt loading was significantly prolonged in the clofibrate-treated group (215 ± 23 d) (Fig. 1B). The mean life span of the treated group was 287 ± 23 d after birth, which corresponded to the mean life span of SHRSP/Izm without salt loading. Based on macroscopic observation and brain weight, three out of five treated rats obviously died of cerebral hemorrhage.

Comparison of Blood Pressure in the Short-Term Experiment

As shown in Fig. 1B, the first death was observed at 22 d after salt loading. The following experiment was carried out at 10 d after salt loading. Before treatment at the age of 9 weeks, the blood pressures of SHRSP/Izm and SHR/Izm were 224 ± 2 ($n=20$) and 203 ± 2 ($n=10$) mmHg, respectively, and thus were significantly different ($p<0.05$). Table 1 shows the blood pressure of each group at 9 d after starting the experiment. The SHRSP/Izm with and without NaCl group had higher blood pressure than the SHR/Izm with and without

Fig. 1. Effect of clofibrate on the development of hypertension and the survival rate in salt-loaded SHRSP/Izm. Systolic blood pressure (A) and survival rate (B) in salt-loaded SHRSP/Izm fed diets with or without 0.25% clofibrate are shown ($n=5$ per group). There was a significant inter-group difference in the survival rate by Kaplan-Meier analysis ($p<0.004$).

NaCl group ($p<0.05$). Clofibrate treatment attenuated the increase of blood pressure in both the SHRSP and salt-loaded SHRSP groups, but there was no significant difference between them.

Table 1. Comparison of Systolic Blood Pressure between SHRSP/Izm and SHR/Izm under Various Feeding Conditions

Treatment	Systolic blood pressure (mmHg)
SHRSP/Izm (5)*	253±6
SHRSP/Izm+clofibrate (5)	241±3
SHRSP/Izm+NaCl (5) [§]	269±5
SHRSP/Izm+NaCl+clofibrate (5)	251±5
SHR/Izm (5)*	222±4
SHR/Izm+NaCl (5) [§]	230±5

(): number of animals. Value: mean±SEM. *[§]There was a significant difference between the groups with the same symbol ($p<0.05$). SHRSP/Izm, stroke-prone spontaneously hypertensive rats; SHR/Izm, spontaneously hypertensive rats.

Expression of CYP Isoforms in Cerebral Cortex Microsomes

EETs produced from arachidonic acid by CYP isoforms play an important role in cerebral vasodilatation. To explore the role of CYP in the cerebral cortex obtained from SHRSP/Izm, CYP expression was examined in the cerebral cortex microsomal fraction. CYP4A1 and 2C11 were detected in the brain, and the levels of these proteins were significantly lower in SHRSP/Izm than in SHR/Izm ($p<0.05$, Fig. 2). Salt loading significantly decreased CYP2C11 expression in SHRSP/Izm, in which it was 56.0% of the level in the SHRSP/Izm control ($p<0.05$). In SHR/Izm, the levels of CYP2C11 protein were almost identical in the cerebral microsomes of the salt-loaded and control groups. CYP4A1 protein levels were slightly, but not significantly, decreased after salt loading in both SHRSP/Izm and SHR/Izm. However, CYP2C23 protein was not detected using the Sigma ProteoQwest™ Colorimetric Western Blotting Kit or ECL plus Western Blotting Detection System. This suggested that CYP2C23 was not the main isoform of CYP2C in the cerebral cortex.

To determine whether clofibrate up-regulates the expression of CYP2C11 and CYP4A1 in cerebral tissue, their expression was measured in the microsomal fraction from cerebral tissue. Clofibrate significantly increased CYP2C11 expression in SHRSP/Izm with and without salt loading ($p<0.05$).

Cerebral Arteriole Diameter and Blood Flow

Physiological changes in the cerebral vasculature were assessed by measurements of blood vessel diameters in the branches of the middle cerebral artery as shown in Fig. 3.

Pial arteriole diameters in the group fed the clofibrate-containing diet with a 1% salt solution were significantly larger than those in the group without clofibrate in the diet in the A1 ($p<0.02$) and A2 ($p<0.005$) branches. Furthermore, the

Fig. 2. Clofibrate up-regulates cerebral CYP2C11 expression in SHRSP/lzm. *A:* Representative Western blot pattern of CYP isoforms and β -actin in microsomal fraction. *B:* Each column represents the average value of five experiments. * $p < 0.05$ between groups.

Fig. 3. Effect of clofibrate on the diameter of cerebral arterioles and cerebral blood flow in salt loaded SHRSP/Izm. **A:** Vessel diameter. Branches of the middle cerebral artery were defined as sections A1 and A2 in each of 5 animals. Data shown are the mean $\pm$ SEM ($n=1$ and 2 at each point for A1 and A2, respectively). * $p<0.01$, ** $p<0.0005$ between the groups. **B:** Cerebral blood flow. Cerebral blood flow was measured using a laser Doppler blood flow meter. Data shown are the mean $\pm$ SEM ($n=5$ at each point). * $p<0.0001$ between the groups.

cerebral blood flow was significantly greater in the clofibrate group than in the group without clofibrate ($p<0.0001$). There was no significant relationship between the blood pressure and the vessel diameter or blood flow.

Discussion

The principal findings of this study are as follows: 1) SHR/Izm showed higher levels of cerebral cortex microsomal CYP4A1 and CYP2C11 protein expression than SHRSP/Izm. 2) Salt loading significantly suppressed the expression of cerebral CYP2C11 in SHRSP/Izm but not in SHR/Izm. 3) Clofibrate significantly prolonged the survival of salt-loaded SHRSP/Izm, up-regulated the expression of cerebral CYP2C11, and attenuated the suppression of CYP induced by salt loading in SHRSP/Izm. 4) In salt-loaded SHRSP/Izm, clofibrate enlarged the diameter of cerebral arterioles and resulted in increased cerebral blood flow without affecting blood pressure.

The biological functions of the EETs have been extensively studied in isolated cell and organ preparations, yet our understanding of their physiological significance *in vivo* is limited due to analytical limitations and the lack of stable and selective EET inhibitors and mimics. Moffat and colleagues showed that EETs (either 5,6- or 11,12-EET) protected against ischemia-reperfusion injury in isolated hearts and ventricular myocytes (18). Other effects of EETs include direct inhibition of cell death in culture induced by hypoxia-reoxygenation, hydrogen peroxide, and serum deprivation. In the present study, CYP2C11 in the cerebral cortex, the most common site of stroke, was intrinsically decreased in SHRSP/Izm compared to SHR/Izm. The CYP2C11 expression in the cerebral cortex significantly declined to about half the original value during salt loading in SHRSP/Izm but not in SHR/Izm. Alkayed *et al.* reported that cerebral blood flow was decreased by inhibition of P-450 arachidonic acid epoxygenase (19). They also reported that a preconditioning ischemic stimulus, such as transient ischemic attack, reduced the cerebral infarction size after the occlusion of the right common carotid artery, in which CYP2C11 mRNA and protein expression was significantly up-regulated (20).

An important function of EETs is to increase cerebral blood flow (CBF). Regional CBF (rCBF) is autoregulated by systemic blood pressure. The rCBF in SHRSP/Izm is rapidly decreased to about half of the normal rCBF when the animals develop severe hypertension (over 220 mmHg) (21). Mies *et al.* have reported that the CBF decline in SHRSP with salt loading is significantly correlated with both the severity of functional deficits and the decrease of protein (22). In the current study, the decrease of cerebral CYP2C11 expression in SHRSP/Izm under salt-loaded conditions accelerated the development of stroke, and the up-regulation of its expression by clofibrate prolonged the time before stroke developed. The diameter of the cerebral arterioles was significantly enlarged, and the cerebral blood flow was significantly increased by clofibrate loading. We did not measure EET levels in the brain. However, Ellis *et al.* demonstrated that brain tissues could synthesize EETs, which were potent dilators of cat and rabbit cerebral arterioles *in vivo* (23). Further, fenofibrate

increased the amount of CYP2C11 enzyme with its activity in the renal cortex of obese Zucker rats (24). Combined, these results suggest that the impaired expression of cerebral CYP2C11 involved in EET synthesis is partly related to stroke-proneness in SHRSP/Izm.

As PPAR- α is involved in regulation of lipid metabolism, we observed the effect of clofibrate on the serum cholesterol level in salt-loaded SHRSP/Izm. The serum cholesterol level in salt-loaded SHRSP/Izm was significantly decreased by clofibrate treatment (data not shown). The serum cholesterol level of SHRSP is commonly lower than SHR. This result suggests that the change in serum cholesterol level after clofibrate treatment is not directly related to stroke development.

In rodent experiments, most results support the hypothesis that PPAR- α agonists play a neuroprotective role in ischemic cerebral diseases (25). On the other hand, clofibrate increased the risk for fatal strokes in the meta-analysis on cholesterol reduction and the risk for stroke in men (26). More experiments are needed before the results are applied to human stroke.

References

- Rubattu S, Volpe M, Kreutz R, Ganten U, Ganten D, Lindpaintner K: Chromosomal mapping of quantitative trait loci contributing to stroke in a rat model of complex human disease. *Nat Genet* 1996; **13**: 429–434.
- Deplanque D, Gele P, Petraut O, et al: Peroxisome proliferator-activated receptor- α activation as a mechanism of preventive neuroprotection induced by chronic fenofibrate treatment. *J Neurosci* 2003; **23**: 6264–6271.
- Inoue H, Jiang XF, Katayama T, Osada S, Umehono K, Namura S: Brain protection by resveratrol and fenofibrate against stroke requires peroxisome proliferator-activated receptor α in mice. *Neurosci Lett* 2003; **352**: 203–206.
- Ishizuka T, Ito O, Tan L, et al: Regulation of cytochrome P-450 4A activity by peroxisome proliferator-activated receptors in rat kidney. *Hypertens Res* 2003; **26**: 929–936.
- Fleming I: Cytochrome P450 and vascular homeostasis. *Circ Res* 2001; **89**: 753–762.
- Hoagland KM, Maier KG, Roman RJ: Contributions of 20-HETE to the antihypertensive effects of Tempol in Dahl salt-sensitive rats. *Hypertension* 2003; **41**: 697–702.
- Muller DN, Theuer J, Shagdarsuren E, et al: A peroxisome proliferator-activated receptor- α activator induces renal CYP2C23 activity and protects from angiotensin II-induced renal injury. *Am J Pathol* 2004; **164**: 521–532.
- Alkayed NJ, Birks EK, Narayanan J, Petrie KA, Kohler-Cabot AE, Harder DR: Role of P-450 arachidonic acid epoxygenase in the response of cerebral blood flow to glutamate in rats. *Stroke* 1997; **28**: 1066–1072.
- The Physiological Society of Japan: Guiding principles for the care and use of animals in the field of physiological sciences. *J Physiol Soc Jpn* 1998; **60**: vii–viii.
- Ikeda K, Nara Y, Yamori Y: Indirect systolic and mean blood pressure determination by a new tail cuff method in spontaneously hypertensive rats. *Lab Anim* 1991; **25**: 26–29.
- Krapfenbauer K, Berger M, Lubec G, Fountoulakis M: Changes in the brain protein levels following administration of kainic acid. *Electrophoresis* 2001; **22**: 2086–2091.
- Ying CJ, Xu JW, Ikeda K, Takahashi K, Nara Y, Yamori Y: Tea polyphenols regulate nicotinamide adenine dinucleotide phosphate oxidase subunit expression and ameliorate angiotensin II-induced hyperpermeability in endothelial cells. *Hypertens Res* 2003; **26**: 823–828.
- Morii S, Ngai AC, Winn HR: Reactivity of rat pial arterioles and venules to adenosine and carbon oxide: with detailed description of the closed cranial window technique in rat. *J Cereb Blood Flow Metab* 1986; **6**: 34–41.
- Noguchi T, Ikeda K, Sasaki Y, et al: Effects of vitamin E and sesamin on hypertension and cerebral thrombogenesis in stroke-prone spontaneously hypertensive rats. *Hypertens Res* 2001; **24**: 735–742.
- Ley K, Pries AR, Gaetgens P: Topological structure of rat mesenteric microvessels networks. *Microvasc Res* 1986; **32**: 315–332.
- Morita-Tsuzuki Y, Bouskela E, Hardebo JE: Vasomotion in the rat cerebral microcirculation recorded by laser-Doppler flowmetry. *Acta Physiol Scand* 1992; **146**: 431–439.
- Gustavsson M, Mallard C, Vannucci SJ, Wilson MA, Johnston MV, Hagberg H: Vascular response to hypoxic preconditioning in the immature brain. *J Cereb Blood Flow Metab* 2007; **27**: 928–938.
- Moffat MP, Ward CA, Bend JR, Mock T, Farhangkhoei P, Karmazyn M: Effects of epoxyeicosatrienoic acids on isolated hearts and ventricular myocytes. *Am J Physiol* 1993; **264**: H1154–H1160.
- Alkayed NJ, Birks EK, Hudetz AG, Roman RJ, Henderson L, Harder DR: Inhibition of brain P-450 arachidonic acid epoxygenase decreases baseline cerebral blood flow. *Am J Physiol* 1996; **271**: 1541–1546.
- Alkayed NJ, Goyagi T, Joh HD, et al: Neuroprotection and P450 2C11 upregulation after experimental transient ischemic attack. *Stroke* 2002; **33**: 1677–1684.
- Yamori Y, Horie R: Developmental course of hypertension and regional cerebral blood flow in stroke-prone spontaneously hypertensive rats. *Stroke* 1977; **8**: 456–461.
- Mies G, Hermann D, Ganten U, Hossman KA: Hemodynamics and metabolism in stroke-prone spontaneously hypertensive rats before manifestation of brain infarcts. *J Cereb Blood Flow Metab* 1999; **19**: 1238–1246.
- Ellis EF, Police RJ, Yancey L, McKinney JS, Amruthes SC: Dilation of cerebral arterioles by cytochrome P-450 metabolites of arachidonic acid. *Am J Physiol Heart Circ Physiol* 1990; **259**: 1171–1177.
- Zhao X, Quigley JE, Yuan J, Wang MH, Zhou Y, Imig JD: PPAR- α activator fenofibrate increases renal CYP-derived eicosanoid synthesis and improves endothelial dilator function in obese Zucker rats. *Am J Physiol Heart Circ Physiol* 2006; **290**: H2187–H2195.
- Bordet R, Ouk T, Petraut O, et al: PPAR: a new pharmacological target for neuroprotection in stroke and neurodegenerative diseases. *Biochem Soc Trans* 2006; **34**: 1341–1346.
- Atkins D, Psaty BM, Koepsell TD, Longstreth WT Jr, Larson EB: Cholesterol reduction and the risk for stroke in men. A meta-analysis of randomized, controlled trials. *Ann Intern Med* 1993; **119**: 136–145.