S-1) Specific Elevation of Thromboxane Synthetase Activity and Leukotriene C Formation in Malignant Hypertension in the Hypothalamus of Stroke-Prone Spontaneously Hypertensive Rats (SHRSP). Eiji Ohtsu, Kumiko Abe, Noriyoshi Akimori*, Hisao Itoh**, Taiju Matsuzawa**, Kimio Yashiro, and Masamichi Sakanoue. Department of Pediatrics, Kitasato University School of Medicine, Kanagawa 228. *Applied Technology Center of Hitachi-Naka Factory, Ibaraki 312. **The Research Institute for Cancer and Tuberculosis, Tohoku University, Miyagi 980.

Summary

The main metabolic alterations in the hypothalamus of SHRSP that the endogenous vaso-dilating prostaglandin I_2 biosynthesis was not so enough adaptively enhanced as in SHRSR and that not only the vaso-constricting thromboxane A_2 biosynthesis but also the edema-causative leukotriene C formation elevated much higher than in SHRSR would reasonably contribute to a cause of malignant hypertension prior to stroke.

Introduction

Thromboxane (TX) A_2 is the most potent vaso-constrictor among agents acting on the cerebral circulation (1). Endogenous formation of the prostaglandin (PG) endoperoxide metabolite, TXB $_2$, by guinea pig and rat cerebral cortex was considerably greater than that of PGE $_2$ or PGF $_{2\alpha}$ (2). Although TXA $_2$ has been reported not to be formed in cerebral vessels (3), Ohtsu et al (4) discovered that endogenous TX-forming capacity (in the prehypertensive state) was specifically lower in hypothalamus of novel stroke-resistant spontaneously hypertensive rats (SHRSR) than in that of both Wistar Kyoto rats (WKY) and stroke-prone spontaneously hypertensive rats (SHRSP) (Vth International Conference on Prostaglandins). Now, it is aimed to clarify whether prostanoid biosynthetic capacity for TXA $_2$ and PGI $_2$ as well as novel leukotriene C formation plays any role in malignant hypertension prior to stroke.

Materials and Methods

For this study on both cyclooxygenase products and lipooxygenase products, the rats (mainly 6 M) maintained in a specific pathogen-free room was used; Male SHRSR (F48-49, substrain C), SHRSP (5) and WKY had been donated by Prof. K. Okamoto. They were anaesthetized by ether and killed by decapitation. Each hypothalamic region was isolated to incubate enzymes as homogenates (~ 0.12 mg) in 50 mM potassium phosphate buffer, pH 7.4 at 24°C (endogenous TX synthetase) (4) or in 50 mM Tris-HCl, pH 7.4 at 37°C (5-lipoxygenase) for 1-2 min. For the lipooxygenase assay 150 μ M arachidonic acid was added in a way, by which our first report on Kawasaki disease had been published (Int. Symp. Leukotrienes and Lipoxygenase Products, Florence, 1981). The reactions were terminated under cooling in an ice box by the addition of 3 volumes of a precooled ethyl ether/methanol/0.2 M citric acid mixture (30/4/1) (6) and 1 μ g of 3, 3', 4, 4'-d $_4$ -labeled 6 keto PGF $_{1\alpha}$ (m/e 512) or PGB $_2$ as an internal standard. Our established mass-fragmentographic quantitation of cyclooxygenase products (4) was reproducibly utilized; briefly, by taking advantage of each deuterated prostanoid including the biosynthesized TX (7) as well as each authentic prostanoid (m/e 508). The typical sensitivity of each prostanoid (TXB $_2$, retention = 3.2 min, 27%; 6 keto PGF $_{1\alpha}$, retention = 3.9 min, 67.6%) was taken in consideration. Each prostanoid content was also confirmed after a method of Goehlert et al (8) or alternatively after a radio-immuno assay.

The biosynthesis of leukocyte-lymphocyte-attractive leukotrienes (LT)B $_4$ was determined by a novel procedure of us using PGB $_2$ as an internal standard for high performance liquid chromatography after a pretreatment procedure with Sep-Pak C $_{18}$ cartridge (9). LTC $_4$, which elevates vascular permeability, was quantitated using 40,000 as the molar extinction coefficient (10) again after a pretreatment procedure with Sep-Pak. Protein determinations were made according to Lowry et al (11).

Results and Discussions

The basal levels and calcium (Ca^{+2})-responsive levels of specific TX synthetase and PGI_2 synthetase activities in hypothalamus of WKY, SHRSP and SHRSP before establishment of hypertension (3 M) were measured time-proportionally.

Fig. 1. The Hypothalamic-Endogenous PGI_2 Synthetase and TXA_2 Synthetase Activity in WKY, SHRSP and SHRSP.

●, TX-synthetase; ○, PGI_2 -synthetase. Each point represents a mean of duplicated determinations of combined materials ($n=5\sim10$). Blood pressure range of each strain (SHRSP, 150 mmHg $\sim$ 170 mmHg; SHRSP, 180 $\sim$ 200 mmHg; WKY, $\sim$ 140 mmHg) was severely selected.

First, it was found that basal TX synthetase was two times higher in SHRSP than in WKY, while the basal level in SHRSP was less than a half that of WKY as initially observed at the prehypertensive stage (within 2 M) (4). TX synthetase activities in SHRSP and SHRSP were diminished to a half by incubation with 2.5 mM of Ca^{+2} , although Ca^{+2} stimulated normal TX synthetase activity exceeding 4-fold in WKY. Secondly, basal PGI_2 synthetase activities were found to be 4-fold higher in SHRSP and 3-fold higher in SHRSP than in WKY. The basal PGI_2 forming capacity in SHRSP was clarified to be not so well adaptively enhanced as that in SHRSP ($0.01 < p < 0.05$, student t test). The PGI_2 synthetase activity in SHRSP was insensitive to Ca^{+2} remaining one fifth of the Ca^{+2} -stimulated level in WKY while the activity in SHRSP was further depressed to a half by Ca^{+2} . PGI_2 , of vascular or nerve-endings (7), would work as a potentially hypotensive counterpart. Phentolamine (α -blocker, 0.5 mg/ml), FPL 55712 (LTC_4 receptor antagonist, 5 $\mu\text{g/ml}$) and calmodulin (0.1 mg/ml) not only improved TX alterations in SHRSP and SHRSP but also recovered PGI_2 synthetase activity in SHRSP (12).

These suppressive effects of Ca^{+2} on the prostanoid biosynthesis in SHR sub-strains (except PGI_2 formations in SHRSP) and a restoring effect of aspirin (2.5 mM) on the hypothalamic deficiency of arachidonate-dependent chemiluminescence (13) prompted us precisely to investigate Ca^{+2} -activatable 5-lipoxygenase pathway (initially measured as a chemotactic LTE_4 -forming unit) and then especially edema-associated LTC_4 formations, since SHRSP was uniquely characterized by malignant hypertension associated with brain-edema which finally led to stroke (14). It was actually clarified that LTC_4 biosynthesis was more than several fold ($p < 0.001$) higher uniquely in the hypothalamus of SHRSP (6 M, 200 mmHg $\sim$) than that of SHRSP (6 M, 160 $\sim$ 180 mmHg) (Table 1), as predicted also as to possible involvement of calmodulin-associated pressor and depressor receptor modulations (7) and their possible Ca^{+2} -desensitizations in Kawasaki disease (15) and obesity.

Table 1. Specific Activity of LTC₄ Formations (nmoles/min/mg)

SHRSR	0.14 ± 0.03	(n=6)
SHRSP	2.32 ± 0.79	(n=6)

LTC₄ (elution time in a precolumn-attached μ Bondapak C₁₈ system = 24 min at 25°C) was identified by a coelution with authentic ³H-LTC₄ (NEN) and ³⁵S-LTC₄ (the most precipitantly labeled in the lymphocytes from a coronary aneurysmal infant of Kawasaki disease, 15).

In addition, the metabolic abnormalities above were characteristically localized in hypothalamus. Recently, it was elucidated that the hypothalamic TXB₂ content in 6 M was age-dependently (over 10-fold exceeding the levels within 2 M in accordance with TX synthetase induction) and significantly ($p < 0.01$, Welch's test) higher in SHRSP (255.7 ± 48.8 ng/100 mg, wet Wt) than in SHRSR (124 ± 33.6 ng/100 mg). The same was with LTC₄ and immuno-suppressive LTB₄ via arachidonyl CoA hydrolase. Furthermore, SHRSP was successively converted into an animal model of Kawasaki disease only when challenged with a newly discovered causative viridans toxin (PI = 7.2, Mol. Wt = 30,000); lymphocytes and vasculatures produced LTC₄ precipitantly just like in presensitized patients (16).

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