METABOLIC ALTERATIONS IN THE ENDOGENOUS FORMATION OF 6 KETO-PROSTAGLANDIN $F_{1\alpha}$, THROMBOXANE B_2 AND PROSTAGLANDIN E_2 IN THE BRAIN-STEM OF STROKE-RESISTANT SPONTANEOUSLY HYPERTENSIVE RATS

Eiji OHTSU and Taiju MATSUZAWA¹

*Department of Radiology and Nuclear Medicine,
The Research Institute for Cancer and Tuberculosis,
Tohoku University, Sendai 980, Japan*

(Received March 31, 1980)

Summary Endogenous biosynthetic capacities for prostaglandin (PG) E_2 , thromboxane (TX) B_2 (a stable degradation product of TXA₂) and 6 keto-PGF_{1 α} (a stable degradation product of PGI₂) in the brain-stem fractions of stroke-resistant spontaneously hypertensive rats (SHRSR) and control Wistar-Kyoto rats (WKR) were determined with novel methods and presented in an original report. In comparison with WKR, it is characteristically found that TXB₂ synthesis is increased in excess of threefold in the pons-medulla oblongata of SHRSR, while being decreased by 75% in the hypothalamic region of SHRSR ($0.01 < p < 0.05$). On the other hand, the biosynthesis of PGE₂ is adaptively elevated in both hypothalamus and pons-medulla oblongata regions of each animal, although the PGI₂/PGE₂ ratio was lowered in both these regions of SHRSR.

Keywords prostaglandins, thromboxane, brain-stem, stroke-resistant spontaneously hypertensive rats, gas chromatography-mass spectrometry

Horton (1) has implied that the natural prostanoids are involved in at least two mechanisms which possibly contribute to the normal control of arterial blood pressure: 1) The first mechanism (neurotransmitter control) stands on the fact that if PGE₂ is administered during nerve stimulation, responses to the nerve stimulation diminish and the amount of noradrenaline released is reduced (2). 2) The second mechanism (vasodilator control) relates to the fact that the vasodilator prostaglandins (PGE, PGA and PGI) will lower systemic arterial pressure (3). Here we propose the data which support both mechanisms mentioned above, by analyzing the metabolic alterations of the endogenous PG and TX biosyntheses in the brain-stem regions between SHRSR, established by Okamoto *et al.* (4), and

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¹ 大津英二, 松沢大樹

normotensive Wistar-Kyoto (WK) control rats.

Recently, biochemical evidence accumulated by us has led to a working hypothesis that a novel malondialdehyde (MDA)-forming and concomitantly proaggregatory TXB₂-forming PGH₂ (a potent antilipolytic factor similar to PGE₂, in contrast to PGD₂, as proposed by Samuelsson's group (5)), as well as plasma lipoperoxide (or MDA value according to a modified procedure (6)) mainly of circulatory systems, must be mobilized during body weight reduction (Symposia of Japan Late Effect Group '77-'78). This has been typically shown in the obese hypertensives rather than in the obese (7). Therefore, in the present work, we examined the endogenous capacity for individual prostanoid biosynthesis in the brain-stem using hypertensive animals. A preliminary report has been described pertaining to abnormal TX distributions (Proc. ISTRY '80, in press).

MATERIALS AND METHODS

1) *Animals.* Male SHRSR (F₄₆-F₄₈, substrain C) and WKR were presented by Prof. Emeritus Kozo Okamoto of Kyoto University and used within 2 months before the establishment of hypertension. They were killed by decapitation. Animals were housed at 21 ± 2°C and 50% relative humidity on a 12 hr light and a 12 hr dark cycle. Water and a commercial rat chow (Funahashi MF) were given *ad libitum*.

2) *Determination of endogenous biosynthetic capacities of prostanoids.* Blocks of the regions of both hypothalamus and pons-medulla oblongata from each rat (7 weeks) were cut into small pieces, washed and homogenized in 10 volumes of ice-cooled 0.05 M Tris-HCl pH 8.0-0.15 M NaCl with a Potter-type homogenizer. Enzymes as homogenated samples of up to 40 µl were incubated in 50 mM potassium phosphate buffer pH 7.4 (100 µl final volume) at 24°C for 2 min and the reaction was stopped by the addition of 0.3 ml of a pre-cooled ethyl ether/methanol/0.2 M citric acid mixture (30/4/1), as described by Miyamoto *et al.* (8). The reaction products were extracted with pre-cooled ethyl ether. The mass spectra (as methyl, methoxy and trimethyl silyl derivatives) of PGE₂, TXB₂, and 6 keto-PGF_{1α} (*m/e* 508) were obtained and quantitated by co-chromatography with each of 3,3,4,4-*d*₄-labeled-6 keto-PGF_{1α}, TXB₂ and PGE₂ (*m/e* 512), respectively (9), as well as with each authentic internal standard if necessary, as shown in Pace-Asciak's mass spectrometric profiles (10). The noise was not significant and no further purification was necessary so far as our reproducible procedures were adopted. No interference by endogenous levels of PGE₁ was observed. The level of recovery of each internal standard was more than 80%. Their derivatives were typically applied to a 1 m × 3 mm glass column containing 2% OV-1 on Chromosorb W (AW, DMCS) 80-100 mesh and were chromatographed at 220°C with helium as the carrier gas at a flow rate of 35 ml/min. Mass fragmentographic analyses with gas-liquid chromatography/mass spectrometry were performed using both Shimadzu-LKB 9000B (Kyoto) and

Hitachi M60 (Katsuta) apparatus with EI ion sources.

3) *The preparation of brain-stem fractional regions.* Animals were killed by decapitation. The anatomical brain-stem identifications were performed by the method of Glowinski and Iversen (11).

4) *Chemicals.* Deuterium-labeled arachidonic acid with precise indications for its application was kindly supplied by Dr. S. Hammarström of the Karolinska Institute. $[1-^{14}\text{C}]\text{PGH}_2$ was obtained from Ran Biochemicals of Israel and PGH_2 from Mr. F. Hirata of Ono Central Research Institute (Osaka). Other materials (reagent grade) were commercially obtained.

RESULTS AND DISCUSSIONS

The biosynthetic activities of each prostanoid at block level were examined in the hypothalamus and pons-medulla oblongata regions of SHRSR and WKR. As typically summarized in Table 1, it was found in SHRSR, in comparison with WKR, that biosynthesis of TXB_2 as a stable form of TXA_2 (a novel potent vasoconstrictor) was accelerated in excess of threefold in the pons-medulla oblongata region in contrast to decreased biosynthesis (by approximately 75%) in the hypothalamus region. These facts lend support to the view that TXA_2 plays a role of pressor mediator *via* glia nutrition in the pons-medulla oblongata region. These TXB_2 patterns were confirmed by monitoring the ions (m/e 256, 260 for TXB_2) for the methyl- and trimethylsilyl derivatives (12). On the other hand, biosynthesis of PGE_2 was elevated almost to the same degree in both regions of SHRSR. Furthermore, biosynthesis of 6 keto- $\text{PGF}_{1\alpha}$ was similarly elevated in SHRSR, although the 6 keto- $\text{PGF}_{1\alpha}/\text{PGE}_2$ ratio in both regions of SHRSR was lowered. If PGI_2 (a novel potent vasodilator) should be produced more abundantly around the regions of the resistance vessels in the brain-stem of SHRSR, it would

Table 1. Endogenous biosynthetic activities of PGE_2 , TXB_2 and 6 keto- $\text{PGF}_{1\alpha}$ in the brain-stems of SHRSR and WKR. The values obtained from 3 rats were combined for each average estimation as shown parentheses.

	PGE_2	TXB_2 (ng/100 mg tissue/min)	6 Keto- $\text{PGF}_{1\alpha}$
SHRSR			
Hypothalamus	40.13*(3)	58.88 (3)	39.63 (3)
Pons-medulla oblongata	34.75*(3)	592.88 (3)	12.75 (3)
WKR			
Hypothalamus	11.63 (3)	232.13*(3)	18.63 (3)
Pons-medulla oblongata	0.50 (3)	160.75*(3)	1.38 (3)

* All matched or comparable data are statistically significant ($0.01 < p < 0.05$), except between PGE_2 levels in SHRSR and between TXB_2 levels in WKR.

increase local blood flow and contribute to blood pressure control. Adaptively accelerated PGE₂ formation *via* PGH₂ (including [1-¹⁴C]- and the deuterium-labeled type) was confirmed and the observations were extended in the brain-washed microsomal fractions using the thin-layer chromatographic method of Sun *et al.* (13). These alterations were considered to be independent of aging, as judged also from certain genetical data on an earlier maturation in the thymus gland of SHRSR (data not shown). The scheduled report will demonstrate that a novel synchronous single photon counting method for chemiluminescence reflects the metabolic abnormality of arachidonic cyclooxygenase using activators and inhibitors and that singlet oxygen participates as an active oxygen as suggested by the regulatory effect of L-tryptophan (International Study Group for Tryptophan Research, Kyoto, 1980).

We express our heartfelt thanks to Prof. O. Hayaishi of Kyoto University and the late Prof. Dr. F. Lynen of the Max-Planck Institute for Biochemistry, for their encouragement. Gratitude is also due to Mrs. R. Kubota of our laboratory for her devoted animal experiments and to Dr. I. Himuro of Chugai Pharmaceutical Company and H. Akimori of Hitachi Company for their accurate GCMS analyses.

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