Antihypertensive Mechanisms of Docosahexaenoic Acid (DHA) - Influences on Vascular Smooth Muscle Cells in Culture.

Masahiko Hirafuji*, Takashi Ebihara*, Fumito Kawahara*, Yukitatsu Kanai*, Kenji Kato*, Akihiko Tanimura**, Hideya Saito*** and Masaru Minami*.

*Department of Pharmacology, Faculty of Pharmaceutical Sciences, and **Department of Pharmacology, School of Dentistry, Health Sciences University of Hokkaido, Ishikari-Tobetsu 061-02, and ***First Department of Pharmacology, Hokkaido University School of Medicine, Sapporo 060, Japan.

We previously reported that the feeding of 6 week-old male stroke-prone spontaneously hypertensive rats (SHRSP) with docosahexaenoic acid (DHA: C22:6n-3) for 14 weeks significantly and dose-dependently suppressed the development of hypertension (1). In the present study, the influences of DHA treatment on functions of rat aortic smooth muscle cells (SMC) in culture was investigated in order to clarify the antihypertensive mechanisms of DHA.

Rat aortic medial SMC were isolated by collagenase and elastase digestion. DHA (Sigma) was added at 30 μ M in serum-containing medium or at 10 μ M in serum-free medium. Relative size of cells treated with angiotensin II (Ang II) and/or DHA was measured by flow cytometric method. The intracellular Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) and Mn^{2+} influx were measured using fura-2 as the fluorescence indicator. The metabolism of inositol phosphates (IPs) were evaluated using *myo*-[^3H]-inositol.

SMC isolated from SHRSP showed the increased cell proliferation as compared with Wistar Kyoto rats (WKY). The treatment of SMC of SHRSP and WKY with DHA (30 μ M) had no significant effects. Also, DHA (10 μ M) had no significant effect on Ang II-induced increase in relative cell size of SMC of SHRSP as measured by flow cytometry. However, DHA (30 μ M) treatment suppressed the increase in $[\text{Ca}^{2+}]_i$ in SMC induced by 5-hydroxytryptamine (5-HT), Ang II and KCl, but not by thapsigargin (TG). DHA treatment also suppressed the Mn^{2+} influx induced by 5-HT, Ang II and KCl. DHA did not suppress the production of IPs.

Vascular hyperplasia and hypertrophy are associated with hypertension. DHA had no effects on the proliferation and hypertrophy of SMC isolated from SHRSP. However, DHA suppressed the agonist-induced Ca^{2+} influx, probably through voltage-dependent Ca^{2+} channel, while had no effect on Ca^{2+} release from the intracellular stores. These results suggest that, in addition to the improvement of renal functions (1), the suppression of Ca^{2+} influx in vascular SMC may be one of the mechanisms for the antihypertensive effect of DHA in SHRSP.

(1) Kimura, S., et al., *Biogenic Amines* 11, 195 (1995).