Sympathetic regulation of vascular endothelial growth factor family expression in brown adipose tissue of rodents

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Brown adipose tissue (BAT) plays a key role in non-shivering thermogenesis in mammals, especially in small rodents. When they are exposed to cold, BAT produces heat by uncoupling of ATP synthesis from fatty acid oxidation in mitochondria. In addition, cold exposure provokes BAT to hyperplasia accompanied by angiogenesis. The heat production and the growth of precursor cells of brown adipocytes have been shown to be directly controlled by sympathetic nerves in this tissue via the β-adrenergic receptors. However, little is known about the mechanism of angiogenesis elicited by cold. Recently, vascular endothelial growth factor (VEGF) and its homologues (VEGF-B and VEGF-C) were cloned, and all of them have been shown to have a potent angiogenic activity. Thus, in the present study, I have explored the expression of VEGF and its homologues in rat BAT, and examined the regulatory mechanism of their expression in vivo and also in vitro using a brown adipocyte cell line HB2 cell.

In rats kept at room temperature, VEGF and VEGF-B mRNA were abundantly expressed in BAT at comparable levels to heart and lung, while VEGF-C mRNA was not detected by the conventional Northern blot analysis. Exposure of rats to cold at 4 ℃ increased VEGF mRNA expression by 2–3 fold only in BAT, but did not affect VEGF-B expression. The increase of VEGF was observed 1–4 hr after the start of cold exposure, but not at 24 hr. The increased VEGF expression in response to cold was found in BAT, but not in lung, heart and kidney. RT-PCR analysis revealed that rat BAT expressed three mRNAs of VEGF splicing variants (VEGF120, VEGF164 and VEGF188). At room temperature the longest isoform (VEGF188) was expressed most abundantly, but after cold exposure the shortest isoform (VEGF120) became dominant. Thus, cold exposure induced a rapid increase in mRNA of VEGF, especially of VEGF 120 lacking heparin-binding region, specifically in BAT. The cold-induced increase of VEGF expression vanished when sympathetic nerves in BAT were surgically excised. Administration of noradrenaline or a specific β3-adrenergic agonist, CL316,243, mimicked the cold-induced VEGF expression in BAT regardless of whether sympathetic nerves were denervated or not. These results suggest that the cold-induced VEGF expression is mediaFted through stimulation of the β-adrenergic pathway by noradrenaline released from sympathetic nerves.

To further clarify the mechanisms of VEGF expression in BAT, an immortal brown adipose cell line, HB2, was establised from p53 tumor suppressor gene-deficient mice. Fibroblast-like HB2 cells, could accumulate lipid droplets in response to insulin and T_3, or troglitazone, which is a ligand of the peroxisome proliferator activated receptor γ type and the most potent inducer of adipose differentiation. The adipocyte characteristics of HB2 cells were also confirmed by expression of aP2 mRNA, a marker of adipocyte. Moreover, mRNA expression of uncoupling protein 1, which is specifically present in brown adipocyte, was induced by treatment of

HB2 adipocytes with troglitazone and noradrenaline. Undifferentiated HB2 cells expressed significant levels of VEGF-B and VEGF-C mRNAs, but a low level of VEGF. After HB2 cells were differentiated into adipocytes, the VEGF mRNA level increased without noticeable change in the VEGF-B and VEGF-C mRNA levels. VEGF mRNA expression increased in undifferentiated but not in differentiated HB2 cells by stimulation through the β-adrenergic pathway. VEGF-B gene expression was not changed by adrenergic stimulation in both undifferentiated and differentiated HB2 cells. In contrast, a marked reduction of VEGF-C mRNA expression was found when HB2 cells were treated with noradrenaline, cAMP analogue and troglitazone. These results suggest a specific role of the β-adrenergic mechanism for regulation of VEGF expression. The regulatory mechanisms of VEGF-B and VEGF-C expression are to be investigated in future studies.

Original papers of this thesis appeared in "Biochem. J.", Vol. 328 : 179–183 (1997) and "J. Vet. Med. Sci.", Vol. 61 : 403–409 (1999).

Pharmacological properties and antihypertensive effects of KRN4884, a novel potassium channel opener

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Original papers of this thesis appeared in "Naunyn-Schmedeberg's Arch Pharmacology", Vol. 354, 460–465 (1996), "Journal of Cardiovascular Pharmacology", Vol. 29, 814–819 (1997), and "Journal of Cardiovascular Pharmacology", Vol. 33, 292–294 (1999).

Role of gastrin in gastric acid secretion and ulcer recurrence, and the effects of a new anti-ulcer drug

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Original papers of this thesis appeared in Journal of Pharmacolgical Experimental Therapeutics, Vol. 277, 28–33 (1996), American Journal of Physiology, Vol. 272, G335–G339 (1997), and Arzneimittel-Forschung/Drug Research, Vol.48, 70–73 (1998).