Endothelin (ET) Receptors in Brain of SHRSP with Cerebral Stroke. Yasuko Sakurai-Yamashita, Kimihiro Yamashita, Kohtaro Taniyama and Masami Niwa
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The endothelin (ET) family of peptides originally isolated from cultured porcine aortic endothelial cells function to increase the mobilization of intracellular Ca2+ and the influx of extracellular Ca2+ in neural cells. There is evidence to support the idea that microglial and astrocytic ET system participate in the pathophysiology of ischemia-related neural tissue damage, as based on findings of delayed neuronal death in an animal model, the stroke-prone spontaneously hypertensive rat (SHRSP), with induced transient forebrain ischemia (Yamashita, K. et al., Cell. Mol. Neurobiol. 13: 15-23, 1993 ; Yamashita, K. et al., J. Neurochem. 63: 1042-1051, 1994). In the present study, we investigated changes of the ET receptor in neural lesions of the brain of SHRSP with cerebral stroke. Furthermore, as nitric oxide (NO) has been thought to be a neurotoxic factor and ET stimulates NO production, we also histochemically studied NO synthase (NADPH-diaphorase) in neural lesions. ET receptors were labelled and characterized using 125I-ET-1, 125I-IRL1620 (a specific radioligand for ETB receptor) and 125I-PD151242 (a specific radioligand for ETA receptor) as ligands for a quantitative receptor autoradiographic experiment. Nitric oxide synthase activity was visualized using the NADPH-diaphorase histochemical method.

Twelve male SHRSPs were decapitated or perfused with 0.1M phosphate buffered saline followed by 4% paraformaldehyde under diethyl ether anesthesia after they showed signs of cerebral stroke; the decrease of body weight and the disruption of behavioral circadian rhythms at 30 to 40 weeks of age. Consecutive, frozen coronal tissue sections (20µm thick) of the damaged brain areas obtained from the non-perfused brains were cut in a cryostat, incubated with 50mM Tris-HCl buffer (pH 7.2) containing 100mM NaCl, 10mM EDTA-Na, 4µg/ml leupeptine, 2µg/ml chymostatin, 10µM phosphoramidon, 0.3% BSA and 1mg/ml bacitracin with 2nM 125I-ET-1, 1nM 125I-IRL1620 or 1nM 125I-PD151242 for 48 hours at 4°C. Non-specific binding study was performed to incubate the sections in the presence of cold 1µM ET-1, IRL1620 or PD151242. Adjacent, related sections were labelled with anti GFAP (CHEMICON), Griffonia simplicifolia B4-isolectin (GSA-IB4; Sigma) or anti ET-1 (Peninsula) and stained with an ABC method. The perfused brains were cut into sections of 100µm thickness and incubated with 100mM Tris-HCl buffer containing 1mM NADP, 0.2mM nitro blue tetrazolium, 15mM sodium malate and 0.2% Triton X-100 at 37°C for 40 min. Some of the sections were further labelled with anti-GFAP or GSA-IB4.

We observed a dramatic increase of 125I-ET-1 binding sites of in the areas corresponding to the neural tissue lesion with cerebrovascular damage. The increase in the binding sites for 125I-IRL1620 and 125I-PD151242 was observed in the all areas where 125I-ET-1 binding sites increased, although the proportion of the increase of ETB and ETA receptors differed in the tissue damages, a finding which suggests that both ETA and ETB receptor participate in the pathophysiology of the cerebrovascular damage. Activated astrocytes and microglia were also present in the areas with the ET receptors. NADPH-diaphorase activity was observed in astrocytes and a few microglia of the cerebrovascular damaged area. Astrocytic ET-like immunoreactivities were found in the damaged areas.

Astrocytes equipped with the ETA and/or ETB receptors were predominantly present in neural lesions of cerebral stroke and these cells had a significant amount of NOS activity. The possibility that astrocytes are activated to drive their own ET system and to produce NO in neural lesions would have to be considered. As in the stage of wound-healing process after damage to neural tissue, the competition between reactive astrocytes and activated microglia are thought to be of importance for survival of neurons and for remodelling of neural tissue, the present finding will pave the way for studies on the pathophysiological significance of glial ET/NO system on neural tissue-repair.