Sympathoinhibitory nNOS Neurons in the Brain of Salt-Sensitive Hypertensive Dahl Rats.

Yasuhiro Nishida[1], Megumi Tandai-Hiruma[1], Jouji Horiuchi[2], Takehito Kemuriyama[1], Haruhisa Hirakawa[1]

[1]National Defense Medical College, Department of Physiology II

[2]University of Sydney, Department of Physiology

Summary

Background: We have previously demonstrated that the nNOS neuronal system in the brainstem was enhanced in salt-sensitive hypertensive Dahl rats. Immunohistochemical study revealed that the number of nNOS containing neurons (nNOS neurons) in the brainstem was greater in hypertensive rats than in normotensive rats.

Objective: To investigate how the above nNOS neuronal system is working in the hypothalamus and medulla oblongata of hypertensive Dahl salt-sensitive (DS) rats, we determined the distribution of nNOS neurons in the hypothalamus and medulla oblongata.

Design and methods: Dahl rats were fed either a regular-salt (0.4% NaCl) or a high-salt (8% NaCl) diet for 4 weeks. ***Immunohistochemical staining*:** Rats were anesthetized with pentobarbital (50 mg/kg, ip) and perfused through the ascending aorta with 300 ml of heparinized saline followed by 400 ml of freshly prepared 4% paraformaldehyde in 0.1M phosphate buffer (pH 7.2). The brain was removed and postfixed at 4ºC overnight. The fixed brain was placed in 10, 20, and finally 30% sucrose in 0.1M phosphate buffer (pH 7.2) at 4ºC overnight. The brain was then frozen in powdered dry ice, blocked in the coronal plane, and sliced a thickness of 40 m on a cryostat. The slices were collected in PBS(-) and free-floating brain slices were undergone to the immunohistochemical procedure using anti-nNOS antibody for the 1st antibody, biotinylated 2nd antibody, and ABC reagent. They were then detected with diaminobenzidine tetrahydrochloride.

Results: nNOS neurons were localized in several nuclei through the brainstem to the hypothalamus; the paraventricular nucleus (PVN), supraoptic nucleus (SON), the dorsolateral periaqueductal gray (DLPAG), pedunculopontine tegmental nucleus (PPT), dorsal raphe nucleus, laterodorsal tegmental nucleus (LDT), lateral parabrachial nucleus (LPB), rostral ventrolateral medulla (RVLM), nucleus tractus solitarius (NTS), raphe magnus. The number of nNOS neurons in these nuclei, except for the two raphes and PVN, was significantly greater in hypertensive than in normotensive DS rats.

Conclusions: These findings suggest that central nNOS neurons in SON, DLPAG, PPT, LDT, LPB, RVLM, and NTS may be the sites for sympathoinhibitory regulation in salt-sensitive hypertensive Dahl rats.