42) Influence of Clonidine, α -Methyldopa and Hydralazine on Noradrenergic and Adrenergic Neuronal Activities in Brainstem Nuclei of Spontaneously Hypertensive Rats. Kazuo Nakamura, Hisae Ishii, Toshikazu Okada, and Keiji Nakamura. Department of Pharmacology, Nippon Roche Research Center, Kamakura 247.

The role of the central nervous system in the maintenance of hypertension has not been clarified. In adult SHR, changes in dopamine β -hydroxylase (DBH) and phenylethanolamine N-methyltransferase (PNMT) activities in some brainstem nuclei have been noted recently. Effects of clonidine and α -methyldopa on both enzyme activities in brainstem nuclei of SHR were examined to clarify the role of catecholaminergic neurons in the maintenance of hypertension as well as the action mechanisms of both drugs using hydralazine as a peripherally active reference.

Methods. Adult SHR and corresponding Kyoto Wistar Rats (KWR) at 25 weeks of age were used. A single oral dose of clonidine (0.1 mg/kg), α -methyldopa (50 mg/kg) and hydralazine (2.5 mg/kg) which lowers aortic BP of conscious SHR by 30 - 40 mmHg was administered twice daily for 4 consecutive days. Animals were sacrificed by decapitation one hour after the last 9th dose on day 5. Nine brainstem nuclei were dissected by the method of Palkovitz. DBH and PNMT activities were measured radioenzymologically by the respective method of Saavedra *et al.* and Moore and Phillipson. Affinity of the three drugs to cerebral α -receptor was measured *in vitro* by a binding assay using ^3H -NE as a radioligand in crude membrane suspension from the brain minus cerebellum.

Results. Density of ^3H -NE binding sites in cerebral crude membranes from SHR (19.5 ± 0.9 fmole/mg protein at 4nM ^3H -NE, $n=8$) was identical to those of KWR (19.7 ± 1.1). Clonidine significantly decreased DBH in LC and increased it in the spinal intermediolateral cell column at T2 level (IML). The drug significantly decreased PNMT in the nucleus hypothalamicus posterior (NHP) and increased it in the nucleus paraventricularis (NPA). α -Methyldopa tended to decrease DBH in NTS, nucleus reticularis lateralis (NRL) and nucleus periventricularis (NPE), while the drug significantly elevated PNMT in NPA. Hydralazine tended to increase DBH in IML, and significantly decreased PNMT in NRL and NHP, and increased it in NPA. In cerebral membrane preparations of SHR, clonidine had the highest affinity ($\text{IC}_{50}=7.0 \pm 1.1$ nM, $n=4$) to ^3H -NE binding sites followed by the affinity (46 ± 8 nM) of α -methyl($\pm$)NE. Hydralazine virtually had no affinity (8.3 ± 2.7 μM).

Discussion. The clonidine-induced decrease in LC DBH might be explained by α -agonistic effects, i.e., highest affinity to ^3H -NE binding sites in crude membrane suspension among three drugs examined. In response to the activation of postsynaptic α -receptor in LC and innervating terminals in NPE and other nuclei except for IML, the decreased DBH presumably serves as a part of compensatory feedback mechanisms which reduces NE availability. The decrease in PNMT in NHP and NPA and the elevated DBH in IML might be also explained by the mechanisms through baroreceptor distension. As compared to clonidine and hydralazine, effects of α -methyldopa on both enzymes are mild. α -Methyldopa significantly elevates PNMT in NPA and slightly lowers DBH in NTS and A1 cell. NTS is the most sensitive site among 16 regions of locally administered α -methyl NE which induces a fall in BP and heart rate (Nikamp, De Jong, 1975). Hydralazine lowers BP by acting on the vasculature by a mechanism independent of sympathetic vasomotor tone and CNS, indicating that changes in brainstem nuclei are most probably caused by autonomic reflexes secondary to reduction of baroreceptor afferent impulses to the NTS and NRL. The reduced baroreceptor impulses to NTS which causes an increase in PNMT in NTS and NPA may decrease epinephrine synthesis in NHP and NRL, and activate NE synthesis in IML, probably resulting in tachycardia.

Conclusion. The CNS-active hypotensive drugs affect noradrenergic and adrenergic neurons in brainstem nuclei through a postsynaptic α -receptor stimulation and the baroreceptor reflex to BP fall. Activation of adrenergic neurons in NRL and NHP of SHR may be results of baroreceptor activation. Density of ^3H -NE binding sites (α -receptor density) in cerebral membrane suspension of SHR is identical to that of KWR.