Selective activation of noradrenergic neurons in the brainstem and spinal cord of young spontaneously hypertensive rats

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Summary. In young spontaneously hypertensive rats (SHR), dopamine β-hydroxylase (DBH) and phenylethanolamine N-methyltransferase (PNMT) activities were examined in the brainstem nuclei. Activation of noradrenergic neurons in the locus coeruleus, A2 and spinal intermediolateral cell areas, resulting in enhanced sympathetic nervous activity in the periphery, initiates hypertension. Adrenergic neurons, unchanged in these and A1 cell areas of young SHR, are not involved in the development of hypertension in SHR.

Trans-synaptic regulation of sympathetic nervous system maturation in the periphery is under control of suprasegmental mechanisms in the central nervous system[1,2]. Young spontaneously hypertensive rats (SHR) were used as a prehypertensive model of essential hypertension. Peripheral circulating levels of noradrenaline and dopamine β-hydroxylase (DBH) as measures of sympathetic nervous activity were markedly elevated in young SHR[3-5]. The elevation was abolished by transection of preganglionic sympathetic fibres to coeliac ganglia[5] or by ganglionectomy[6]. Recent work has demonstrated the presence of noradrenaline and adrenaline containing neurons in the brain and in the spinal cord. The noradrenaline-synthesizing enzyme, DBH, and the adrenaline-synthesizing enzyme, phenylethanolamine N-methyl-transferase (PNMT), have been detected in discrete areas of the brain and the spinal cord by the use of antibodies against bovine adrenal DBH and PNMT, respectively[7,8]. Nerve cell groups containing DBH were found to be densely localized in the rostral area of the nucleus reticularis lateralis (A1) and the dorsal part of the reticular formation ventromedial to the vestibular nuclei (A2)[7], both areas identical to nerve cell groups containing PNMT[8]. The commissural portion of the nucleus tractus solitarius (NTS) has been demonstrated as a site of termination[9] and relay of baroreceptor afferents[10].

Methods. In young SHR, noradrenergic and adrenergic neuronal activites in the A1, A2, NTS, locus coeruleus (LC) and spinal intermediolateral cells (IML) were examined by measuring respective DBH and PNMT activities at the age of 5 weeks, when the blood pressure of SHR did not differ from normotensive controls. Normotensive animals of the Kyoto Wistar rats (KWR), from which SHRs were derived, were used as controls. Systolic blood pressures as measured by a tail plethysmographic method were 108±9 mmHg (mean±SEM; n=5) for young KWR and 110±5 mmHg (n=5) for young SHR. For dissecting the cerebral nuclei, rat brain maps for DBH[7] and PNMT[8] neurons were utilized based on Craigie's rat brain anatomy[11]: A1 and A2 cell areas for PNMT on Craigie's frontal A5 and A6; A1 and A2 cell areas, and NTS for DBH on the frontal A4, and LC on the frontal A8. Spinal IML sections were obtained from the spinal segments T2 because of the abundance of cardioacceleratory IML neurons[12]. The individual nucleus was removed bilaterally with a tube knife (500 μm diameter) from 300 μm cryostat section by microdissection method[13]. DBH and PNMT activities in the homogenates of respective 6 and 1 pairs of nuclei were determined radiometrically[14,15]. The homogenate protein was assayed[16] with bovine serum albumin as standard.

Results and discussion. In young SHR, DBH activities in LC, A2, and IML cells were significantly elevated as compared with those in young KWR (table). DBH activities in the NTS and A1 areas did not differ markedly from those of young KWR. In these young SHRs, PNMT activities in the A1, A2, LC and IML areas were not significantly different from those in young KWRs (table). The present findings on DBH elevation in the LC, A2, and IML areas of young SHR indicate genetic variation in enzyme involved in synthesis of noradrenaline. DBH, tyrosine hydroxylase and PNMT, enzymes involved in catecholamine synthesis, have been shown to be under genetic control[17]. Electrical stimulation of LC was known to elevate blood pressure in anesthetized cats[18,19]. The present findings indicate selective elevation of DBH activity in the A2 region but not in the NTS. The NTS receives DBH-containing axons from closely adjacent A2 cell area[7]. In young SHR at prehypertensive stage, NTS neurons are still not operative. Further, the present results are in good agreement with the previous report[20] describing accumulation of noradrenaline in the A2 area and no change in noradrenaline contents in the NTS of SHR at 16 weeks of age. All this suggests that noradrenergic neuronal activity in the NTS, a terminal of baroreceptor afferents, is functionally independent from the A2 cell area. The elevation of DBH activity found in the A2 cell and LC areas may be identical with the recent finding, the increase in DBH activity in the pons-medulla area of young SHR at 8 weeks of age[21]. The A2 cells containing DBH cell bodies are known to send DBH-containing fibres to the spinal IML[22]. Electrolytic lesions of the LC deplete catecholamine-containing nerve terminals in thoracic spinal ventral column and intermediate grey[23]. The IML region contains the highest concentration of DBH[24] and noradrenaline[25] among the rat spinal areas. These imply that elevation of DBH activity in the IML terminals of young SHR may be closely related to elevation of its activity in DBH cell bodies at the

Enzyme activities in the brainstem and spinal of young spontaneously hypertensive (SHR) and Kyoto Wistar rats (KWR) at 5 weeks of age

Nuclei	Dopamine β-hydroxylase (nmole/h mg protein)		Phenylethanolamine N-methyltransferase (nmole/h mg protein)	
	KWR	SHR	KWR	SHR
Locus coeruleus	8.21±1.28	12.82±1.49*	119±19	98±19
A 2 cell area	2.28±0.28	3.13±0.22*	167±29	164±15
N. tractus solitarius	1.11±0.09	1.18±0.15	1416±60	1404±122
A 1 cell area	2.80±0.35	2.43±0.17	556±70	589±72
Intermediolateral cell area	0.72±0.05	1.04±0.11*	125±17	140±18

Values are mean±SEM of 6 samples, 6 pairs of nuclei/sample for DBH and of 14 pairs of nuclei for PNMT. * $p<0.05$ compared with KWR.

LC and A2 areas. Most neuronal populations in the spinal IML cells are sympathetic preganglionic neurons[26]. Among PNMT-containing neurons in 5 areas examined, we could not find any significant change in both young SHR and KWR. The results are in accordance with the recent report[27] indicating no change in adrenaline contents in A1 and A2 areas of young SHR, since the distribution of PNMT activity in the rat brain correlates with that of adrenaline[28]. However, the early report[29] describes a significant elevation of PNMT activity in the A1 and A2 areas of young SHR. The discrepancy might be explained by strain difference in the onset of hypertension caused by long inbreeding at various sources. Indeed our preliminary study indicates elevation of PNMT activity in the A1 cell area of adult SHR at 25 weeks of age. The present results give no direct evidence suggesting the participation of adrenergic neurons in the brainstem and spinal areas in the development of hypertension in SHR.

The observed activation of noradrenergic neuronal activity in the LC, A2 and IML areas may be related to activation of synaptic neurons in sympathetic ganglia[5] and of peripheral sympathetics[3-5]. Since activation of the peripheral sympathetic nervous system disappeared at adult SHR, causal relationship of both is still doubtful[6]. However, when one can consider labile hypertension as an early sign of essential hypertension[30,31], the activation of the peripheral sympathetic nervous system in young SHR cannot be excluded as one of the causal factors inducing hypertension. It is clear that in young SHR the selective activation of noradrenergic neurons in the LC, A2 and IML areas initiates the peripheral sympathetic nervous activation, as an early sign of hypertension. Adrenergic neurons in the brainstem were not involved in the development of hypertension nor in the activation of the peripheral sympathetic nervous system of young SHR.

1 H. Thoenen, Life Sci. *14*, 223 (1974).
2 I.B. Black, E.M. Bloom and R.W. Hamil, Proc. nat. Acad. Sci. USA *73*, 3575 (1976).
3 H. Grobecker, M.F. Roizen, V. Weise, J.M. Saavedra and I.J. Kopin, Nature *258*, 267 (1975).
4 T. Nagatsu, K. Ikuta, Y. Numata, T. Kato, M. Sano, I. Nagatsu and T. Takeuchi, Science *191*, 290 (1976).
5 K. Nakamura and K. Nakamura, Nature *266*, 265 (1977).
6 K. Nakamura and K. Nakamura, Naunyn-Schmiedebergs Arch. Pharmak. *299*, 143 (1977).
7 L.W. Swanson and B.K. Hartman, J. comp. Neurol. *163*, 467 (1975).
8 T. Hökfelt, K. Fuxe, M. Goldstein and O. Johansson, Brain Res. *66*, 235 (1974).
9 T. Chiba and N. Doba, Brain Res. *102*, 255 (1976).
10 H.R. Kirchheim, Physiol. Rev. *56*, 100 (1976).
11 W. Zeman and J.R.M. Innes; in: Craigie's Neuroanatomy of the Rat. Academic Press, New York 1963.
12 J.L. Henry and F.R. Calaresu, Am. J. Physiol. *222*, 700 (1972).
13 M. Palkovits, Brain Res. *59*, 449 (1973).
14 J.M. Saavedra, M. Brownstein, M. Palkovits, S. Kizer and J. Axelrod, J. Neurochem. *23*, 869 (1974).
15 K.E. Moore and O.T. Phillipson, J. Neurochem. *25*, 289 (1975).
16 D.H. Lowry, N.J. Rosebrough, A.L. Farr and R.J. Randall, J. biol. Chem. *193*, 265 (1951).
17 R.D. Ciaranello and J. Axelrod, J. biol. Chem. *248*, 5616 (1973).
18 D.G. Ward and C.G. Gunn, Brain Res. *107*, 401 (1976).
19 H. Przuntek and G. Philippu, Naunyn-Schmiedebergs Arch. Pharmak. *276*, 119 (1973).
20 D.H. Versteeg, M. Palkovits, J. Van der Gugten, H.L.J.M. Wijnen, G.W.M. Smeets and W. de Jong, Brain Res. *112*, 429 (1976).
21 A. Nagaoka and W. Lovenberg, Eur. J. Pharmac. *43*, 297 (1977).
22 A. Dahlström and K. Fuxe, Acta physiol. scand. *62*, suppl. 232, 1 (1964).
23 L.-G. Nygren and L. Olson, Brain Res. *132*, 85 (1977).
24 J.A. Zivin, J.L. Reid, M.L. Tappaz and I.J. Kopin, Brain Res. *105*, 151 (1976).
25 J.A. Zivin, J.L. Reid, J.M. Saavedra and I.J. Kopin, Brain Res. *99*, 293 (1975).
26 J.M. Chung, K. Chung and R.D. Wurster, Brain Res. *91*, 126 (1975).
27 H.J.L.M. Wijnen, D.H.G. Versteeg, M. Palkovits and W. de Jong, Brain Res. *135*, 180 (1977).
28 S.H. Koslow and M. Schlumpf, Nature *251*, 530 (1974).
29 J.M. Saavedra, H. Grobecker and J. Axelrod, Science *191*, 483 (1975).
30 V. DeQuatro and Y. Miura, Am. J. Med. *55*, 363 (1973).
31 S. Julius and M. Esler, Am. J. Cardiol. *36*, 685 61975).

The effects on thermoregulation of intracerebroventricular injections of L-aspartic acid in the sheep

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Summary. L-Aspartic acid was injected into a lateral cerebral ventricle of the sheep at ambient temperatures between 0° and 40 °C. Doses of 100 or 500 nmoles · kg^{-1} caused a rise in heat production and/or a decrease in heat loss; rectal temperature rose. Atropine sulphate attenuated or prevented these effects.

We have recently reported[1] that the injection of L-glutamic acid into the cerebral ventricular system of the sheep produces regular changes in the thermoregulatory effectors which result in a rise in rectal temperature (T_{re}). In view of the accumulating evidence[2,3] that L-aspartic acid may be involved in central nervous transmission we have tested the effect of intracerebroventricular (ICV) injections of this substance in similar experiments. A total of 30 experiments were done on 12 adult castrated male Welsh Mountain sheep, each fitted with a cannula aseptically implanted into one lateral cerebral ventricle. At least 7 days were allowed after surgery before experiments were begun. These were conducted in a temperature-controlled chamber at 0°, 7°, 20° or 40 °C. A dose of 100 or 500 nmoles · kg^{-1} L-aspartic acid was injected through the cannula in a volume of 0.15 ml sterile, pyrogen-free 0.9% saline; the drug was washed in with a further injection of 0.25 ml saline. In some experiments an injection of 20 or 40 nmoles · kg^{-1} atropine sulphate (in 0.15 ml) was given 10 min before the L-aspartic acid.

Rectal temperature (T_{re}), respiratory frequency (RF) and the temperature of the skin of the ears and flank (indicative