21) Myocardial Parasympathetic Nervous System in SHR: Choline Acetyltransferase Activity in Heart by HPLC-ECD.

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Introduction: Several recent studies have presented evidence that a CNS cholinergic mechanism may be involved in the pathogenesis of the elevated blood pressure in spontaneously hypertensive rats (SHR). In this study, peripheral cholinergic function in heart of SHR was assessed by the measurement of choline acetyltransferase (CAT) activity in discrete areas of heart of SHR and Wistar Kyoto rats (WKY).

Materials and Methods: Male SHR and WKY were examined at the ages of 4 and 12 weeks. The blood pressure and heart rate were measured by tail-cuff method before sacrifice. The animals were decapitated and the hearts were taken out to divided into four parts such as right atrium (RA), left atrium (LA), right ventricle (RV) and left ventricle (LV). Each specimen were frozen on dry ice and stored in -40°C until analysis. The enzyme solution for measurement of CAT activity was prepared from frozen tissue by homogenization in 12.5 ml of 25 mM sodium phosphate buffer (pH 7.4), per g weight, using an ultrasonic probe homogenizer (SONIFIER Model 185, Branson, U. S. A.) at a setting of 5 (20 kHz, 4w, 30 sec). An assay for CAT activity was performed according to Nagatsu's method. The acetylcholine (ACh) enzymatically formed was quantified by F-Y method using a highly sensitive and specific high performance liquid chromatography-electrochemical detection (HPLC-ECD) with a immobilized fixed enzyme column system.

Results: 1) Blood pressures were significantly higher in SHR at the ages of 4 and 12 weeks (107 ± 1 mmHg, 172 ± 3 mmHg) compared with age matched WKY (87 ± 4 mmHg, 129 ± 1 mmHg). Heart rate was significantly heigher in SHR (400 ± 14 beats/min) compared with in WKY (315 ± 14 beats/min) at the age of 12 weeks, but not at the age of 4 weeks (424 ± 3 beats/min, 423 ± 5 beats/min).

2) The CAT activities in RA were extremely higher than the other portions of heart in SHR as well as in WKY. At the age of 4 weeks, the CAT activities in RA, LA, RV and LV of SHR were significantly heigher than those of WKY. At the age of 12 weeks, the CAT activity of SHR remained to be heigher only in RA than that of WKY.

Conclusion: The present study revealed that RA had an extremely strong cholinergic innervation compared with LA, RV and LV in both WKY and SHR, which was estimated by CAT activity, and that the myocardial CAT activity in SHR was significantly higher not only at hypertensive stage but also at prehypertensive stage than that in age matched WKY.

Choline Acetyltransferase Activity in Hearts of WKY and SHR
[formed ACh p mol/min/mg tissue]

	n	Atrium		Ventricle	
		right	left	right	left
4w	WKY (12)	5.67 ± 0.60	1.35 ± 0.17	1.15 ± 0.09	0.76 ± 0.07
	SHR (12)	$9.07 \pm 0.93^{**}$	$2.23 \pm 0.25^{**}$	$1.97 \pm 0.10^{***}$	$1.00 \pm 0.09^{*}$
12w	WKY (6)	7.07 ± 0.33	2.35 ± 0.25	1.74 ± 0.14	0.71 ± 0.11
	SHR (5)	$12.63 \pm 0.55^{***}$	2.97 ± 0.59	1.94 ± 0.24	0.72 ± 0.08

Mean $\pm$ S.E. $^{*}p < 0.05$ $^{**}p < 0.01$ $^{***}p < 0.001$ vs. WKY