Acta Medica et Biologica
Vol. 38, No. 2, 69-74 1990

Changes in Cardiac α_1-and β_1-adrenergic Receptors and Ca^{2+} Binding Sites in the Myocardium of SHR

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Received March 15, 1990

Summary. **Values of Kd and Bmax in ^{3}H-prazosin, ^{125}I-iodocyanopindolol and ^{3}H-nitrendipine bindings to α_1- and β_1-adrenergic receptors and Ca^{2+} binding sites of the myocardium of 2, 6, and 16 week-old Wistar Kyoto normotensive Rats (WKY) and spontaneously hypertensive rats (SHR) were compared using a radioligand binding assay method. The catecholamine concentration in the myocardium of both WKY and SHR was also determined. Results obtained as follows: (1) decrease in the Bmax values in α_1- and β_1-adrenoceptors of WKY and SHR during growth was observed; (2) values of Bmax in β_1-adrenoceptors of 16 week-old SHR higher than those of WKY were observed, and in contrast low Kd values of α_1-adrenoceptors in 16 week-old SHRs were observed; (3) no alterations in the Ca^{2+} binding sites in myocardium of both groups were observed during growth and between WKY and SHR; (4) in addition, the increase of norepinephrine concentration in the myocardium of both WKY and SHR during growth was observed and that of 16 week-old SHR was higher than that of WKY. The present study therefore implies that significant high norepinephrine concentration in the myocardium of 16 week-old SHR results in changes in β_1-adrenoceptors binding sites, suggesting that defects of membranes of myocardium of SHR may occur.**

INTRODUCTION

Several properties of the myocardial cell membrane (sarcolemma) are altered during spontaneous hypertension.[1)] Recent evidence indicates that the cell membrane of the myocardium contains α_1- and β_1-adrenoceptors.[2)] Characteristics of these receptors of spontaneously hypertensive rats (SHR) changed along with increased sympathetic nerve activity in SHR. However, conflicting evidence has been reported in regard to α_1- and β_1-adrenoceptors of SHR; the β_1-adrenergic receptor number has been reported to either decrease,[3-8)] remain the same[9,10)] or increase in SHR or hypertrophied myocardium.[11,12)] Thus, to elucidate the discrepancy in the characteristics of α_1- and β_1-adrenoceptors and Ca^{2+} channel receptors of SHR, we compared the binding properties of ^{3}H-prazosin, ^{125}I-iodocyanopindolol (^{125}I-ICYP) and ^{3}H-nitrendipine to the membrane of the myocardium. The catecholamine concentrations in hearts of SHR at different ages and Wistar Kyoto normotensive rats (WKY) were also determined.

MATERIALS AND METHODS

Radioligands: ^{3}H-prazosin (30Ci/mmole), (−)-^{125}I-ICYP (2200Ci/mmole) and ^{3}H-nitrendipine (87Ci/mmole) were purchased from New England Nuclear Corp.

Rats: Male WKY and SHR at the ages of 2, 6 and 16 weeks were supplied by the Charles Rivers Corp. (Japan). All the results were compared with their normotensive controls (WKY).

Preparation of Membrane-enriched Fraction of Myocardium: The membrane-enriched fraction from

the myocardium of SHR and WKY were prepared by using the method described previously by the authors.[13,14] The myocardium was removed, frozen in the liquid nitrogen and stored at −80°C. Defrosted myocardium was weighed, minced and homogenized in 10 volumes of 10 mM Tris-HCl (pH 7.4), 0.25 M sucrose with a polytron homogenizer. The homogenates were filtered through 4 layers of gauze and the filtrate was centrifuged at 40,000 g for 30 min. The resultant pellets were immediately rinsed with 120 mM Tris-HCl (pH 7.4) and 40 mM $MgCl_2$, and then homogenized in 20 ml of the same buffer. ^{3}H-nitrendipine binding was assayed on the same day, but α_1- and β_1-adrenoceptors bindings were done at a later date on the membrane-enriched fraction frozen in liquid nitrogen and stored at −80°C. Protein concentrations were determined by the method of Lowry et al.[15] using bovine serum albumin as a standard.

Binding Assay: The α_1-, β_1- and Ca^{2+}-binding assays were performed in duplicate with ^{3}H-prazosin, ^{125}I-ICYP and ^{3}H-nitrendipine binding. The membrane suspension (0.25 mg of protein) was incubated for 30 min at 23°C in a total volume of 0.5 ml containing 60 mM Tris-HCl (pH 7.4) and 20 mM $MgCl_2$. Values of Bmax and Kd were calculated using Scatchard analysis, with each radioligand concentration being 0.1-0.8 nM (^{125}I-ICYP), 0.1-1.0 nM (^{3}H-prazosin) and 0.1-1.6 nM (^{3}H-nitrendipine), respectively. At the end of the incubation period, the reaction mixture was immediately filtered through a Whatman GF/C glass fiber filter using an improved cell harvester LM-101 (Labo Science, Tokyo). In the case of ^{3}H-prazosin and ^{3}H-nitrendipine binding, the filter was added to 5 ml of Tt 76 scintillation fluid and the radioactivity of the filter in a tube was determined by scintillation counting. ^{125}I-ICYP binding was determined with an auto-well gamma counter. The difference in mean values between total and non-specific binding determined in the presence of 10 μM l-propranolol (^{125}I-ICYP binding), 10 μM phentolamine (^{3}H-prazosin binding) and 10 μM nitrendipine (^{3}H-nitrendipine binding) was taken as the specific binding.

Scatchard analysis of the kinetic studies was carried out on an NEC PC-9801F computer system that performed iterative nonlinear regression, as described previously.[14] The significant differences were analyzed by using Student's t-test.

Determination of Catecholamine Concentration in Myocardium: High Performance Liquid Chromatography (HPLC) with electrochemical detection (ECD) for the determination of catecholamine concentration in the defrosted myocardium was employed in the

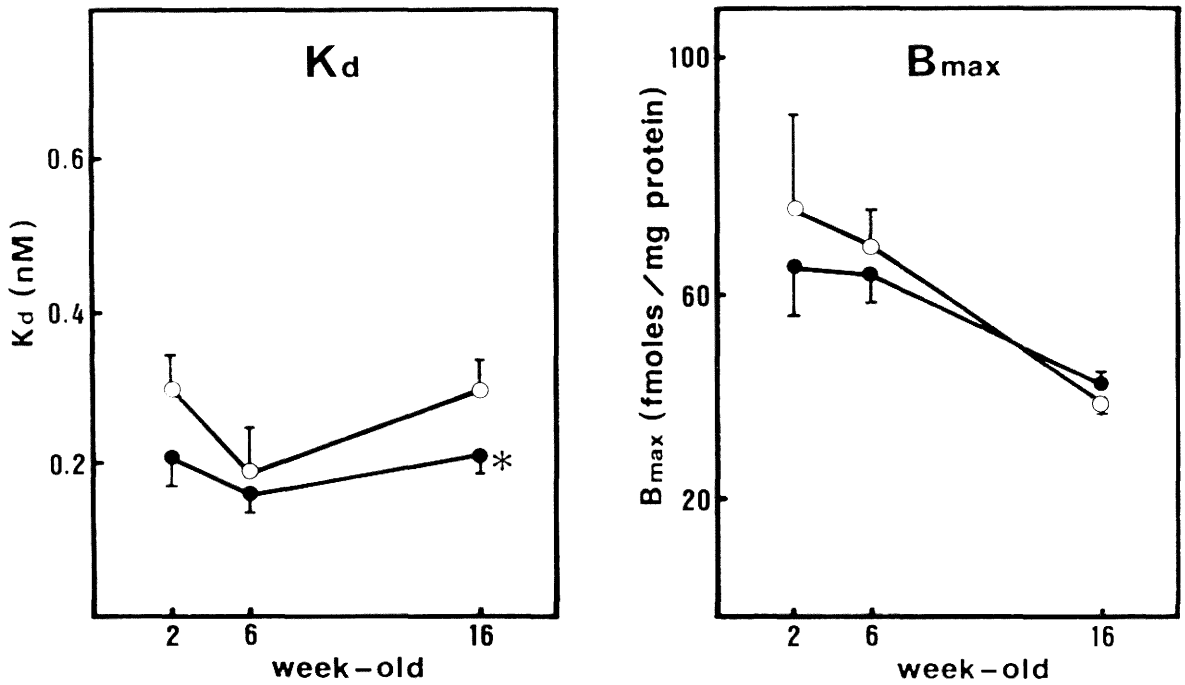

Fig. 1. Changes with age in Kd and Bmax values of α_1-adrenoceptors in the myocardium of WKY (○—○) and SHR (●—●). *Significant differences ($p<0.02$) as compared with WKY.

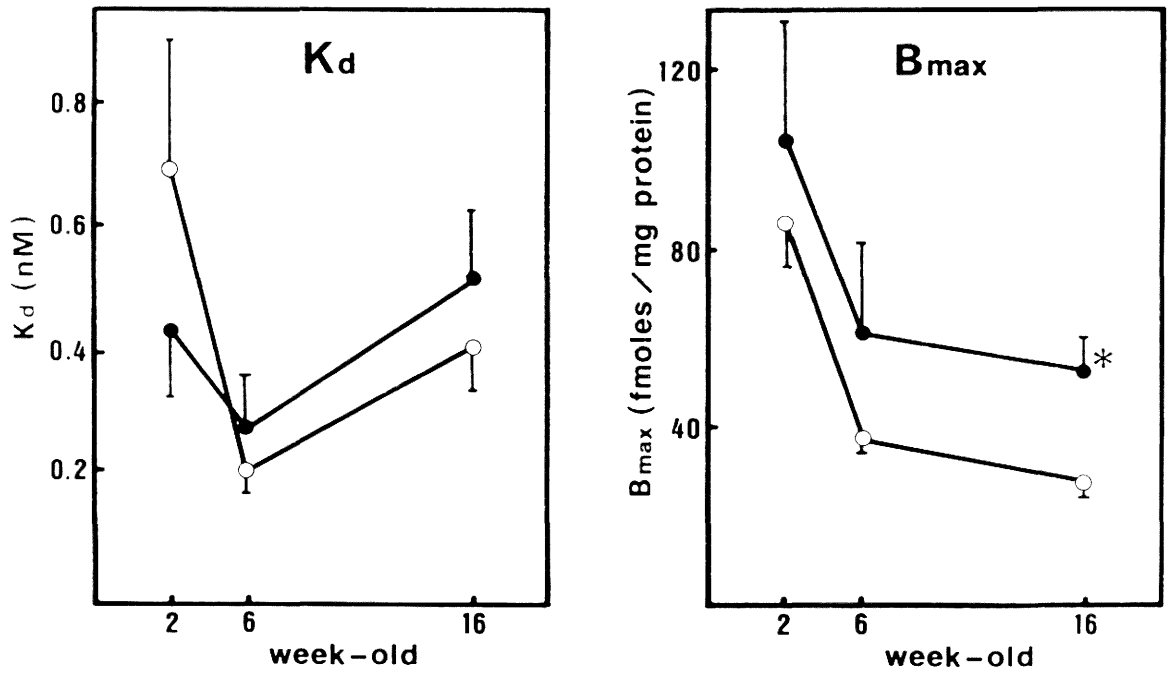

Fig. 2. Changes with age in Kd and Bmax values of β_1-adrenoceptors in the myocardium of WKY (○—○) and SHR (●—●). *Significant differences ($p<0.02$) as compared with WKY.

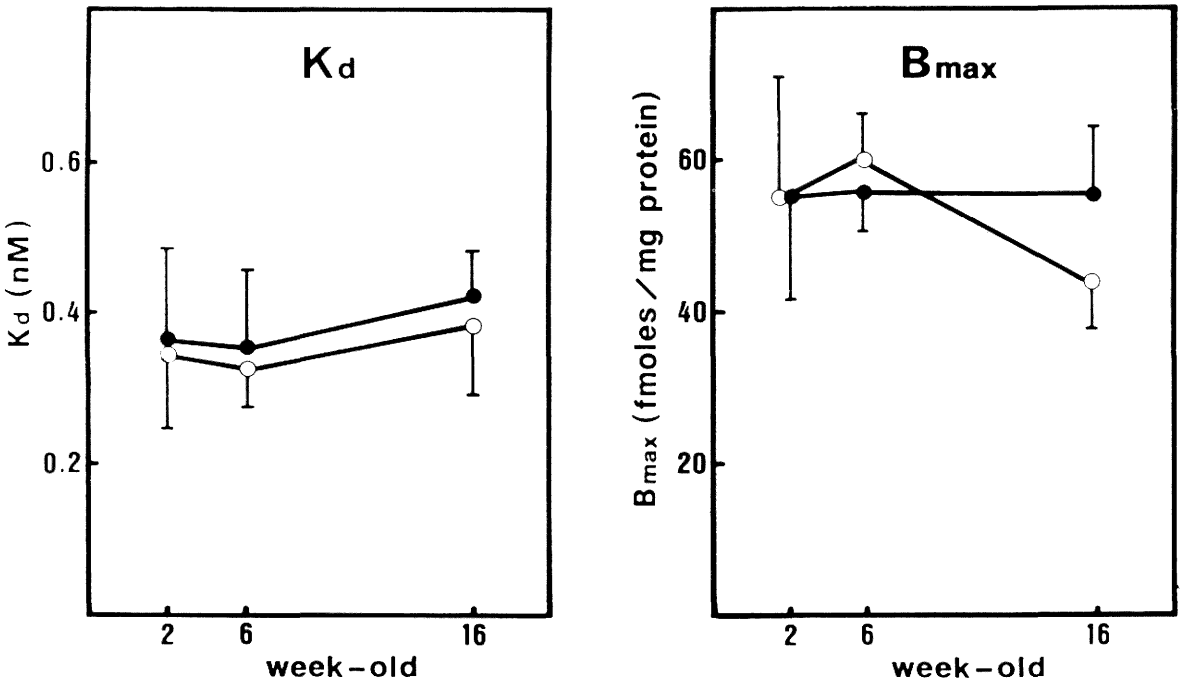

Fig. 3. Changes with age in Kd and Bmax values of Ca^{2+} channel receptors in myocardium of WKY (○—○) and SHR (●—●).

present study.[16] In brief, approximately 10 mg of heart muscle was defrosted and suspended in 1 ml of the 0.1 M $HClO_4$ solution containing 10 nM internal standard, 3, 4-dihydroxybenzylamine (DHBA), and 0.25 mM sodium meta-sulfite. This suspension was homogenized using a glass-homogenizer and the homogenate was centrifuged at 3,500 rpm for 10 min at 4°C. An aliquot (0.8 ml) of the resultant supernatant was poured into a Sepacol Minicolumn (Seikagaku Kogyo, Inc.) containing 20 mg of activated alumina (Wako Junyaku), and gently rotated at 4°C for 20 min to adsorb the catecholamine to this alumina. The mixture was removed using a water-jet pump, and the alumina was washed three times with the cold double-distilled water. Dehydration of the alumina was carried out by 1,000 rpm centrifugation for 1 min; the addition of 200 μl 0.2 M $HClO_4$ to the alumina induced the release of the catecholamine from this. An aliquot of 100 μl of 0.2 M $HClO_4$ solution was injected into the HPLC system (Shimadzu LC-6A, Shimadzu, Inc., Japan) for the determination of catecholamine concentration in the myocardium. The conditions of HPLC-ECD for the determination of catecholamine are as follows: column (Shimpack CLC-ODS, 150×6 mm I.D.), column temperature (25°C), mobile phase (0.1 M citrate buffer (pH 4.4): MeOH (85:15), 0.2 mM EDTA, 0.07% sodium octyl sulfate), flow rate (1.3 ml/min). Yanaco VMD-101A (Yanagimoto Seisaku, Inc.) was employed for ECD when applied volts were +0.7v vs Ag/AgCl. The concentration of catecholamines in the myocardium was calculated from the ratio of the peak height of DHBA to those of the samples. Under these experimental conditions, the detection limit was 5 pg for norepinephrine, 15 pg for epinephrine and 20 pg for dopamine, respectively.

Table 1. Changes in values of Kd and Bmax of α_1-, β_1-adrenoceptors and Ca^{2+}-channel receptors in myocardiums of WKY and SHR

Age (weeks)		α_1-adrenoceptors		β_1-adrenoceptors		Ca^{2+}-channel	
		Kd(nM)	Bmax (fmoles/mg protein)	Kd(nM)	Bmax (fmoles/mg protein)	Kd (nM)	Bmax (fmoles/mg protein)
2	WKY	0.30±0.04(5)	73.5±16.3(5)	0.66±0.19(5)	84.2±17.0(5)	0.35±0.11(4)	55.5±15.6(4)
	SHR	0.21±0.02(5)	63.3± 9.0(5)	0.41±0.10(4)	101.2±27.0(4) *	0.37±0.12(4)	55.2±13.2(4)
6	WKY	0.20±0.05(4)	66.8± 5.0(4)	0.21±0.03(4)	36.0± 2.2(4) ***	0.33±0.06(4)	59.9±5.5(4)
	SHR	0.16±0.02(5)	61.7± 5.3(5) *	0.27±0.08(5)	59.5±19.6(5)	0.36±0.10(5)	55.9±5.2(5)
16	WKY	0.30±0.03(4)	39.5± 1.6(4)	0.39±0.07(6)	26.8± 2.4(6)	0.39±0.10(6)	43.8±6.2(6)
	SHR	0.21±0.02(6) **	40.9± 2.1(6)	0.49±0.11(6)	51.3± 7.1(6) **	0.43±0.05(6)	55.3±8.9(6)

Values are mean±S.E. Numbers in parentheses represent number of experiments.
Significant differences: *$p<0.05$. **$p<0.02$, ***$p<0.01$.

Table 2. Changes in catecholamine concentrations in hearts of WKY and SHR

		2 weeks	6 weeks	16 weeks
WKY	NE(10)	249±12	322±17	350±24
	E (10)	48±5	31±3	38±4
	DA(10)	11±1	5±1	7±1
SHR	NE(10)	285±17	362±16	527±22*
	E (10)	38±8	39±5	32±3
	DA(10)	11±1	7±1	13±2

Data are shown as ng/gram wet tissue. Results are means±S.E. Significant differences (WKY vs SHR); *$p<0.01$. NE; norepinephrine, Numbers in parentheses represent the number of animals. E; epinephrine, DA; dopamine.

RESULTS

Our previous report[16,17] showed higher values for the blood pressure and heart weights of SHR than those of WKY. As depicted in Figures 1-3, the changes with age in Kd and Bmax values of α_1- and β_1-adrenoceptors and Ca^{2+}-channel receptors were shown. Although Kd values of those receptors were not changed during growth, the decrease in Bmax values of α_1- and β_1-adrenoceptors with age were observed, and these changes coincided for both groups.

Table 1 shows the changes in α_1- and β_1-adrenoceptors and Ca^{2+}-channel receptors in the myocardium of SHRs. Lower Kd values in α_1-adrenoceptors or higher Bmax values in β_1-adrenoceptors of 16 week-old SHR than those of the same age of WKY were observed. In addition, Table 2 shows higher norepinephrine concentration in the myocardium of 16 week-old SHRs than that of WKY.

DISCUSSION

Baker et al.[18] proposed that the number of the receptors/unit area of WKY remains constant during growth because the cell surface area (external sarcolemma/g tissue) and number of β_1-adrenoceptors declines with age, and thus cell surface area is the major factor determining normal numbers of receptors per cardiocyte. In the present study, changes with age in the Bmax values of α_1- and β_1-adrenoceptors of myocardium of WKY were in accordance with the paper reported by Baker et al.[18] Thus, it is of interest that both WKY and SHR had the same inclination in both the number and affinity of adrenoceptors with age.

Enhanced sympathetic activity has been ascribed to the development and maintenance of human and experimental hypertension, and despite enhanced sympathetic activity in SHRs, contractile responsiveness to β_1-adrenergic stimuli is consistently diminished. Recently, evidence has also accumulated for changes in α_1-[7,8] and β_1-adrenoceptors,[3-7,9-12] under hypertension. Some of these changes are due to alterations in the concentration of norepinephrine available to bind the receptor, the increases in concentrations leading to a decreased number of receptors (down-regulation).[3] We and others,[8,11,12,19] however, found an increase in receptor numbers of β_1-adrenoceptors and a higher affinity of α_1-adrenoceptors. The reason for the conflicting results in these experiments is not clear, but may be due in part to methodological differences or differences in the radioligand used.

Also, changes in α_1- and β_1-adrenoceptors presented here may be due to the membrane dysfunction of a "feedback suppression mechanism"[20] and/or "down-regulation"[3] or alterations in the rate of synthesis or a breakdown of receptors of the myocardium of SHR because of high concentrations of norepinephrine in the myocardium of 16 week-old SHR, as presented here. Therefore, a sarcolemmal defect which cannot control many factors like adenyl cyclase activities or the signal transduction processes from the β-adrenergic receptor to the catalytic unit of the cyclase may have occurred. In fact, several studies have already showed that myocardial cell membranes of SHR differed from those of WKY in many ways: a decrease in Na^+-K^+-ATPase activities,[21] high affinity for calcium binding,[22] high phospholipid metabolism[23] and abnormal membrane fluidity.[24] Thus, changes in properties or dysfunction of the heart muscle membrane of SHR may occur, and adrenoceptors themselves or the microenvironment of that may be altered, reflecting the affinity or density of α_1- and β_1-adrenoceptors.

Chatelain et al.[25] reported that ^{3}H-nitrendipine binding was impaired in 24 week-old SHR, whereas no alteration in the binding properties was observed in 9 week-old SHR. Our observations, in the present study, also showed no alteration in ^{3}H-nitrendipine binding between 2 and 16 week-old SHR. This implies that the dysfunction of Ca^{2+} channel was not present in SHR until approximately the 24th week.

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