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REDUCED NUMBER OF β-ADRENERGIC RECEPTORS IN THE MYOCARDIUM OF SPONTANEOUSLY HYPERTENSIVE RATS

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SUMMARY: Inotropic response to β-adrenergic stimulation of the myocardium is decreased in hypertension. A biochemical basis for this decrease was provided by the observation that the number of β-adrenergic receptors - as reflected in specific [^{3}H]dihydroalprenolol binding - was diminished in the myocardium of spontaneously hypertensive rats without a change in the affinity of dihydroalprenolol for the binding sites or in the capacity of isoproterenol to displace dihydroalprenolol. The decline in β-adrenergic receptor numbers is not secondary to blood pressure elevation and may be related to increased sympathetic drive in spontaneously hypertensive rats.

INTRODUCTION: Impaired sympathetic control of cardiac performance has been described in both human and experimental hypertension and is characterized by decreased chronotropic and inotropic responses to β-adrenergic stimulation (1,2). In addition, Hamrell and Alpert (3) have reported slow isometric relaxation rates for papillary muscles of spontaneously hypertensive rats and reduced responsiveness to isoproterenol. In agreement with their findings, we have recently described (4) decreased cyclic AMP-dependent enhancement of calcium transport by cardiac sarcoplasmic reticulum of hypertensive rats.

The biochemical basis for altered mechanical performance of the hypertensive myocardium is not clear; reorganization of structural elements occurs in the heart during establishment of hypertension chiefly as a result of cardiac hypertrophy and has been proposed (5) as a major determinant of hemodynamic alterations. Alternatively, differences in the interactions of catecholamines with their receptor sites on the myocardium might be responsible. The present report gives data in support of a deficiency of β-adrenergic receptors as a major factor in the impairment of sympathetic control of the heart in hypertension.

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MATERIAL AND METHODS: Experiments were carried out on male spontaneously hypertensive rats of the Aoki-Okamoto strain and age-matched Wistar-Kyoto normotensive controls. Animals were obtained at age 3 weeks from Taconic Farms, Inc., Germantown, N.Y. and were housed and fed under identical conditions. Systolic blood pressure was measured weekly by a tail-cuff plethysmographic technic.

Cardiac membranes for identification of β-adrenergic receptors were prepared essentially as described by Alexander et al. (6). Ventricles were minced in 4 volumes of cold buffer containing 0.25 M sucrose-5 mM tris-HCl (pH 7.4)- 1 mM $MgCl_2$ and were homogenized with a Polytron PT-20 at a setting of 3 for 15 sec. twice. The homogenates were filtered through four layers of gauze and spun at 700 x g for 15 min. The supernatant was centrifuged at 10,000 xg for 15 min. and the pellet was discarded. The supernatant was spun at 40,000 xg for 30 min. and the pellet was resuspended in 2.5 ml of 50 mM tris-HCl (pH 7.4)-10 mM $MgCl_2$. The yield of cardiac membranes was 7.2 ± 0.3 mg/g wet weight in the hypertensive and 7.9 ± 0.4 mg/g in the control animals.

For (-)[^{3}H]dihydroalprenolol binding, 100 µl of membrane suspension (0.6 -1.0 mg protein) and 10 nM [^{3}H]dihydroalprenolol (New England Nuclear Company, sp. activity 58.5 Ci/mmol) were incubated with shaking for 15 min. at 37°C in a total volume of 150 µl of 50 mM tris-HCl (pH 8.0)-10 mM $MgCl_2$. At the end of the incubation period, 100 µl aliquots were placed into 2 ml of ice-cold buffer and immediately filtered through GF/C glass fiber filters; the filters were washed with 10 ml of cold buffer, dried, added to 10 ml of Triton-toluene based scintillation fluid and counted. Nonspecific binding was determined by filtering aliquots of membranes which had been incubated in the presence of 10^{-5}M (±)propranolol.

The relative purity of cardiac membranes was checked by determining the activities of Na^+, K^+-ATPase (7), 5'-nucleotidase (8), and cytochrome c oxidase (9) in both homogenates and membrane preparations.

RESULTS AND DISCUSSION: [^{3}H]Dihydroalprenolol binding to cardiac membranes was a saturable process with half-maximal saturation occurring at 10 nM dihydroalprenolol for both hypertensive and normotensive animals. A Scatchard plot of specific [^{3}H]dihydroalprenolol binding to cardiac membranes from 5-week old animals is shown in Figure 1. The intercept in the abscissa indicates the number of binding sites whereas the negative reciprocal of the slope provides an estimate of the equilibrium dissociation constant (K_d) for the interaction of dihydroalprenolol with the binding sites. At saturating dihydroalprenolol concentrations (50 nM), specific binding accounted for 60% of the total while, at lower concentrations, it accounted for 80%. Although the K_d values were essentially the same for hypertensive (11.2 ± 0.4 nM) and normotensive (10.8 ± 0.3 nM) animals, the maximum number of binding sites was significantly lower in the myocardium of the hypertensive rats (62 ± 4 fmol/mg vs. 89 ± 7 fmol/mg in Wistar-Kyoto controls, $p<0.01$).

The ability of isoproterenol to compete with [^{3}H]dihydroalprenolol for binding sites was tested (Figure 2); despite differences in the number of binding sites between the two groups of animals, the concentration of isoproterenol required

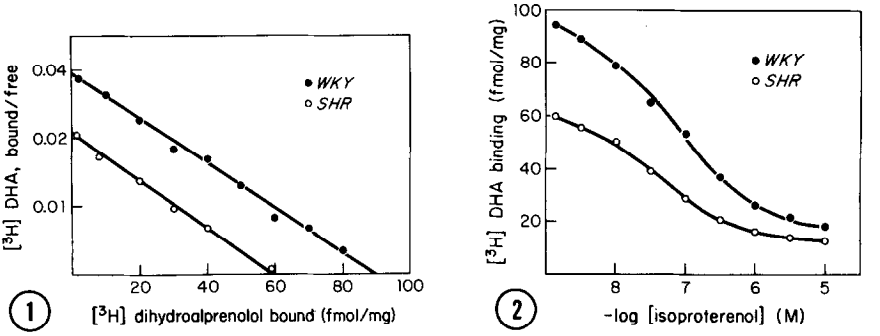

Figure 1: Scatchard plot of (-) [^{3}H]dihydroalprenolol binding to cardiac membranes from 5-week old spontaneously hypertensive (○) and WKY normotensive rats.

Figure 2: Competition for (-) [^{3}H]dihydroalprenolol binding sites by isoproterenol in cardiac membranes of 5-week old control (●) and hypertensive (○) rats; membranes (3 mg/ml) were incubated in the presence of 10 nM [^{3}H]dihydroalprenolol and varying concentrations of isoproterenol and specific binding was measured as described in the text.

for half-maximal inhibition of [^{3}H]dihydroalprenolol binding was virtually identical (2.1×10^{-7}M in hypertensive and 1.3×10^{-7}M in normotensive rats). Similar results were obtained with a number of other β-adrenergic agonists (not shown) and indicate that, in hypertension, there is a quantitative but not qualitative change in myocardial β-receptors.

The age-dependency of this change is shown in Table 1. Most of the difference between normotensive and hypertensive animals was already evident at 5 weeks of age and did not change appreciably thereafter. Since the decline in β-receptor number antedates the rapid rise in blood pressure it cannot be ascribed merely to the mechanical effects of increased intraluminal pressure and may, instead, reflect genetically determined differences. We have recently observed a similar decrease in the density of β-adrenoreceptors in arteries and veins from neonatal spontaneously hypertensive rats and consider this as further evidence of a genetically determined change. It is tempting, in this regard, to implicate the increase in sympathetic drive which accompanies and, perhaps, determines the development of hypertension in these animals (10,11). It has been shown (12,13) that occupancy of β-receptors determines their number and that prolonged exposure

Table 1: Age-dependency of specific (-) [^{3}H]dihydroalprenolol binding to cardiac membranes from normotensive (WKY) and hypertensive (SHR) animals. Results represent mean + SEM for seven experiments in each age group.

Age (weeks)	Systolic blood pressure (mm Hg)	[^{3}H]Dihydroalprenolol binding (fmol/mg prot.)	K_d (nM)
5 WKY	102 ± 6	89 ± 7	10.7 ± 0.3
SHR	134 ± 5	62 ± 4	11.2 ± 0.4
10 WKY	110 ± 7	90 ± 6	10.1 ± 0.3
SHR	171 ± 8	60 ± 7	11.0 ± 0.4
20 WKY	122 ± 8	84 ± 3	10.6 ± 0.4
SHR	192 ± 9	56 ± 3	10.8 ± 0.2
30 WKY	125 ± 9	86 ± 4	11.2 ± 0.3
SHR	198 ± 10	54 ± 5	11.4 ± 0.4

Table 2: Distribution of "marker" enzymes in homogenates and cardiac membranes from control (WKY) and hypertensive (SHR) animals, 5 weeks of age; results represent mean ± SEM from seven experiments in each group. [a] µmoles P_i/mg/min [b] nmoles P_i/mg/min [c] nmoles cytochrome oxidized/mg/min.

		Na^+,K^+-ATPase[a]	5'-nucleotidase[b]	cytochrome oxidase[c]
Homogenates:	WKY	0.81 ± 0.07	10.7 ± 1.9	241 ± 16
	SHR	0.69 ± 0.08	10.1 ± 1.6	235 ± 10
Membranes :	WKY	2.48 ± 0.10	76.2 ± 5.8	43 ± 6
	SHR	2.33 ± 0.20	79.1 ± 6.0	40 ± 4

to catecholamines results in "desensitization" and depletion of receptors. Our observations would, then, reflect increased sympathetic stimulation during the establishment of hypertension.

Consideration should be given to the possibility that decreased [^{3}H]dihydroalprenolol binding to cardiac membranes from hypertensive rats is secondary to changes in the yield of receptor-carrying membranes rather than a reflection of reduced receptor density. The following considerations argue against this possibility: (a) There was no significant difference in the distribution of "marker" enzymes in crude homogenates or membranes between the two groups of

animals (Table 2). (b) We compared [^{3}H]dihydroalprenolol binding to crude homogenates obtained by centrifuging cardiac homogenates at 40,000 xg for 60 min. and resuspending the pellet in the incubation medium; although binding was lower than with cardiac membranes , the difference between hypertensive (26 $\pm$4 fmol/mg) and normotensive (43 $\pm$ 6 fmol/mg) animals was preserved. (c) Decreased numbers of β-receptors was seen prior to the development of significant cardiac hypertrophy which accompanies (14) the progressive rise in blood pressure beyond 5 weeks of age. We feel, therefore, that our results indicate a true decline in myocardial β- receptor numbers which may provide a partial explanation for the decreased responsiveness of the hypertensive myocardium to β-adrenergic stimulation.

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