INTRACELLULAR DISTRIBUTION OF CARDIAC BETA-ADRENOCEPTORS IN SHR AND WKY

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To compare the intracellular distribution of beta-adrenoceptors in isolated myocytes of SHR with that of age-matched WKY and to examine changes in the distribution with aging, we measured the number of cell surface and total beta-adrenoceptors in 6-(young groups) and 37-week old (old groups) SHR and WKY.

The number of surface beta-adrenoceptors was significantly lower in 6-week old SHR compared with that in age-matched WKY. But there was no difference in the number of total beta-adrenoceptors between the two groups.

The number of surface beta-adrenoceptors in old groups was significantly reduced compared with that in the young groups, in both SHR and WKY. However, the number of total beta-adrenoceptors in the old groups did not show any difference from that in the young groups.

Isoproterenol-stimulated c-AMP formation in 6-week old SHR was significantly lower than that of WKY of the same age. Isoproterenol-stimulated c-AMP formation in the old groups was significantly reduced than that of the young groups in both SHR and WKY.

These results suggested that (1) there may be a difference in the intracellular distribution of cardiac beta-adrenoceptors between 6-week old SHR and WKY, (2) the intracellular distribution of cardiac beta-adrenoceptors may be changed with aging, and (3) intracellular distribution might be related to the difference in c-AMP formation to beta-agonist.

IT is known that the inotropic response to beta agonist decreases in the myocardium of spontaneously hypertensive rat (SHR).^{1,2} This decrease is associated with a decrease in the number of beta-adrenoceptors and isoproterenol-stimulated adenylate cyclase activity in the myocardium of SHR.^{3,4}

Beta-adrenoceptors were dynamically regu-

lated by synthesis, degradation, internalization (from plasma membrane to cytosol), recycle (from cytosol to plasma membrane) under physiological conditions.⁵⁻⁷

Short-term exposure to isoproterenol and ischemia induced a redistribution of beta-adrenoceptors between the surface plasma membrane and cytosol without any change in the number of total cardiac beta-adrenoceptors.^{8,9} These results suggest the possibility that the reduced number of cardiac beta-adrenoceptors in the surface plasma membrane in SHR may be due to an abnormal intracellular distribution of beta-adrenoceptors. Only one report has been

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published on the intracellular distribution of beta-adrenoceptors in the myocardium of SHR,¹⁰ while the change in intracellular distribution with aging has been seldom investigated.

³H-CGP12177 is a hydrophilic beta-adrenoceptor ligand, which is not taken up by cells and therefore binds only to cell surface receptors in intact myocytes. However, ³H-CGP12177 binds not only surface beta-adrenoceptors but also beta-adrenoceptors of the cytosol in lysed myocytes.

We measured the number of surface beta-adrenoceptors and total beta-adrenoceptors by the methods mentioned above. The aim of this study is (1) to compare the intracellular distribution in SHR with that in WKY and (2) to examine the changes in intracellular distribution of beta-adrenoceptors with aging.

MATERIALS AND METHODS

Animals

Six- (young group) and 37-week-old (old group) male SHR from the Japanese strain of Okamoto-Aoki¹¹ and WKY control rats were used.

Measurement of blood pressure

Blood pressure was measured by the tail-cuff method using an electro-sphygmomanometer (PE-300, Tokai Irika).

Myocyte isolation

Myocytes were isolated according to the method described by Haworth et al¹² with some modifications. Perfusion HEPES buffer (120 mM NaCl, 5 mM KCl, 1.2 mM MgSO₄, 5 mM NaHCO₃, 10 mM glucose, 20 mM HEPES, pH 7.4) was gassed at room temperature with 95% O₂/5% CO₂. Rats were anesthetized with 50 mg/kg B.W. sodium pentobarbital intraperitoneally. The heart was taken out immediately and was perfused retrogradely in a Langendorff apparatus. When the heart was devoid of blood, the perfusion medium was replaced with HEPES buffer containing 0.06 mg/ml of collagenase (type 1, Sigma Chemical Co.) and 1 mg/ml bovine serum albumin (BSA). The enzymic perfusion medium was recirculated at 37°C for 20 min or until the heart became flaccid. The atria together with any connective tissue were then trimmed away and the ventricles were placed in 20 ml HEPES buffer containing 0.001% trypsin, 0.5 mM CaCl₂, 1 mg/ml BSA, divided into 20–30 pieces, and

incubated for 10 min at 37°C with constant shaking and gassing. The resulting cell suspensions were filtered through gauze and centrifuged at 50g for 3 min. The preparation was visually homogeneous and 85–90% of the cells were rod-shaped, with clear striations, sharp edges and excluded trypan blue. There was no difference in the percentage of rod cells among 4 groups.

Beta-adrenoceptor assay

To quantitate beta-adrenoceptors, ³H-CGP 12177; ³H-(±)-4-(3-*t*-butylamino-2-hydroxypropoxy)-benzimidazol-2-one, (Amersham, 40 Ci/mmol), was used.^{13,14} The myocytes, suspended in 120 mM NaCl, 5 mM KCl, 1.2 mM MgSO₄, 5 mM NaHCO₃, 0.5 mM CaCl₂, 10 mM glucose, 20 mM HEPES, pH 7.4, were incubated with varying concentrations (0.1 nM–5 nM) of ³H-CGP12177 at 4°C for 16 hours. At the end of the incubation, a 10-fold excess of ice-cold incubation buffer was added to each tube, and the samples were filtered through Whatman GF/F filters. The filters were washed 3 times with 5 ml of ice-cold buffer and placed in 10 ml of ACS-II (Amersham). The radioactivity retained on the filter was counted with a scintillation counter (B 460C, Packard. Inst. Co.). Non-specific binding was detected in the presence of 10 μM propranolol. Binding curves and Scatchard plots were produced to determine the maximum number of sites (B_{max}) and affinity (K_d) of ligand binding.¹⁵

It is known that ³H-CGP12177 specifically labels cell surface beta-adrenoceptors in intact cells,^{13,14} so we can measure only surface beta-adrenoceptors in myocytes according to the method above. We also measure the number of total beta-adrenoceptors, according to the method of Limas.⁵ Namely myocytes were resuspended in 10 mM Tris-HCl (pH 7.4)-1.2 mM MgCl₂ to induce lysis. They were then homogenized and centrifuged at 100,000g and the pellets, resuspended in 120 mM NaCl, 5 mM KCl, 1.2 mM MgSO₄, 5 mM NaHCO₃, 0.5 mM CaCl₂, 10 mM glucose, 20 mM HEPES, pH 7.4, were incubated with ³H-CGP12177 at 4°C for 16 hours.

Adenylate cyclase activity

Adenylate cyclase activity were measured according to Limas's method.⁵ Myocytes suspended in HEPES buffer were incubated with 10⁻⁹M–10⁻⁵M isoproterenol at 37°C for 2 min in the presence of 0.5 mM 3-isobutyl-1-methylxanthine. Reactions were stopped by adding 6%

TABLE I BLOOD PRESSURE, HEART RATE, URINARY NOREPINEPHRINE EXCRETION

	Young group		Old group	
	SHR (n = 7)	WKY (n = 7)	SHR (n = 7)	WKY (n = 7)
Systolic Blood Pressure (mmHg)	180 ± 14	114 ± 8	204 ± 11	119 ± 8
I	$p < 0.001$		$p < 0.001$	
II			$p < 0.01$	NS
Heart Rate /min	389 ± 39	364 ± 23	363 ± 30	370 ± 30
I	NS		NS	
II			NS	NS
Urinary Norepinephrine Excretion (μg/day)	245 ± 43	224 ± 33	133 ± 24	124 ± 23
I	NS		NS	
II			$p < 0.001$	$p < 0.001$

Results represent as mean ± S.D. (n = 7).

In I, the p values denote statistical significance from WKY of the same age.

In II, the p values denote statistical significance from each young group.

Fig.1. Binding of ³H-CGP12177 to intact myocytes or lysed myocytes in young WKY. The data are means of duplicated determinations in a single representative experiment and are represented as fmoles of ³H-CGP12177 bound/mg protein. Inset; Scatchard analysis is consistent with a single site. Bound (B); specific ³H-CGP12177 bound (fmol/mg prot). Free (F); concentration of free ³H-CGP12177.

TABLE II TOTAL AND SURFACE β -ADRENERGIC RECEPTORS ON MYOCYTES OF SHR AND WKY

	Young groups				Old groups			
	SHR (n = 7)		WKY (n = 7)		SHR (n = 7)		WKY (n = 7)	
	Surface	Total	Surface	Total	Surface	Total	Surface	Total
B max (f mol/mg prot.)	23.1 $\pm$ 3.9	42.4 $\pm$ 3.8	32.3 $\pm$ 4.4	45.3 $\pm$ 4.6	13.5 $\pm$ 2.6	40.3 $\pm$ 4.0	15.7 $\pm$ 3.3	42.5 $\pm$ 6.7
I	p < 0.01	NS			NS	NS		
II					p < 0.001	NS	p < 0.001	NS
Kd (nM)	0.60 $\pm$ 0.10	0.57 $\pm$ 0.07	0.57 $\pm$ 0.10	0.64 $\pm$ 0.10	0.69 $\pm$ 0.18	0.60 $\pm$ 0.10	0.84 $\pm$ 0.12	0.69 $\pm$ 0.06
I	NS	NS			NS	NS		
II					NS	NS	NS	NS

Results represent as mean $\pm$ S.D. (n = 7).
 In I, the p values denote statistical significance from WKY of the same age.
 In II, the p values denote statistical significance from each young group.

Fig. 2. Isoproterenol-stimulated c-AMP accumulation. Results represent as mean $\pm$ S.D. (n = 7). Symbols denote statistical significance from WKY of the same age. (*p < 0.01, **p < 0.001)

trichloroacetic acid (TCA), followed by centrifugation at 2,000g for 10 min. After extraction with ether, the amount of c-AMP in the supernatant was determined with Yamasa c-AMP kit according to Honma's method⁶

Urinary norepinephrine excretion

Twenty-four hour urine, collected in a flask containing 1 ml of 6N-HCl, was centrifuged for 5 min at 2000g to remove solid particles. The supernatant was stored at -80°C for subsequent determination of norepinephrine. A 5 ml portion of the urine sample was hydrolyzed by heating to 100°C for 20 min in the presence of 2N-HCl, and norepinephrine was assayed by high-performance liquid affinity chromatography (DR-3, Shimazu) with electrochemical detector.

Protein assay

Protein was assayed by Lowry's method¹⁷

Statistics

Results are expressed as mean $\pm$ S.D.. The statistical significance of difference was calculated using unpaired Student's t test and analysis of variance. A p values less than 0.05 was considered statistically significant.

TABLE III ISOPROTERENOL-STIMULATED c-AMP

	Young group		Old group	
	SHR (n = 7)	WKY (n = 7)	SHR (n = 7)	WKY (n = 7)
Isoproterenol (10^{-5} M)-stimulated c-AMP accumulation (% increase)	56.9 $\pm$ 5.4	78.4 $\pm$ 6.3	32.3 $\pm$ 4.4	35.4 $\pm$ 4.8
I	$p < 0.001$		NS	
II			$p < 0.001$	$p < 0.001$

Results represent as mean $\pm$ S.D. (n = 7).

In I, the p values denote statistical significance from WKY of the same age.

In II, the p values denote statistical significance from each young group.

Fig.3. Numbers of total and surface beta-adrenoceptors and isoproterenol-stimulated c-AMP formation.

Results represent as mean $\pm$ S.D. (n = 7). The p values denote statistical significance from age-matched WKY. The p values in () denote statistical significance from each young rat.

RESULTS

Blood pressure and heart rate (Table I)

In both young and old groups, blood pressure in SHR was significantly elevated than that in age-matched WKY, with no significant difference in heart rate. There was a significant difference in blood pressure between young and old SHR. No significant difference was found between young and old WKY, in both heart rate and blood pressure.

Urinary norepinephrine excretion (Table I)

There was no difference between SHR and WKY in urinary norepinephrine excretion, in either young or old groups. The values of old groups were significantly decreased than those of young groups in both SHR and WKY.

Cardiac beta-adrenoceptors (Fig. 1, Table II)

Figure 1 demonstrated that ^3H -CGP12177 bound to intact myocytes or lysed myocytes in a saturable manner, and that Scatchard plots of the binding data revealed a single class of sites.

(1) Young groups: The decreased number of surface beta-adrenoceptors was found in the

myocytes of SHR with no change in affinity; Bmax is 23.1 ± 3.9 fmol/mg prot. in SHR and 32.3 ± 4.4 fmol/mg prot. in WKY. In contrast, the number of total beta-adrenoceptors in SHR was not different from that of WKY (SHR; 42.4 ± 3.8 fmol/mg prot., WKY; 45.3 ± 4.6 fmol/mg prot.).

(2) Old groups: no difference was found in either the number of surface or total beta-adrenoceptors, between SHR and WKY (SHR; 13.5 ± 2.6 fmol/mg prot., 40.3 ± 4.0 fmol/mg prot., WKY; 15.7 ± 3.3 fmol/mg prot., 42.5 ± 6.7 fmol/mg prot., respectively).

Adenylate cyclase activity (Fig. 2, Table III)

Isoproterenol-stimulated c-AMP accumulation was expressed as percentage increase over non-stimulated basal values. Figure 2 shows that incubation with isoproterenol caused concentration-dependent increase in the formation of c-AMP in both young and old groups. Isoproterenol (10^{-5} M)-stimulated c-AMP formation is shown in Table III.

(1) Young groups: The percentage increase in c-AMP of SHR was $56.9 \pm 5.4\%$, showing significant decrease than that of WKY ($78.4 \pm 6.3\%$).

(2) Old groups: no significant difference between SHR and WKY was found in isoproterenol-stimulated c-AMP.

Isoproterenol-stimulated c-AMP in old groups was significantly reduced than that in young groups in both SHR and WKY.

Figure 3 demonstrates the number of total and surface beta-adrenoceptors and isoproterenol-stimulated adenylate cyclase. The decrease in isoproterenol-stimulated c-AMP formation was almost parallel to the decrease in the number of surface beta-adrenoceptors.

DISCUSSION

It is apparent that exposure of cells to beta-agonist leads to a shift of beta-adrenoceptors from the cell surface plasma membrane to cytosol (internalization)⁵⁻⁷. Removal of the agonist results in rapid reappearance of the beta-adrenoceptors on cell surface (recycle)⁵. As described above, beta-adrenoceptors were regulated dynamically, suggesting that the changes in the number of surface beta-adrenoceptors may be dependent on the changes in the metabolism of beta-adrenoceptors.

Limas reported that, after the preincubation with isoproterenol, the number of beta-adreno-

ceptors measured with ^3H -CGP12177 decreased in intact myocytes but was not changed in lysed myocytes.⁵ After intact cells were incubated with ^3H -CGP12177, beta-adrenoceptors were separated on sucrose density gradient, showing that ^3H -CGP12177 binds only to heavy peak containing high amounts of plasma membrane.¹³ In addition, ^3H -CGP12177 binding in intact cells could be completely displaced by hydrophilic agonist.¹⁴ These studies demonstrate that ^3H -CGP12177 binds only to surface beta-adrenoceptors in intact cells, but that it binds not only the surface beta-adrenoceptors but also beta-adrenoceptors of cytosol in the lysed myocytes. Therefore we can measure the cell surface beta-adrenoceptors only in intact myocytes and the total (cytosol + surface) beta-adrenoceptors in lysed myocytes, using ^3H -CGP12177 binding assay. There is also possibility that ^3H -CGP12177 might be taken up by cells if cell surface membrane is injured with collagenase. After isolated myocytes were incubated with ^3H -CGP12177 at 4°C for 16 hours, membrane fractions and soluble fraction were obtained by centrifugation.¹⁰ There was little ^3H -CGP12177 count in the soluble fractions (data not shown), indicating that ^3H -CGP12177 was not taken up by myocytes isolated with collagenase.

In young groups, the absolute number of surface beta-adrenoceptors decreased in SHR. This result is consistent with previous reports in which beta-adrenoceptors were assayed using membrane fractions.^{3,4} SHR also have a reduced number of surface beta-adrenoceptors, with no difference in total number. This result is similar to the Limas reports¹⁰ and suggests the altered intracellular distribution of beta-adrenoceptors in myocardium of SHR.

The number of surface beta-adrenoceptors of old groups decreased more than that of young groups, in both SHR and WKY. But the number of total beta-adrenoceptors was not decreased. These results suggest that the intracellular distribution of cardiac beta-adrenoceptors may be different between young groups and old groups.

The number of total beta-adrenoceptors was not different in 4 groups. Therefore, the change in the number of surface beta-adrenoceptors probably result from the changes in the intracellular distribution of beta-adrenoceptors. Intracellular distribution of beta-adrenoceptors depends on the balance between internalization and recycle, which are generally regulated by changes

in sympathetic nerve activity.

We and others previously reported that internalization of beta-adrenoceptors was impaired in the myocardium of young SHR and aged rats.¹⁸⁻²⁰ In our experiments, sympathetic nerve activity did not differ between young SHR and WKY or decreased in old rats. But according to previous reports, sympathetic nerve activity in young SHR increased when compared to that in WKY of the same age.^{21,22} Even if sympathetic nerve activity increased before 6-weeks old, the number of surface beta-adrenoceptors must be higher in both 6 week-old SHR (than that in WKY of the same age) and old rats (than that in each young rat), in presence of impaired internalization of beta-adrenoceptors, when sympathetic nerve activity is not different between 6-week old SHR and WKY or decreased with aging. However, the number of surface beta-adrenoceptors decreased in young SHR but not in young WKY or decreased in old rats than in each young rat. Therefore, the reduced number of surface beta-adrenoceptors in 6-week old SHR is thought to be due to impaired recycling to cell surface of beta-adrenoceptors, rather than to internalization. These impaired recycling in SHR have already been found at a relatively young age and before the establishment of hypertension, suggesting the possibility that these impairments may be genetic. But to confirm genetic effect, further investigations at a younger age will be necessary.

Considering the impaired internalization of beta-adrenoceptors in old rats,^{18,19} the changes in intracellular distribution are also thought to be due to impaired recycling of beta-adrenoceptors, as in young SHR. But these changes may be related to aging, and probably not the result of blood pressure elevation, because these changes are found not only in SHR but also in WKY. Therefore, a reduced number of beta-adrenoceptors is thought to be related to impaired recycling, in both SHR and old rats, although the basal cause of impaired recycling may be different; one may be genetic and the other may be related to aging.

In old groups, there was no difference in number of surface beta-adrenoceptors but mean value was decreased in SHR. Mean values in old groups were relatively small with a large standard deviation, so that no difference might be detected between SHR and WKY.

The decrease in isoproterenol-stimulated c-AMP formation in the myocardium of young

SHR and aged rats was almost proportional to the decrease in the number of surface beta-adrenoceptors, because only surface beta-adrenoceptors are coupled to adenylate cyclase; the internalized beta-adrenoceptors are not coupled to adenylate cyclase.^{23,24} It has been known that inotropic response and chronotropic response to beta agonist, mediated through c-AMP formation, are reduced in the myocardium of SHR,^{1,2} and that those responses are also diminished with aging.²⁵ Therefore, the difference in intracellular distribution of beta-adrenoceptors is thought to result in a difference between inotropic and chronotropic response to beta-agonist.

These results suggest that (1) the difference in the intracellular distribution of cardiac beta-adrenoceptors is found between 6-week old SHR and WKY, (2) the intracellular distribution of cardiac beta-adrenoceptors changes with age. (3) these differences in the intracellular distribution may be related to the difference in c-AMP formation to beta-agonist.

REFERENCES

1. FUJIWARA M, KUCHII M, SHIBATA S: Differences of cardiac reactivity between spontaneously hypertensive and normotensive rats. *Eur J Pharmacol* 19: 1, 1972
2. SARAGOCA M, TARAZI RC: Impaired cardiac contractile response to isoproterenol in the spontaneously hypertensive rat. *Hypertension* 3: 380, 1981
3. KUMANO K, UPSHER ME, KHAIRALLAH PA: Beta adrenergic receptor response coupling in hypertrophied hearts. *Hypertension* 5 (Suppl I): I-175, 1983
4. BAKER SP, KATOVICH MJ: Chronic propranolol treatment decreases cardiac β -adrenoceptors in spontaneously hypertensive rats. *Eur J Pharmacol* 78: 479, 1982
5. LIMAS CJ, LIMAS C: Rapid recovery of cardiac β -adrenergic receptors after isoproterenol-induced "down"-regulation. *Circ Res* 55: 524, 1984
6. HARDEN TK: Agonist-induced desensitization of the β -adrenergic receptor-linked adenylate cyclase. *Pharmacol Rev* 35: 5, 1983
7. STADEL JM, STRULOVICI B, NAMBI P, LAVIN TN, BRIGGS MM, CARON MG, LEFKOWITZ RJ: Desensitization of the β -adrenergic receptor of frog erythrocytes. *J Biol Chem* 258: 3032, 1983
8. KARLINER JS, SIMPSON PC, HONBO N, WOLOSZYN W: Mechanisms and time course of beta₁ adrenoceptor desensitization in mammalian cardiac myocytes. *Cardiovasc Res* 20: 221, 1986
9. MAISEL AS, MOTULSKY HJ, INSEL PA: Externalization of β -adrenergic receptors promoted by myocardial ischemia. *Science Wash DC* 230: 183, 1985
10. LIMAS CJ, LIMAS C: Altered intracellular adreno-

- ceptor distribution in myocardium of spontaneously hypertensive rats. *Am J physiol* 253: H904, 1987
11. OKAMOTO K, AOKI K: Development of a strain of spontaneously hypertensive rats. *Jpn Circ J* 27: 282, 1963
 12. HAWORTH RA, HUNTER DR, BERKOFF HA: The isolation of Ca^{2+} -resistant myocytes from the adult rat. *J Mol Cell Cardiol* 12: 715, 1980
 13. HERTEL C, MULLER P, PORTENIER M, STAEHELIN M: Determination of the desensitization of β -adrenergic receptors by ^3H -CGP-12177. *Biochem J* 216: 669, 1983
 14. STAEHELIN M, SIMONS P, JAEGGI K, WIGGER N: CGP-12177; A hydrophilic β -adrenergic receptor radioligand reveals high affinity binding of agonists to intact cells. *J Biol chem* 258: 3496, 1983
 15. SCATCHARD G: The attraction of proteins for small molecules and ions. *Ann NY Acad Sci* 51: 660, 1949
 16. HONMA M, SATOH T, TAKEZAWA J, UIM: An ultrasensitive method for the simultaneous determination of cyclic AMP and cyclic GMP in small volume samples from blood and tissue. *Biochem Med* 18: 257, 1977
 17. LOWRY OH, ROSENBROUGH NJ, FARR AL, RANDALL RJ: Protein measurement with folin phenol reagent. *J Biol Chem* 193: 265, 1951
 18. MATSUMORI Y, OHYANAGI M, KAWAMOTO H, SHIBATA R, IWASAKI T: The changes of cardiac α_1 and β adrenergic receptors with antihypertensive therapy by hydralazine. *J Jap Coll Angiol* 27: 1175, 1987 (in Japanese)
 19. BLASI AD, FRATELLI M, WIELOSZ M, LIPARTITI M: Regulation of beta adrenergic receptors on rat mononuclear leukocytes by stress; receptor redistribution and down-regulation are altered with aging. *J Pharmacol Exp Therap* 240: 228, 1987
 20. LIMAS CJ, LIMAS C: Decreased isoproterenol-induced "down"-regulation of beta-adrenergic receptors in the myocardium of SHR. *Hypertension* 6 (Suppl I): I-31, 1984
 21. JUDY WV, WATANABE AM, HENRY DP, BESCH HR, MURPHY WR, HOCKEY GM: Sympathetic nerve activity. Role in regulation of blood pressure in spontaneously hypertensive rat. *Circ Res* 38: (Suppl II): 21, 1976
 22. NAGAOKA A, LOVENBERG W: Plasma norepinephrine and dopamine- β -hydroxylase in genetic hypertensive rats. *Life Sci* 19: 29, 1976
 23. STRULOVICI B, STADEL JM, LEFKOWITZ RJ: Functional integrity of desensitized β -adrenergic receptors. *J Biol Chem* 258: 6410, 1983
 24. STRASSER RH, STILES GL, LEFKOWITZ RJ: Translocation and uncoupling of the β -adrenergic receptor in rat lung after catecholamine promoted desensitization in vivo. *Endocrinology* 115: 1392, 1984
 25. LAKATTA EG, GERSTENBLITH G, ANGELL CS, SHOCK NW, WEISFELDT ML: Diminished inotropic response of aged myocardium to catecholamines. *Circ Res* 36: 262, 1975