56) α_1 receptor in isolated myocytes of SHR

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α_1 binding experiments have been analysed using hydrophobic radioligands and the membrane fractions. But these methods have some problems ; 1) the interstitial cells are involved in the membrane fractions, 2) the hydrophobic radioligands probably dissolve in the plasma membrane and enter the cells and may interact with the internalized receptors, 3) it is difficult to correlate the result of the binding assay using the membrane fractions with the estimations of the receptor functioning in physiological conditions. In this report, cardiac α_1 receptor of isolated myocytes was assayed using hydrophobic radioligand ^3H -bunazosin.

Method Eight-week-old male SHR and WKY were used. Myocytes were isolated according to the method described by Haworth et al. with some modifications. After the isolation of myocytes, the myocytes were suspended in the 10 mM Tris-HCl, 10 mM MgCl_2 to induce lysis and the membrane fraction were prepared. The intact myocyte suspensions or the membrane fractions were incubated with various concentrations (0.1 nM-5 nM) of ^3H -bunazosin at 4°C for 14-16 hours. After the incubation, the samples were filtered through Whatman GF/F filters. The radioactivities retained on the filters were counted. Non-specific binding was the count detected in the presence of $1\mu\text{M}$ phentolamine.

Result Competition-inhibition experiments with various antagonists indicated that relative potencies of antagonists for ^3H -bunazosin binding were prazosin>phentolamine>yohimbine. This was consistent with the notion that ^3H -bunazosin was binding selectively to α_1 -adrenergic receptors. Compared competition-inhibition data for these antagonists between the two strains, the potencies and IC_{50} values in SHR are similar to those in WKY. We examined the inhibition of ^3H -bunazosin by ℓ -norepinephrine. The Hill's coefficient for norepinephrine inhibition were nearly close to 1.0 in both SHR and WKY, indicating that one affinity site for norepinephrine. The norepinephrine concentrations which inhibited 50% of ^3H -bunazosin binding (IC_{50}) in SHR ($7.9 \pm 0.6 \times 10^{-6}\text{M}$) are similar to those in WKY ($7.1 \pm 0.9 \times 10^{-6}\text{M}$). The similarity of competition for ^3H -bunazosin binding suggests that the affinity of the α_1 receptor to norepinephrine in SHR is identical with that in WKY. Scatchard analysis of the binding data showed straight lines. The number of α_1 receptor expressed as the receptor number per 10^6 cells were significantly increased in SHR ($529 \pm 21 \text{ fmol}/10^6 \text{ cells}$) than in WKY ($405 \pm 19 \text{ fmol}/10^6 \text{ cells}$, $p < 0.01$). The K_d values were $0.78 \pm 0.07 \text{ nM}$ in WKY and $0.83 \pm 0.03 \text{ nM}$ in SHR and there was no significance between the values of the two strains. To investigate whether the increase in the number of α_1 receptor per 10^6 cells in SHR was due to the increase in the number of α_1 receptor or the increase in the size of SHR myocytes, the number of α_1 receptor per membrane protein was examined. The number of α_1 receptor per membrane protein were also significantly increased in SHR ($150 \pm 8 \text{ fmol}/\text{mg prot.}$) than in WKY ($134 \pm 7 \text{ fmol}/\text{mg prot.}$)

Conclusions (1) We investigated α_1 receptor of isolated myocytes of 8-week-old male SHR and WKY using ^3H -bunazosin. (2) The affinities of the α_1 adrenergic receptor to norepinephrine and various α antagonists in SHR are identical to those in WKY. (3) The number of α_1 receptor of isolated myocytes was significantly increased in SHR than in WKY.