Effects of Chinese herb medicines (Dai-saiko-to, Choto-san and Sairei-to) on ^{3}H-prazosin, ^{125}I-ICYP and ^{3}H-nitrendipine binding to α_1-and β-adrenergic receptors and Ca^{2+} binding sites in the myocardium of spontaneously hypertensive rats

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Abstract

Effects of 3 kinds of blended traditional Chinese herb medicines (Dai-saiko-to, Choto-san and Sairei-to) on ^{3}H-prazosin, ^{125}I-iodocyanopindolol (^{125}I-ICYP) and ^{3}H-nitrendipine binding to α_1-and β-adrenergic receptors and Ca^{2+} binding sites in the myocardium of spontaneously hypertensive rats (SHR) were examined using the radioligand binding assay method. The myocardial norepinephrine (NE) concentration of both Wistar Kyoto normotensive rats and SHR was also determined. Rats given the herb medicines had less myocardial NE. Also, B_{max} values of β-adrenoceptors and Ca^{2+}-binding sites in SHRs myocardium given Chinese herb medicine were returned to normalcy. These results suggest that changes in the number of β-adrenoceptors, and in NE concentration may be related to stabilization of blood pressure or inhibition of left ventricular hypertrophy.

Key words Choto-san (Tyô-tô-san), Dai-saiko-to (Dai-saiko-tô), Sairei-to (Sairei-tô), α_1-adrenoceptor, β-adrenoceptor, Ca^{2+} binding site, myocardium, spontaneously hypertensive rats.

Abbreviations SHR, spontaneously hypertensive rat ; WKY, Wistar-Kyoto rat ; Choto-san (Diao-Teng-San), 釣藤散 ; Dai-saiko-to (Da-Chai-Hu-Tang), 大柴胡湯 ; Sairei-to (Chai-Ling-Tang), 柴苓湯.

Introduction

Chinese herb medicines have been shown to be clinically effective in cases of mild hypertension.[1-3] Still, their effects are not as potent as those of known diuretics, β-adrenoceptor blockers or Ca^{2+}-channel blockers. Recent evidence indicates that the myocardial cell membrane contains α_1- and β-adrenoceptors.[4] Characteristics of these receptors in spontaneously hypertensive rats (SHR) have been shown to change along with the increased sympathetic nerve activity in SHR. Our previous report also showed higher values of B_{max} in SHR myocardium than in Wistar Kyoto normotensive rats (WKY).[5] In the present study, we examined the effects of 3 kinds of blended traditional Chinese herb medicines on ^{3}H-prazosin, ^{125}I-iodocyanopindolol (^{125}I-ICYP) and ^{3}H-nitrendipine binding to α_1- and β-adrenoceptors and Ca^{2+} channel receptors in the SHR myocardium. We also measured the norepinephrine (NE) concentration in the SHR myocardium.

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Materials and Methods

Radioligands : ^{3}H-prazosin (30 Ci/mmole), (−)-^{125}I-ICYP (2,200 Ci/mmole) and ^{3}H-nitrendipine (87 Ci/mmole) were purchased from New England Nuclear Co.

Rats : Male WKY and SHR (5 weeks old) were supplied by Charles River Japan, Inc. (Japan).

Drugs : Three kinds of Chinese herb medicines were used in this study. All were provided by Tsumura & Co. (Japan). The drugs were dissolved in water, which the SHRs drank freely for 10 weeks, starting from 6 weeks of age. The animals drank dissolved Dai-saiko-to (900 mg/kg/day), Choto-san (900 mg/kg/day) and Sairei-to (1,200 mg/kg/day).

Preparation of membrane-enriched fraction of myocardium : The membrane-enriched fractions from the myocardium of SHR and WKY were prepared by using the method described previously by Nagatomo *et al*.[6,7] The myocardium was removed, frozen in liquid nitrogen and stored at −80℃. After thawing, the tissue 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 rinsed at once with 120 mM Tris-HCl (pH 7.4), 40 mM $MgCl_2$, and homogenized in 20 ml of the same buffer. ^{3}H-Nitrendipine binding was assayed on the same day. Part of the tissue homogenate was stored at −80℃ for later assay of α_1- and β-adrenoceptor binding. Protein concentrations were determined by the method of Lowry *et al*.[8] using bovine serum albumin as a standard.

Binding assay : The α_1-, β- and Ca^{2+}-binding assays were carried out in duplicate using ^{3}H-prazosin, ^{125}I-ICYP and ^{3}H-nitrendipine binding as ligands, respectively. The membrane suspension (0.25 mg of protein) was incubated at 23℃ for 30 min in a total volume of 0.5 ml containing 60 mM Tris-HCl (pH 7.4) and 20 mM $MgCl_2$. B_{max} and K_d were calculated using Scatchard analysis. The radioligand concentrations were 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). 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 was determined by a scintillation counter. ^{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 1-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, by iterative nonlinear regression as described previously.[7] The data were analyzed by one way ANOVA followed by Tukey test.

Determination of catecholamine concentration in myocardium : High performance liquid chromatography (HPLC) with electrochemical detection (ECD) was used to detect myocardial catecholamines. In brief, the heart muscle (approximately 10 mg) was homogenized using the glass-homogenizer with 1 ml of 0.1 M $HClO_4$ solution containing 10 nM internal standard, 3,4-dihydroxybenzylamine (DHBA), and 0.25 mM sodium meta-sulfite. This homogenate was centrifuged at 1,500 *g* at 4℃ for 10 min. An aliquot (0.8 ml) of the supernatant was poured into a Sepacol Minicolumn (Seikagaku Kogyo, Inc.) which contained 20 mg of activated alumina (Wako Junyaku, Japan), and was gently rotated at 4℃ for 20 min to adsorb the catecholamine into the alumina. The mixture was removed using a water-jet pump and the alumina was washed with cold double-distilled water. The alumina was dehydrated by 750 *g* centrifugation for 1 min, and the addition of 200 μl of 0.2 M $HClO_4$ to the alumina induced the release of catecholamine. An aliquot of 100 μl of 0.2 M $HClO_4$ solution was injected into the HPLC system (Shimadzu LC-6A,

Shimadzu, Inc., Japan). The analytical conditions were as follows : column (Shim-pack CLC-ODS, 150×6 mm I.D.), column temperature (25℃), 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 used for ECD when applied volts were +0.7 v *vs.* Ag/AgCl. The concentrations of catecholamines in myocardia were calculated from the ratios of the peak heights of DHBA to those of the samples.

Results

NE concentration in SHR myocardium was higher than in WKY. SHR given Chinese herb medicines had lower myocardial NE concentrations than those given only water (Table I). The rank order of NE concentrations was : Sairei-to < Choto-san = Dai-saiko-to.

As shown in Table II, there were no differences in K_d or B_{max} of α_1-adrenoceptors. In contrast, there was tendency for decrease of the B_{max} values of β-adrenoceptors and Ca^{2+}-binding sites and the group given Sairei-to had a relatively low β-adrenoceptor K_d.

Table I Effects of Dai-saiko-to, Choto-san and Sairei-to on cardiac norepinephrine concentration in spontaneously hypertensive rat.

	Norepinephrine (ng/g w.t)
WKY	342±53**
SHR	636±151
Dai-saiko-to	479±105*
Choto-san	477±62*
Sairei-to	390±41**

Values are mean±S.D. of 8 rats. *$p<0.05$ and **$p<0.01$ *vs.* SHR.

Discussion

It is well known that Chinese herb medicines can induce the decrease in blood pressure clinically and this hypotensive effects are mild. It has already been reported that these agents also showed mild hypotensive effects on 16 week-old SHRs.[10] In addition, the hypotensive effect of Chinese herb medicines may be due in part to their diuretic action. They have been shown to increase urinary volume and sodium excretion.[11]

On the other hand, enhanced sympathetic activity has been implicated in the development and maintenance of human and experimental hypertension. Recently, we and others[5,12-15] found that ^{125}I-ICYP can bind to both β-adrenergic and serotonergic (5-HT_{1B}) receptors and that SHRs have more myocardial β-adrenoceptors and also a higher affinity of α_1-adrenoceptors. The SHRs high myocardial NE concentration may reflect a dysfunction of a membrane feedback suppression mechanism and/or alteration of the rate of synthesis or breakdown of receptors. The present study indicates that after drinking Chinese herb medicines SHRs had less myocardial NE. In addition, one of these drugs also induced a decrease in the number of myocardial β-adrenoceptors. Thus, it is possible that Chinese herb medicines can prevent the SHR myocardial membrane dysfunction.

Chatelain *et al.*[16] reported that ^{3}H-nitrendipine binding was impaired in 24 week-old SHR,

Table II Effects of Chinese medicines on the binding of ^{3}H-prazosin, ^{125}I-ICYP and ^{3}H-nitrendipine to α_1- and β-adrenergic receptors and Ca^{2+}-channel in the myocardium of SHR.

	α_1-adrenoceptors		β-adrenoceptors		Ca^{2+} binding sites	
	K_d (nM)	B_{max} (fmoles/mg protein)	K_d (nM)	B_{max} (fmoles/mg protein)	K_d (nM)	B_{max} (fmoles/mg protein)
WKY	0.24±0.03(5)	34.8±6.3(5)	0.40±0.05(10)	28.3±2.1(10)**	0.45±0.09(10)	35.8± 5.2(10)
SHR	0.19±0.05(8)	36.9±8.6(8)	0.45±0.07(11)	47.6±5.9(11)	0.34±0.05(6)	49.5± 6.8(6)
Dai-saiko-to	0.18±0.02(4)	37.4±4.0(4)	0.43±0.13(4)	32.4±3.5(4)	0.56±0.21(4)	29.3±12.8(4)
Choto-san	0.21±0.02(4)	40.7±4.2(4)	0.32±0.10(4)	29.6±6.8(4)	0.46±0.09(4)	37.8±11.5(4)
Sairei-to	0.13±0.03(4)	33.9±3.3(4)	0.19±0.03(4)*	27.5±1.6(4)	0.29±0.02(4)	34.8± 1.4(4)

Values are means±S.E. Numbers in parentheses represent number of experiments.
Significant differences (*vs.* SHR) : *$p<0.05$, **$p<0.01$.

whereas no alteration in the binding properties was observed in 9 week-old SHR. On the other hand, Ishii *et al.*[17] presented evidence that Choto-san decreased B_{max} values of Ca^{2+} binding sites in the brain. The present study also showed that the Chinese herb medicines affected only a slight decrease in B_{max} values of Ca^{2+} binding sites. Thus, these herb medicines may affect Ca^{2+} transport across the cell membrane and their hypotensive effects may be due in part to this action.

In conclusion, the Chinese herb medicine changed the number of β-adrenoceptors, suggesting that this drug may alleviate high blood pressure or left ventricular hypertrophy in SHRs.

和文抄録

大柴胡湯，釣藤散，柴苓湯がSHRラット心筋のα_1，β，Ca受容体における効果を^{3}H-prazosin，^{125}I-ICYP，^{3}H-nitrendipineを用いたラジオリガンドバインディングアッセイ法にて検討した。心筋カテコラミン濃度も同時に測定し，WKYと対比検討した。SHRに3剤投与後，SHR心筋のβ，Ca受容体数は正常化し，心筋ノルエピネフリン濃度も低下した。以上から漢方3剤の血圧低下作用や左室肥大抑制作用はβ，Ca受容体数の正常化作用と密接に関係すると思われた。

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