ELSEVIER

European Journal of Pharmacology 344 (1998) 31–36

ejp

Alteration of α_1-adrenoceptor subtypes in aortas of 12-month-old spontaneously hypertensive rats

Kaiming Xu, Zhizhen Lu, Hong Wei, Youyi Zhang, Chide Han *

Institute of Vascular Medicine, Third Hospital, Beijing Medical University, Beijing 100083, PR China

Received 3 July 1997; revised 26 November 1997; accepted 28 November 1997

Abstract

Alterations in α_1-adrenoceptor subtypes in aortas from 12-month-old spontaneously hypertensive rats (SHR) were studied in functional studies and RNase protection assays. The norepinephrine-induced contraction, including maximum response and pD_2 values, was not significantly different between the SHR and age-matched Kyoto Wistar (WKY) rats. The pA_2 values of the α_{1D}-adrenoceptor subtype-selective antagonist BMY7378 (8-(2-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl)-8-azaspiro(4.5)decane-7,9-dionedihydrochloride) were increased from 8.10 ± 0.12 in WKY rats to 8.45 ± 0.13 in SHR ($P < 0.05$). The pA_2 values of the α_{1A}-adrenoceptor subtype-selective antagonist RS-17053 (*N*-[2-(2-cyclopropylmethoxyphenoxy)ethyl]-5-chloro-α,α-dimethyl-1H-indole-3-ethanamine hydrochloride) were reduced from 8.52 ± 0.20 in WKY rats to 7.82 ± 0.18 in SHR ($P < 0.05$), whereas the pA_2 values of the α_{1A}/α_{1D}-adrenoceptor subtype-selective antagonist WB4101 (2-(2,6-dimethoxphenoxyethyl)-aminomethyl-1,4 benzodioxane) were not significantly different between WKY rats and SHR (9.05 ± 0.22 versus 9.27 ± 0.15, $P > 0.05$). Preincubation of preparations in 50 μM chloroethylclonidine for 30 min irreversibly inhibited the norepinephrine-induced response more profoundly in aortas from SHR than in aortas from WKY rats. The results of RNase protection assays showed that mRNAs for α_{1A}- and α_{1B}-adrenoceptor subtypes were decreased and that mRNA for the α_{1D}-adrenoceptor subtype was increased in aortas from SHR compared with WKY rats. The results suggested that the α_{1A}-adrenoceptor subtype was decreased and the α_{1D}-adrenoceptor subtype was increased in aortas of 12-month-old SHR. © 1998 Elsevier Science B.V.

Keywords: α_1-Adrenoceptor; Subtype; Aorta; Spontaneously hypertensive rat (SHR)

1. Introduction

The α_1-adrenoceptor in vascular smooth muscle plays a vital role in the modulation of sympathetic nervous system activity, regulation of blood pressure and maintenance of homeostasis. Molecular cloning techniques and pharmacological studies have identified the existence of three subtypes of α_1-adrenoceptor, that is, α_{1A}, α_{1B} and α_{1D} (Graham et al., 1996). mRNA for all the three α_1-adrenoceptor subtypes is expressed in rat aorta (Piascik et al., 1994; Xu and Han, 1996). Recent functional studies further showed that α_1-adrenoceptor agonist-induced contraction of rat aorta was mainly mediated by the α_{1D}-adrenoceptor subtype (Saussy et al., 1994; Testa et al., 1995).

Hypertension is accompanied by an elevated sympatho-adrenal tone, which is frequently associated with desensitization of adrenoceptors. In cultured rat and rabbit aortic smooth muscle cells, adrenergic stimuli can induce down-regulation of the α_{1B}-adrenoceptor subtype (Izzo et al., 1990; Chen et al., 1995). In cultured neonatal cardiac myocytes, adrenergic stimuli differentially regulated the three α_1-adrenoceptor subtypes (Rokosh et al., 1996). Therefore, in the aorta of spontaneously hypertensive rats (SHR), the expression of α_1-adrenoceptor subtypes may be affected by a raised sympathetic tone, but this has not yet been proved. In addition, the alteration of α_1-adrenoceptor-mediated contraction in SHR aorta is still controversial. Our previous experiments showed that norepinephrine-induced contraction in SHR aorta was unchanged compared with that in aorta from WKY rats (Han et al., 1992). But some other researchers reported that the aortic contraction induced by norepinephrine was decreased (Holck, 1986) or increased (Brown et al., 1994). Therefore, in the present studies we compared the contractile responses induced by α_1-adrenoceptor subtypes and mRNA

* Corresponding author. Tel.: +86-10-62092306; fax: +86-10-62017700; e-mail: hanqd@mail.bjmu.edu.cn

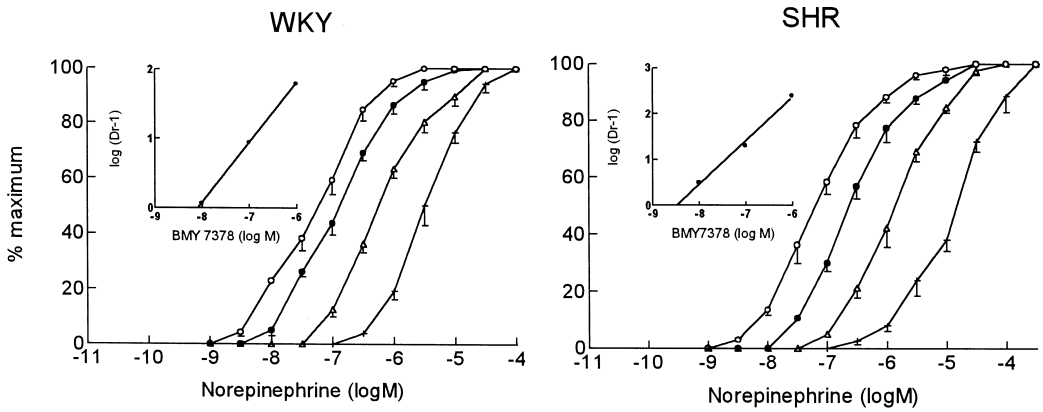

Fig. 1. Effect of BMY7378 on the norepinephrine-induced contraction in aortas from WKY rats and SHR. Concentration–response curves for norepinephrine in the absence (open circle) and presence of BMY7378: 0.01 μM (filled circle), 0.1 μM (open triangle), 1 μM (cross). WKY rats ($n = 8$), SHR ($n = 4$).

levels for the three α_1-adrenoceptor subtypes in aortas from 12-month-old SHR and WKY rats.

2. Materials and methods

2.1. Materials

Compounds were obtained from the following sources: WB4101 (2-(2,6-dimethoxphenoxyethyl)-aminomethyl-1,4 benzodioxane), BMY7378 (8-(2-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl)ethyl)-8-azaspiro(4.5)decane-7,9-dionedihydrochloride), norepinephrine (Research Biochemicals, Natick, MA); yohimbine, desmethylimipramine, normetanephrine, (±)-propranolol (Sigma Chemical Corp., St. Louis, MO); RS-17053 (*N*-[2-(2-cyclopropylmethoxyphenoxy)ethyl]-5-chloro-α,α-dimethyl-1H-indole-3-ethanamine hydrochloride) (Roche Bioscience, USA).

2.2. Functional experiments

Male WKY and spontaneously hypertensive rats aged 12 months were killed by cervical dislocation. The full length of aorta was dissected and placed in an ice-cold Krebs–Ringer bicarbonate buffer (KRB) containing (in mM) 120 NaCl, 5.5 KCl, 2.5 $CaCl_2$, 1.2 NaH_2PO_4, 1.2 MgCl, 20 $NaHCO_3$, 11 glucose and 0.029 Na_2EDTA equilibrated with 95% O_2:5% CO_2. The vessels were cleaned of fat and connective tissue. The proximate end of the thoracic aorta was cut into rings, 3 mm long, for in vitro contractile studies. The remaining vessels were immediately stored in liquid nitrogen for total RNA isolation.

The KRB used for the functional study contained 0.1 μM desmethylimipramine and 1 μM normetanephrine to block the neuronal and extraneuronal uptake of norepinephrine, 1 μM (±)-propranolol to block β-adrenoceptors and 0.1 μM yohimbine to block α_2-adrenoceptors. The ring segments were mounted at 37°C in 10 ml organ

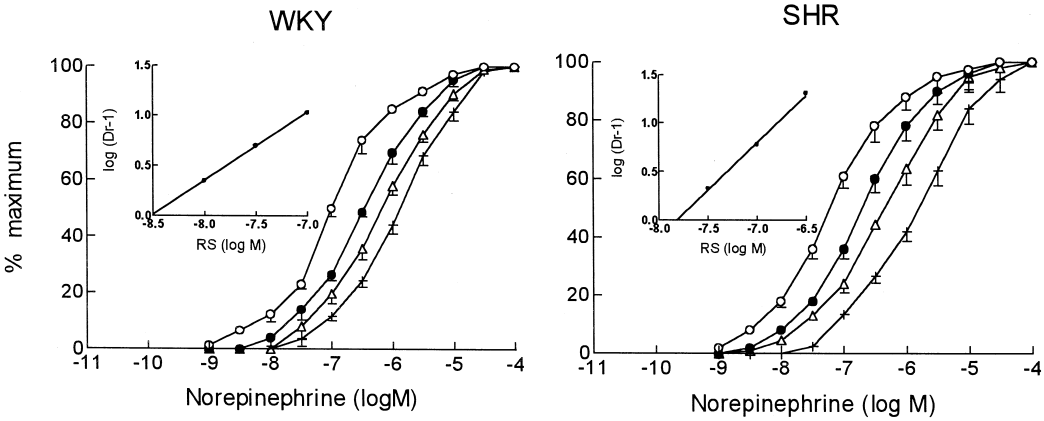

Fig. 2. Effect of RS-17053 on the norepinephrine-induced contraction in aortas from WKY rats and SHR. Concentration–response curves for norepinephrine in the absence (open circle) and presence of RS-17053: 0.01 μM for WKY rat and 0.3 μM for SHR (filled circle), 0.3 μM for WKY rats and 0.1 μM for SHR (open triangle), 0.1 μM for WKY rats and 3 μM for SHR. WKY rats ($n = 4$), SHR ($n = 4$).

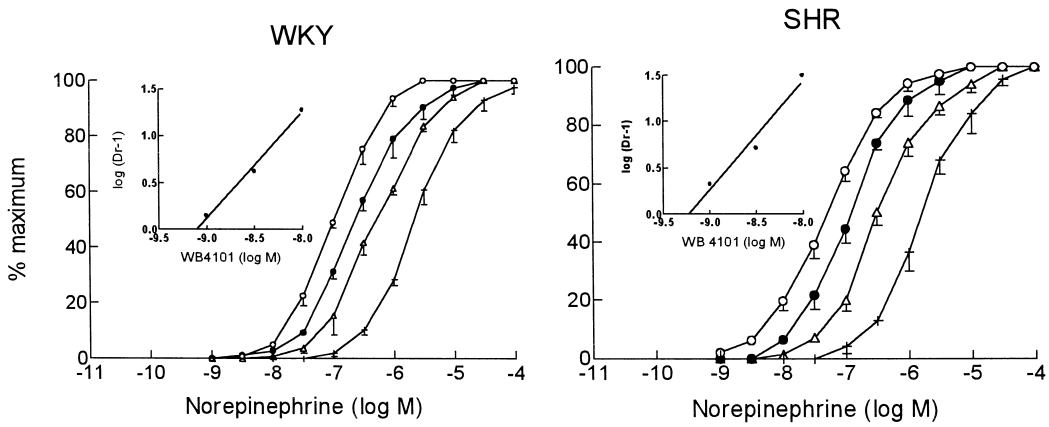

Fig. 3. Effect of WB4101 on the norepinephrine-induced contraction in aortas from WKY rats and SHR. Concentration–response curves for norepinephrine in the absence (open circle) and presence of BMY7378: 1 nM (filled circle), 3 nM (open triangle), 10 nM (cross). WKY rats ($n = 4$), SHR ($n = 5$).

baths containing KRB that was continuously equilibrated with 95% O_2:5% CO_2 and were attached to force-displacement transducers. The preparations were equilibrated for 1 h at an optimal resting tension of 1.0 mg and primed twice with 10 μM norepinephrine. After thorough washing, a cumulative norepinephrine concentration–response curve was generated, followed by another 30 min wash. The preparations were then incubated with increasing amounts of BMY7378, WB4101 or RS-17053 for 45 min, after which a second norepinephrine concentration–response curve was generated in the presence of the antagonists. pA_2 values were calculated by Schild plot. In chloroethylclonidine experiments, the preparations were preincubated with 50 μM chloroethylclonidine for 30 min, then washed for 45 min and the norepinephrine concentration–response curve was repeated. ED_{50} values and 95% confidence limits were calculated for all dose–response curves.

2.3. RNase protection assays

2.3.1. Isolation of total RNA

Frozen vessel tissues were divided into three pools, then ground into a powder in liquid nitrogen and homogenized with a high-speed homogenizer. The total RNA was then extracted by the single-step method of Chomczynski and Sacchi (1987). RNA samples were then quantified using a spectrophotometer at 260/280 nm and aliquoted in 30 μg. The samples were stored at -70°C for later use.

2.3.2. RNA probe labeling

Antisense RNA probes were transcribed with T_3 or T_7 RNA polymerase from DNA templates in the presence of [α-^{32}P]UTP and purified on an 8 M urea–6% polyacrylamide gel prior to use. The DNA templates for antisense RNA synthesis were as follows: for α_{1A}-adrenoceptor subtype, a 487 bp (*Hin*dIII/*Xho*I) fragment of α_{1A} cDNA; for α_{1B}-adrenoceptor subtype, a 306 bp (*Bam*HI/*Pst*I) fragment of α_{1B} cDNA; for α_{1D}-adrenoceptor subtype, a 414 bp (*Eco*RV/*Sma*I) fragment of α_{1D} cDNA. The above three fragments were all cloned into pBluescript SK(−). For GAPDH (glyceraldehyde-3-phosphate dehydrogenase), a 316 bp fragment corresponding to 369–685 of GAPDH cDNA (Ambion); for cyclophilin A, a 318 bp fragment corresponding to 98–416 of cyclophilin A cDNA (Danielson et al., 1988). The fragment was produced by

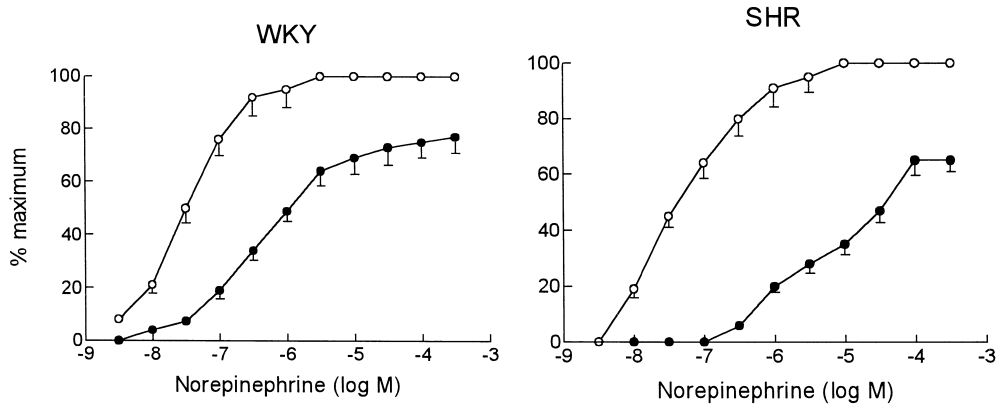

Fig. 4. Effect of chloroethylclonidine alkylation on the norepinephrine-induced contraction in aortas from WKY rats and SHR. Concentration–response curves for norepinephrine in the absence (open circle) and presence of chloroethylclonidine (50 μM, filled circle).

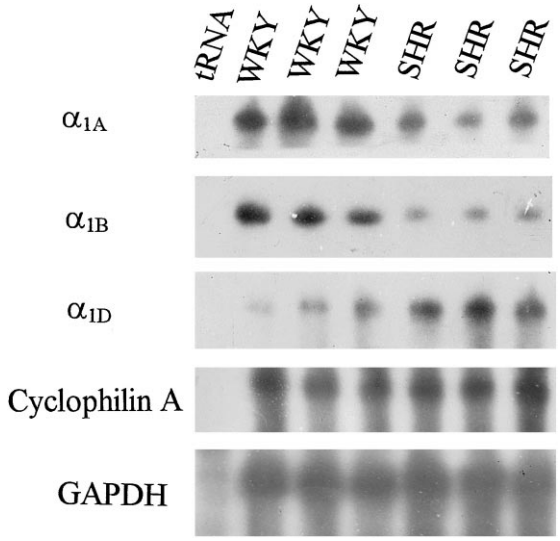

Fig. 5. Determination of mRNA levels for the three α_1-adrenoceptor subtypes by RNase protection assays in aortas from WKY rats and SHR. Yeast tRNA was used as a control for nonspecific hybridization. GAPDH and cyclophilin A were used as controls for input RNA. The similar results were obtained in additional two experiments.

reverse transcription-polymerase chain reaction, then cloned into pGEM-TEASY (Promega), and finally subcloned into the EcoRI sites of pBluescript SK(−). The insertion was oriented by restriction enzyme (*Sty*I) analysis. The size of the probes/protected fragments was as follows: α_{1A} subtype, 572/487; α_{1B} subtype, 356/306; α_{1D} subtype, 459/414; GAPDH, 355/316; cyclophilin A, 396/318.

2.3.3. RNase protection assays

30 μg of total RNA was used in RNase protection assay with probes specific for each of the three rat α_1-adrenoceptor subtype mRNA as described and validated previously (Xu and Han, 1996). 20 μg of total RNA from aortas was used in the RNase protection assay with probes for GAPDH and cyclophilin A. The probes for GAPDH and cyclophilin A were used as internal control. Autoradiographic bands were quantified by imaging analysis (LEICA Q550 IW, Germany).

2.4. Data analysis

Data are presented as the means ± S.D. Statistical analysis was performed by analysis of variance and *t*-test.

3. Results

3.1. Contractile response

3.1.1. Contractile response induced by norepinephrine

Norepinephrine induced contraction in a concentration dependent manner. Neither pD_2 values (7.18 ± 0.36 versus 7.09 ± 0.28, $n = 11$) nor maximal contractions (1391 ± 314 mg/mg tissue versus 1141 ± 229 mg/mg tissue, $n = 11$, $P > 0.05$) were significantly different between preparations isolated from SHR and WKY rats.

3.1.2. Effect of α_1-adrenoceptor subtype-selective antagonists on norepinephrine-induced aortic contraction

RS-17053, BMY7378 or WB4101 competitively inhibited norepinephrine-induced contraction, i.e. shifting the curve to the right without causing a significant change in the maximum contraction, in a concentration-dependent manner in aortas isolated from SHR and WKY rats. The pA_2 values of BMY7378 were increased from 8.10 ± 0.12 (slope = 0.87 ± 0.11, $n = 8$) in WKY rats to 8.45 ± 0.13 (slope = 1.06 ± 0.07, $n = 4$) in SHR ($P < 0.05$, Fig. 1). The pA_2 values of RS-17053 were reduced from 8.52 ± 0.20 (slope = 0.68 ± 0.10, $n = 4$) in WKY rats to 7.82 ± 0.18 (slope = 0.98 ± 0.11, $n = 4$) in SHR ($P < 0.05$, Fig. 2). The pA_2 values of WB4101 were not significantly different between WKY rats and SHR (9.05 ± 0.22, slope = 0.99 ± 0.13, $n = 4$ versus 9.27 ± 0.15, slope = 1.15 ± 0.14, $n = 5$, $P > 0.05$, Fig. 3).

3.1.3. Effects of chloroethylclonidine on norepinephrine-induced aortic contraction

Chloroethylclonidine irreversibly inhibited the norepinephrine-induced response in aortas from WKY rats and SHR. As shown in Fig. 4, norepinephrine-induced responses were affected by chloroethylclonidine more profoundly in the SHR aortas than in the WYK rat aortas. The EC_{50} values were increased by 211 ± 11 fold ($n = 5$) and 30 ± 6 fold ($n = 5$) in aortas from SHR and WKY rats, respectively. Maximum contractions were decreased more

Table 1
Quantification of mRNA levels for three α_1-adrenoceptor subtypes, GAPDH and cyclophilin A in aortas from 12-month-old SHR and aged-matched WKY rats. Values are expressed as means ± S.D.

	n	α_{1A}	α_{1B}	α_{1D}	Cyclophilin A	GAPDH
WKY	3	2719 ± 366	1872 ± 268	842 ± 225	2560 ± 233	4277 ± 466
SHR	3	1372 ± 265[a]	767 ± 117[a]	1772 ± 169[a]	2489 ± 142[b]	3743 ± 235[b]

[a] $P < 0.05$ compared with WKY rats.
[b] $P > 0.05$ compared with WKY rats.

markedly in the SHR aorta (decreased by 35 ± 2.7%) than in the WKY rat aortas (decreased by 28 ± 1.2%).

3.2. RNase protection assays

The mRNA levels for three α_1-adrenoceptor subtypes were determined in aortas from 12-month-old SHR and aged-matched WKY rats. A representative autoradiography of RNase protection assay is shown in Fig. 5. Quantification of mRNAs showed that the mRNA levels for α_{1A}- and α_{1B}-adrenoceptor subtype were decreased whereas the mRNA level for α_{1D}-adrenoceptor subtype was increased in aortas from SHR. The mRNA levels for the 'housekeeping' genes, GAPDH and cyclophilin A, were similar in aortas of WKY rats and SHR (Table 1).

4. Discussion

Recent studies indicate that BMY7378 is a selective α_{1D}-adrenoceptor subtype antagonist which exhibits a 100-fold higher affinity for the cloned α_{1D}-adrenoceptor subtype than for the cloned α_{1A}- and α_{1B}-adrenoceptor subtypes (Goetz et al., 1995). RS-17053 is a selective α_{1A}-adrenoceptor subtype antagonist which exhibits a 30 to 100-fold higher affinity for the cloned α_{1A}-adrenoceptor subtype than for the cloned α_{1B}- and α_{1D}-adrenoceptor subtypes (Ford et al., 1996). WB4101 exhibits higher affinity for the α_{1A}- and α_{1D}-adrenoceptor subtypes than for the α_{1B}-adrenoceptor subtype (Minneman and Esbenshade, 1994). Although chloroethylclonidine can inactivate the three α_1-adrenoceptor subtypes, there is a wide variation in sensitivity, with the α_{1A}-adrenoceptor subtype being least sensitive and the α_{1B}- and α_{1D}-adrenoceptor subtypes being decidedly more sensitive (Han et al., 1995). In the present studies, we used BMY7378, RS-17053, WB4101 and chloroethylclonidine as tools to assess the specific contribution of α_1-adrenoceptor subtypes to norepinephrine-induced contraction in aortas from WKY rats and SHRs at the age of 12 months.

Our experiments showed that a α_{1D}-adrenoceptor subtype-selective antagonist, BMY7378, more potently antagonized, whereas a α_{1A}-adrenoceptor subtype-selective antagonist, RS-17053, less potently antagonized the norepinephrine-induced responses in the SHR aorta than in the WKY rat aorta. This suggests that the α_{1D}-adrenoceptor subtype plays more, and the α_{1A}-adrenoceptor subtype plays less, of a role in aortic contraction in SHR than in WKY rats. Since WB4101 has high affinity for both α_{1A}- and α_{1D}-adrenoceptor subtypes, the influence of an increase in the α_{1D}-adrenoceptor subtype and a decrease in the α_{1A}-adrenoceptor subtype was balanced out. Therefore, the pA_2 values of WB4101 were unchanged in SHR and WKY rats. The observation that the SHR aortas were affected more profoundly by chloroethylclonidine than were WKY rat aortas further supports the above conclusion. The alteration in α_1-adrenoceptor subtype mediated-response may reflect either changes at a receptor level or changes in post-receptor mechanisms. Because there was too little tissue to allow determination of receptor protein by radioligand binding assay, we detected mRNAs for the three α_1-adrenoceptor subtypes in aortas from SHR and WKY rats. The results of RNase protection assays showed that mRNAs for α_{1A}- and α_{1B}-adrenoceptor subtypes were decreased in the aortas from SHR, whereas mRNA for the α_{1D}-adrenoceptor subtype was increased compared with that in the WKY rats. It is known that the mRNAs for the three α_1-adrenoceptors are localized specifically in the medial layer of the aorta (Piascik et al., 1994). Therefore, mRNA levels detected in the present studies basically reflect the change in mRNAs for the three α_1-adrenoceptor subtypes in vascular smooth muscle, although total RNA was extracted from intact vessels containing not only smooth muscle cells but also adventitia and endothelium. These results are in a good agreement with our functional data. Therefore, we infer that in the aorta from SHR, there are fewer α_{1A} and α_{1B} subtype receptors and more α_{1D} subtype receptor than in aorta from age-matched WKY rats.

It has been well documented that α_1-adrenoceptor agonist-induced aortic contraction is mainly mediated by the α_{1D}-adrenoceptor subtype in young rats (Saussy et al., 1994; Testa et al., 1995). However, the α_{1A}-adrenoceptor subtype become increasingly important in aortic contraction with aging (Gurdal et al., 1995). Our previous functional studies also revealed that in aortas from 12-month-old WKY rats α_{1A}- and α_{1D}-adrenoceptor subtypes jointly mediated aortic contraction (Lu et al., 1997). The results of the present studies show that in the aortas isolated from 12-month-old WKY rats, the pA_2 values of RS-17053 were higher, while the pA_2 values of BMY7378 were lower than the pA_2 values obtained for aortas from young rats (10-week-old) in our previous experiments (7.85 ± 0.20 for RS-17053 and 8.68 ± 0.20 for BMY7378, Lu et al., 1997) and Ford's experiments (7.7 ± 0.1 for RS-17053 and 8.5 ± 0.2 for BMY7378, Ford et al., 1996). In addition, the Schild plot slopes were significantly less than unity. These results further suggest that in aortas from 12-month-old WKY rats, not only the α_{1D}-adrenoceptor subtype, but also the α_{1A}-adrenoceptor subtype mediates norepinephrine-induced contraction. However, in the aortas from 12-month-old SHR the sensitivity to BMY7378 was increased and the sensitivity to RS-17053 was decreased significantly. The pA_2 values of the two antagonists were close to those in young WKY rats (Lu et al., 1997) and the slopes were not significantly different from unity. These results suggest the effect of age-related changes in α_1-adrenoceptor subtypes is probably 'buffered' in SHR.

The pathophysiological significance in the change in α_1-adrenoceptor subtypes in vascular smooth muscle is still unclear. The different α_1-adrenoceptor subtypes may

play different roles in mediating proliferation and hypertrophy of smooth muscle cells (Yu et al., 1996; Chen et al., 1995) and even have the opposite influence on the hypertrophy (Chen et al., 1995). Thus, changes in α_1-adrenoceptor subtypes in blood vessels may be involved in the pathogenesis of hypertension. However, our preliminary data showed that the subtypes of α_1-adrenoceptor present in aortas from 3-month-old SHR were not different from those in aortas of age-matched WKY rats, although the systemic blood pressure is significantly elevated. Therefore, the change in α_1-adrenoceptor subtypes seems to be a link in the vicious circle rather than the original cause of the hypertension.

References

Brown, L., Amos, G., Miller, B., 1994. Disease-induced changes in alpha-adrenoceptor-mediated cardiac and vascular responses in rats. Clin. Exp. Pharmacol. Physiol. 21, 721–728.

Chen, L.Q., Xin, X.B., Eckhart, A.D., Yang, N.Y., Faber, J.E., 1995. Regulation of vascular smooth muscle growth by α_1-adrenoceptor subtypes in vitro and in situ. J. Biol. Chem. 270, 30980–30988.

Chomczynski, P., Sacchi, N., 1987. Single step method of RNA isolation by acid guanidium thiocyanate–phenol–chloroform extraction. Anal. Biochem. 162, 156–198.

Danielson, P.E., Forss-Petter, S., Brow, N.A.D., Calavetta, L., Douglass, J., Milner, R.J., Sutcliffe, J.G., 1988. A cDNA clone of the rat mRNA encoding cyclophilin. DNA 7, 261–267.

Ford, A.P.D.W., Arredondo, N.F., Blue, D.R., Bonhaus, D.W. Jr., Jasper, J., Shannon Kava, M., Lesnick, J., Pfister, J.R., Amy Shieh, I., Vimont, R.L., Williams, T.J., Mcneal, J.E., Stamey, T.A., Clarke, D.E., 1996. RS-17053 (*N*-[2-(2-cyclopropylmethoxyphenoxy)ethyl]-5-chloro-α,α-dimethyl-1H-indole-3-ethanamine hydrochloride), a selective α_{1A}-adrenoceptor antagonist, displays low affinity for functional α_1-adrenoceptors in human prostate: Implications for adrenoceptor classification. Mol. Pharmacol. 49, 209–215.

Goetz, A.S., King, H.K., Ward, S.D., True, T.A., Rimele, T.J., Saussy, D.L.J., 1995. BMY 7378 is a selective antagonist of the D subtype of alpha 1-adrenoceptors. Eur. J. Pharmacol. 272, R5–R6.

Graham, R.M., Perez, D.M., Hwa, J., Piascik, M.T., 1996. Alpha 1-adrenergic receptor subtypes. Molecular structure, function, and signaling. Circ. Res. 78, 737–749.

Gurdal, H., Cai, G., Johnson, M.D., 1995. α_1-Adrenoceptor responsiveness in the ageing aorta. Eur. J. Pharmacol. 274, 117–123.

Han, C., Li, J.L., Chen, Y.M., 1992. Changes of subtypes of alpha 1-adrenoceptor in blood vessels of spontaneously hypertensive rats. Sheng Li Hsueh Pao 44, 229–236.

Han, C., Hollinger, S., Theroux, T.L., Espenshade, T.A., Minneman, K.P., 1995. ^{3}H-tamsulosin binding to cloned α_1-adrenergic receptor subtypes expressed in human embryonic kidney 293 cells: Antagonist potencies and sensitivity to alkylating agents. Pharmacol. Commun. 5, 117–126.

Holck, M.I., 1986. α_1-adrenoceptor reserve and effects of Ca^{2+} entry-block (Ro 18–3981) on aorta of spontaneously hypertensive rats. Eur. J. Pharmacol. 148, 9–15.

Izzo, N.I. Jr., Seidman, C.E., Collins, S., Colucci, W.S., 1990. Alpha 1-adrenergic receptor mRNA level is regulated by norepinephrine in rabbit aortic smooth muscle cells. Proc. Natl. Acad. Sci. USA 87, 6268–6271.

Lu, Z.Z., Zhang, Y.Y., Han, C., 1997. Comparison of functional α_1-adrenoceptor subtypes in aortae between 12 month- and 10-week-old rats. Acta Physiol. Sin. 49, 361–365.

Minneman, K.P., Esbenshade, T.A., 1994. α_1-Adrenergic receptor subtypes. Annu. Rev. Pharmacol. Toxicol. 34, 117–133.

Piascik, M.T., Smith, M.S., Soltis, E.E., Perez, D.M., 1994. Identification of the mRNA for the novel alpha 1D-adrenoceptor and two other alpha 1-adrenoceptors in vascular smooth muscle. Mol. Pharmacol. 46, 30–40.

Rokosh, D.G., Stewart, A.F., Chang, K.C., Bailey, B.A., Karliner, J.S., Camacho, S.A., Long, C.S., Simpson, P.C., 1996. Alpha1-adrenergic receptor subtype mRNAs are differentially regulated by alpha1-adrenergic and other hypertrophic stimuli in cardiac myocytes in culture and in vivo. Repression of alpha1B and alpha1D but induction of alpha1C. J. Biol. Chem. 271, 5839–5843.

Saussy, D.L., Goetlz, A.S., King, H.K., True, T.A., 1994. BMY7378 is a selective antagonist of α_{1D}-adrenoceptors: Further evidence that vascular α_1-adrenoceptors are the α_{1D}-subtype. Can J. Pharmacol. 72, 323p.

Testa, R., Destefani, C., Guarneri, L., Poggesi, E., Simonazzi, I., Taddei, C., Leonardi, A., 1995. The α_{1D}-adrenoceptor subtype is involved in the noradrenaline-induced contractions of rat aorta. Life Sci. 57, PL159–PL163.

Xu, K., Han, C., 1996. Quantification of mRNAs for three alpha1-adrenoceptor subtypes in rat aorta by solution hybridization. Life Sci. 59, 343–347.

Yu, S.M., Tsai, S.T., Guh, J.H., Ko, F.N., Teng, C.M., Ou, J.T., 1996. Mechanism of catecholamine-induced proliferation of vascular smooth muscle cells. Circulation 94, 547–554.