ELSEVIER

European Journal of Pharmacology 298 (1996) 257–263

ejp

α_1-Adrenoceptors mediating contraction in arteries of normotensive and spontaneously hypertensive rats are of the α_{1D} or α_{1A} subtypes

Rafael Villalobos-Molina *, Maximiliano Ibarra

Sección de Terapéutica Experimental, Departamento de Farmacología y Toxicología, CINVESTAV-IPN, Apartado Postal 22026, México D.F. 14000, Mexico

Received 7 August 1995; revised 6 November 1995; accepted 14 November 1995

Abstract

α_1-Adrenoceptor subtypes mediating contraction in carotid, aorta, mesenteric and caudal arteries from both Wistar Kyoto (WKY) normotensive and spontaneously hypertensive (SHR) rats were investigated by using the α_{1A}-adrenoceptor agonist methoxamine and antagonized with selective, competitive antagonists WB-4101, 5-methyl urapidil or BMY 7378 (8-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl)-8-azaspiro(4,5)decane-7,9-dione dihydrochloride). Isometric tension changes were recorded after methoxamine addition to the arterial rings, and the effects of the antagonists determined. All the antagonists shifted to the right the concentration-response curve to methoxamine. pA_2 values indicate that all arteries but caudal express the α_{1D}-adrenoceptor subtype, since BMY 7378 values were high in these arteries. Due to the high pA_2 values for 5-methyl urapidil and WB-4101 and the low values for BMY 7378 we conclude that the tail artery expresses the α_{1A} and not the α_{1B} subtype. No differences were found between both strains of rats, suggesting that hypertension does not modify the α_1-adrenoceptors in conductance arteries.

Keywords: α_{1D}-Adrenoceptor; α_{1A}-Adrenoceptor; BMY 7378; Spontaneously hypertensive rat (SHR); Artery; Wistar Kyoto rat (WKY)

1. Introduction

In hypertension, increased contractile responses to agonists have been correlated with an enhanced protein kinase C activity (Turla and Webb, 1987; Silver et al., 1992), an augmented phosphoinositide metabolism (Turla and Webb, 1990), and an increased Ca^{2+} mobilisation (Thorin-Trescases et al., 1994). Whether these alterations could be related to a specific α_1-adrenoceptor subtype in spontaneously hypertensive rat (SHR) vasculature, different to the one present in normotensive Wistar Kyoto (WKY) rats, is not known. However, Michel et al. (1989) reported an increase in α-adrenoceptor density in kidney from hypertensive rats, and Suzuki et al. (1994) found an increase in the number of α_1-adrenoceptors along with an increase in the affinity for the antagonist, in mesenteric vasculature of deoxycorticosterone-salt hypertensive rats. Abdul Sattar and Johns (1994a, b) reported that the α_1-adrenoceptor involved in vasoconstriction in kidney of stroke prone-spontaneously hypertensive rats and two models of induced hypertension is of the α_{1A} subtype; nonetheless information about α_1-adrenoceptor subtypes in other SHR blood vessels is scarce.

To date the existence of three α_1-adrenoceptor subtypes (namely α_{1A}, α_{1B}, and α_{1D}), based on both pharmacological and cloning studies, is well documented (Minneman, 1988; Perez et al., 1991; Schwinn and Lomasney, 1992; Faure et al., 1994). Vascular α_1-adrenoceptors were classified as α_{1A} in rat renal artery, α_{1B} in rat aorta and a mixture of α_{1A} and α_{1B} in rat mesenteric artery (Han et al., 1990); later Piascik et al. (1994) showed that the novel α_{1D} subtype is expressed in several rat blood vessels and Tsujimoto et al. (1994) communicated (in abstract form) that the adrenoceptor mediating contraction in rat tail artery is of the α_{1c} subtype, which is actually the α_{1A} subtype (Laz et al., 1994; Perez et al., 1994).

Very recently Goetz et al. (1995) described a new α_{1D}-adrenoceptor selective antagonist named BMY 7378 (8-(2-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl)-8-azaspiro(4.5)decane-7,9-dione dihydrochloride) which has been tested in cloned as well as pharmacological models, showing high affinity for the α_{1D} subtype; however, Burt et al. (1995) reported a low affinity of the α_{1A}-adrenocep-

* Corresponding author. Tel.: (525) 675-6248; fax: (525) 675-9168.

SSDI 0014-2999(95)00781-4

tor in rat vas deferens and an intermediate affinity of the α_{1B} subtype in rat spleen for this antagonist. Taking together, these elements prompted us to characterize the α_1-adrenoceptor subtypes that mediate contraction in arteries of WKY and SHR rats. Experiments were designed to compare the pA_2 values from different competitive antagonists to the methoxamine-induced contraction.

2. Materials and methods

2.1. Determination of isometric tension changes

Male normotensive (WKY, 458 ± 9 g) and spontaneously hypertensive (SHR, 359 ± 5 g) rats of 6 months of age (± 1 week) were reared in our animal facility and fed ad libitum on a standard diet (Purina, México). Systolic blood pressure and heart rate were measured in the conscious state by a tail-cuff method (average values for blood pressure were 134 ± 1 (WKY) and 183 ± 5 mm Hg (SHR), and for heart rate, 357 ± 11 (WKY) and 398 ± 9 beats/min (SHR), n = 10–13). Rats were anesthetized with ether and carotid, aorta, mesenteric and caudal arteries dissected and cleaned of surrounding tissue. Arterial rings (3–5 mm in length) were denuded of endothelium and placed in 10 ml chambers filled with Krebs solution (NaCl, 118 mM; KCl, 4.7 mM; $CaCl_2$, 2.5 mM; $MgSO_4$, 1.2 mM; KH_2PO_4, 1.2 mM; $NaHCO_3$, 25 mM; EDTA, 0.026 mM and glucose, 11.1 mM at 37°C and pH 7.4 with constant oxygenation (O_2/CO_2, 19:1)), attached to the bottom of the chamber and to an isometric FT03 Grass Force displacement transducer, connected to a 7D Grass polygraph. Arterial rings were subjected to an initial optimal tension of 3 g (mesenteric, carotid and aorta) or 2 g (caudal), obtained from preliminary experiments using increments in initial tension, up to reach the optimal. The tissue was challenged with methoxamine (3.1×10^{-6} M), and washed every 30 min for 2 h. Then, reproducible cumulative concentration-response curves to methoxamine (10^{-7} M to 3.1×10^{-5} M) were obtained for each artery. In order to avoid fatigue of the arterial preparation, a 60 min recovery period was allowed between methoxamine curves. To rule out any alteration on the tissue sensitivity to the agonist due to the duration of these experiments, simultaneous assays were performed, i.e., control arteries in the presence of the agonist and arteries in the presence of the agonist plus the antagonist (either, 5-methyl urapidil, WB-4101 or BMY 7378). The antagonist was present 15–30 min before agonist addition and throughout the experiment. The absence of endothelium was confirmed by the lack of a relaxing response to acetylcholine (10^{-6} M).

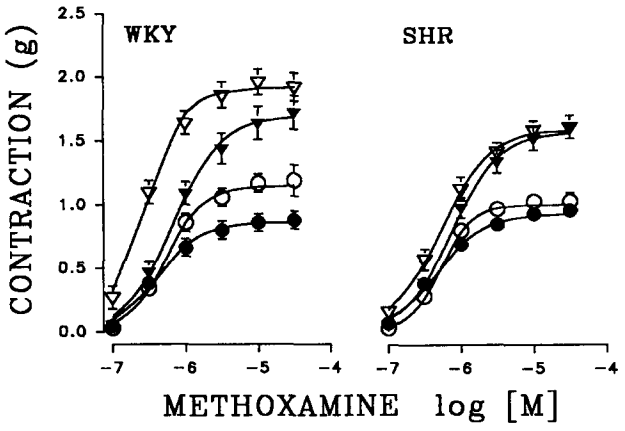

Fig. 1. Concentration-response curves for methoxamine in aorta (▽), mesenteric (▼), caudal (○) and carotid (●) arteries from WKY or SHR rats (n = 10–13).

Table 1
pA_2 values for the selective antagonists of α_1-adrenoceptors in the vasculature of WKY and SHR rats

	5-MU	WB-4101	BMY 7378
WKY			
Artery			
Mesenteric	8.28	9.41	8.33
	(7.95–8.69)	(9.23–9.58)	(8.09–8.57)
Carotid	8.02	9.23	8.66
	(7.77–8.29)	(9.07–9.39)	(8.23–9.08)
Aorta	7.72	9.17	8.72
	(7.57–7.87)	(8.85–9.49)	(8.39–9.05)
Caudal	8.19	9.08	6.90
	(7.97–8.41)	(8.69–9.36)	(6.60–7.20)
SHR			
Mesenteric	8.37	9.63	8.68
	(8.03–8.69)	(9.48–9.77)	(8.44–8.92)
Carotid	8.29	9.31	8.51
	(7.67–8.91)	(9.09–9.53)	(8.14–8.89)
Aorta	7.63	8.98	8.27
	(7.47–7.78)	(8.62–9.35)	(8.08–8.46)
Caudal	8.56	9.14	6.88
	(8.27–8.84)	(9.02–9.25)	(6.67–7.09)

Limits of confidence are indicated in parentheses. Data from 4–7 animals in each condition.

2.2. Analysis of data

Contraction is given in g for an easy comparison among the experiments. Data are the means of 4–7 different animals for each group. The pA_2 and slope (m) values were calculated according to the method of Arunlakshana and Schild (1959). Statistical analysis was done by using the Student's t-test.

2.3. Chemicals

Methoxamine was a generous gift of Burroughs Wellcome (NJ, USA), 5-methyl urapidil, WB-4101 and BMY 7378 were from Research Biochemicals Int. (MA, USA); all the other reagents were of analytical grade.

3. Results

Methoxamine elicited reproducible concentration-dependent contractions in all four arteries from both WKY and SHR rats (not shown). The potency (pD_2) of the agonist and the maximal effect (E_{max}) were similar when arteries of WKY and SHR were compared (pD_2, 6.22 ± 0.01; 6.33 ± 0.03; 6.56 ± 0.06; 6.16 ± 0.01 vs. 6.33 ± 0.05; 6.33 ± 0.06; 6.28 ± 0.07; 6.10 ± 0.04; E_{max}, 1.17 ± 0.07; 0.88 ± 0.07; 1.96 ± 0.10; 1.72 ± 0.13 vs. 1.02 ± 0.06; 0.95 ± 0.06; 1.58 ± 0.07; 1.60 ± 0.09 g, in caudal, carotid, aorta and mesenteric WKY vs. SHR, respectively, Fig. 1).

Preincubation of the arteries with BMY 7378, 5-methyl urapidil or WB-4101 caused a shift to the right in the contraction elicited by methoxamine, in both WKY and SHR (Figs. 2–5). Since the maximal effect was attained with increasing concentrations of the agonist, the competitive antagonism was demonstrated for all three agents tested. High pA_2 values were obtained for WB 4101, 5-methyl urapidil and BMY 7378 in all arteries, except for the BMY 7378 in the caudal of both WKY and SHR (Table 1). Slopes of the Schild plots were not statistically different from the theoretical value of 1, except for aorta from SHR with 5-methyl urapidil ($m = 1.44$, $P = 0.05$) (data not shown).

4. Discussion

The α_1-adrenoceptor subtypes mediating contraction in rat vasculature have been characterized by use of antago-

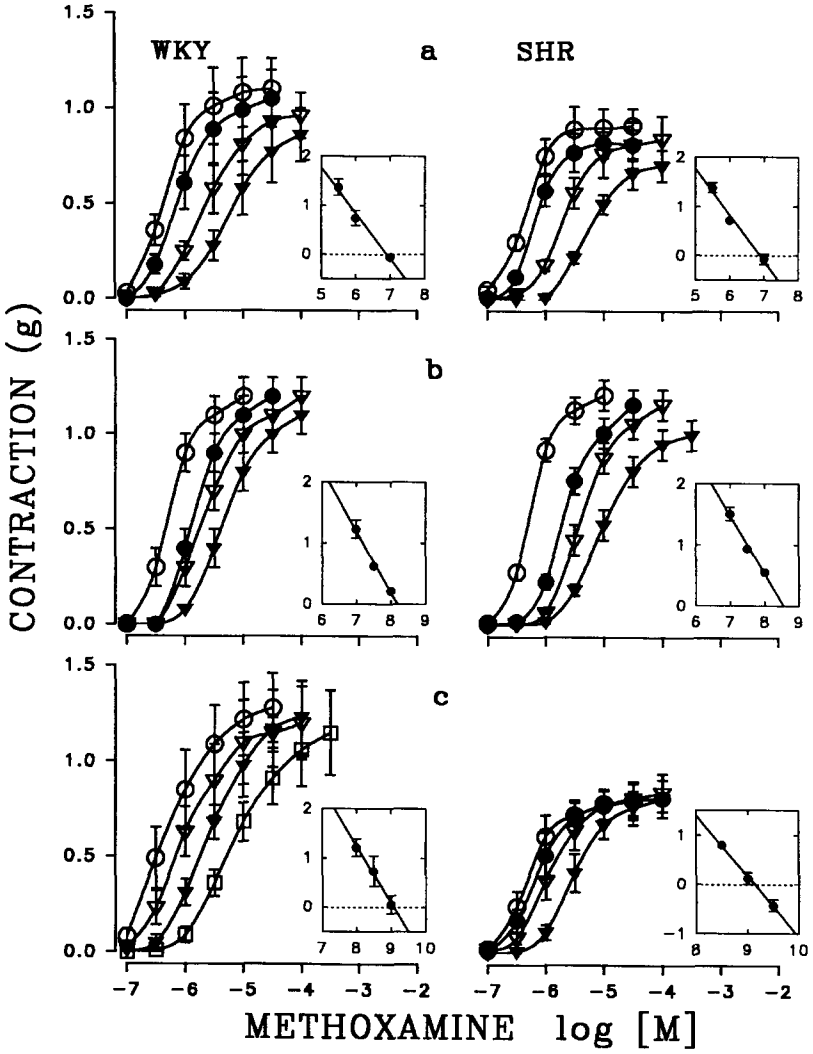

Fig. 2. Antagonism of methoxamine-induced contractions in caudal arteries from WKY and SHR rats. (a) BMY 7378, Control (○); 10^{-7} M (●); 10^{-6} M (▽); 3.1×10^{-6} M (▼). (b) 5-Methyl urapidil, 10^{-8} M (●); 3.1×10^{-8} M (▽); 10^{-7} M (▼). (c) WB-4101, 3.1×10^{-10} M (●); 10^{-9} M (▽); 3.1×10^{-9} M (▼); 10^{-8} M (□). Insets represent the Schild plot, where y axis is log (CR − 1) and x axis is concentration of antagonist. Data represent the means ± S.E.M. of 4–7 different animals.

nists that have some subtype specificity when used in functional (Han et al., 1990; Suzuki et al., 1994; Goetz et al., 1995; Burt et al., 1995), binding (Piascik et al., 1994; Suzuki et al., 1994) and molecular (Perez et al., 1994; Piascik et al., 1994) experiments. In contrast, a small amount of research has been done regarding the identification of α_1-adrenoceptor subtypes expressed in essential hypertension that could explain, at least in part, the increase in blood pressure.

To our knowledge, this is the first report that comparatively characterizes the α_1-adrenoceptor subtypes that mediate contraction in several arteries from WKY and SHR rats, based upon the recent knowledge on α_1-adrenoceptor classification (see Watson and Girdlstone, 1995; Hieble et al., 1995). Our results show that WB-4101 and 5-methyl urapidil were not useful to differentiate between the α_1-adrenoceptors mediating contraction in all four arteries studied from both WKY or SHR rats, since their respective pA_2 values were similar and high (Table 1). These high pA_2 values indicate that the α_{1B}-adrenoceptor subtype does not seem to participate in contraction in these arteries, even though the presence of the mRNA for this receptor has been demonstrated in rat vasculature (Piascik et al., 1994). pA_2 values for BMY 7378 were high in aorta, mesenteric and carotid arteries from both WKY and SHR rats; according to Piascik et al. (1994) the first two arteries contain the mRNA for the α_{1D} subtype and Goetz et al. (1995) reported a pA_2 value of 8.9 for aorta which is similar to the one we found (Table 1) with this antagonist. Based on these results, we suggest that the α_{1D}-adrenoceptor subtype mediates contraction in aorta, mesenteric and carotid arteries in normotensive and hypertensive rats, which is in disagreement with a report of Fujimoto (1994) where he suggests that aorta and femoral arteries (from

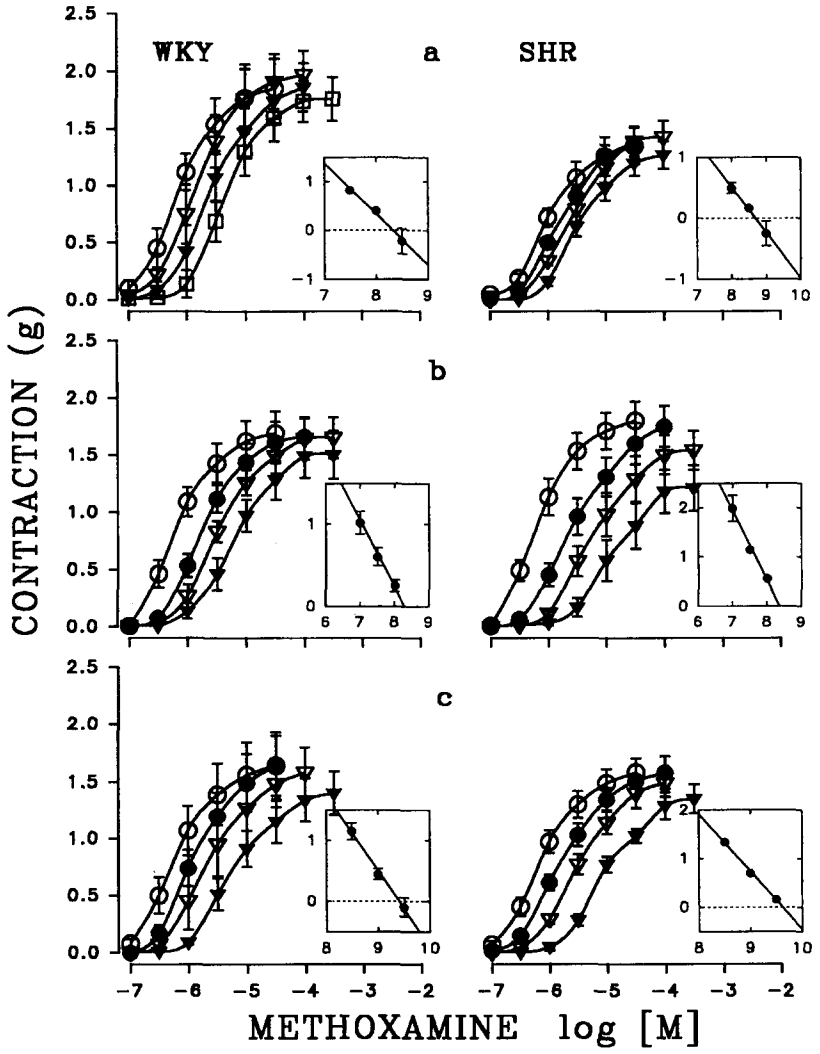

Fig. 3. Antagonism of methoxamine-induced contractions in carotid arteries from WKY and SHR rats. (a) BMY 7378, Control (○); 3.1×10^{-9} M (●); 10^{-8} M (▽); 3.1×10^{-8} M (▼). (b) 5-Methyl urapidil, (c) WB-4101 and insets as in Fig. 2. Data represent the means ± S.E.M. of 4–7 different rats.

WKY and SHR) present the α_{1A} subtype (α_{1H}, in his nomenclature).

On the contrary, pA_2 values for BMY 7378 were about 6.9 in caudal artery from both WKY and SHR rats, indicating that this vessel expresses the α_{1A}-adrenoceptor subtype since the reported pK_i value in membranes expressing this adrenoceptor is 6.7 (Goetz et al., 1995) and the pA_2 value for contraction in rat vas deferens is 6.7 (Burt et al., 1995) for the antagonist. Our results support a previous report from Tsujimoto et al. (1994) that the rat tail artery contains the α_{1C}-adrenoceptor subtype (which is actually the α_{1A} subtype) and is the one responsible for contraction in this artery.

On the other hand, our results indicate that conductance arteries do not present differences, regarding the α_1-adrenoceptor subtypes, between the WKY and SHR rats that could explain the observed high blood pressure. However, they do suggest that an increased coupling between the α_1-adrenoceptor and the intracellular machinery may exist that could account for the increase in the protein kinase C activity and the second messenger generation observed during hypertension. Evidently, more experiments are needed to confirm this suggestion.

Acknowledgements

The authors thank Mr. J. Javier López-Guerrero for his skillful technical assistance and Mr. Julio Sánchez for his help in the blood pressure measurements. This work was

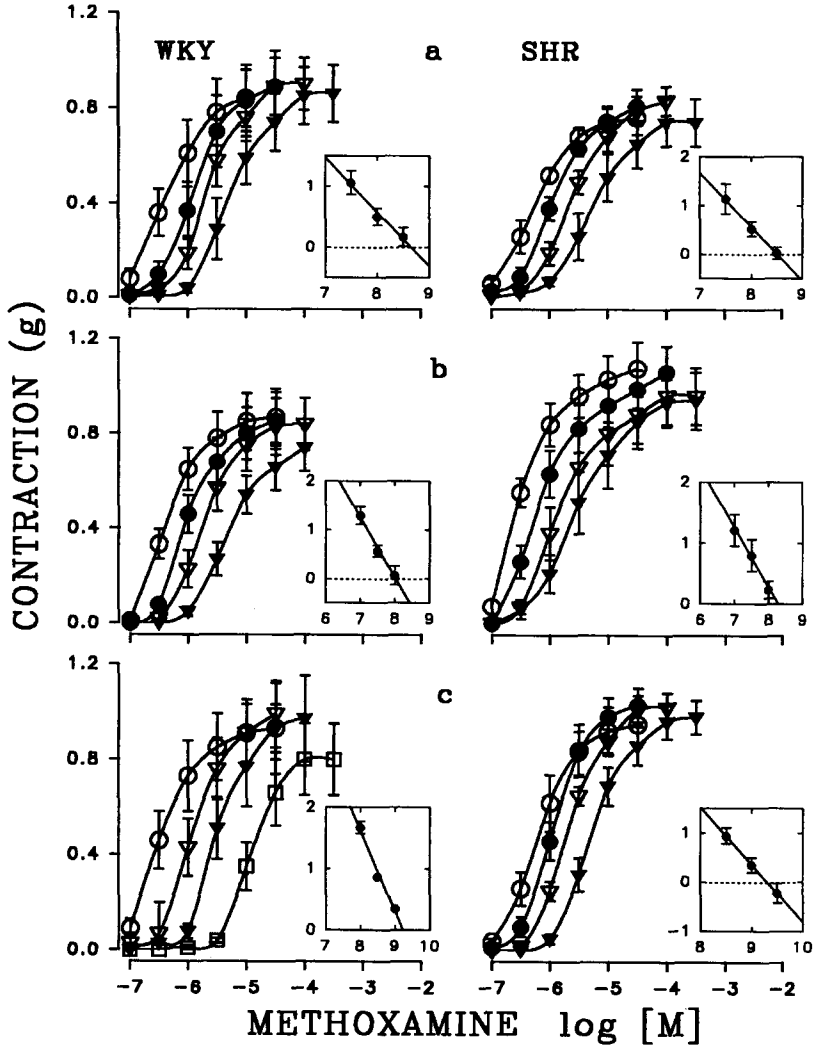

Fig. 4. Antagonism of methoxamine-induced contractions in mesenteric arteries from WKY and SHR rats. (a) BMY 7378, Control (○); 10^{-9} M (●); 3.1×10^{-9} M (▽); 10^{-8} M (▼); 3.1×10^{-8} M (□). (b) 5-Methyl urapidil, (c) WB-4101 and insets as in Fig. 2. Data represent the means ± S.E.M. of 4–7 different rats.

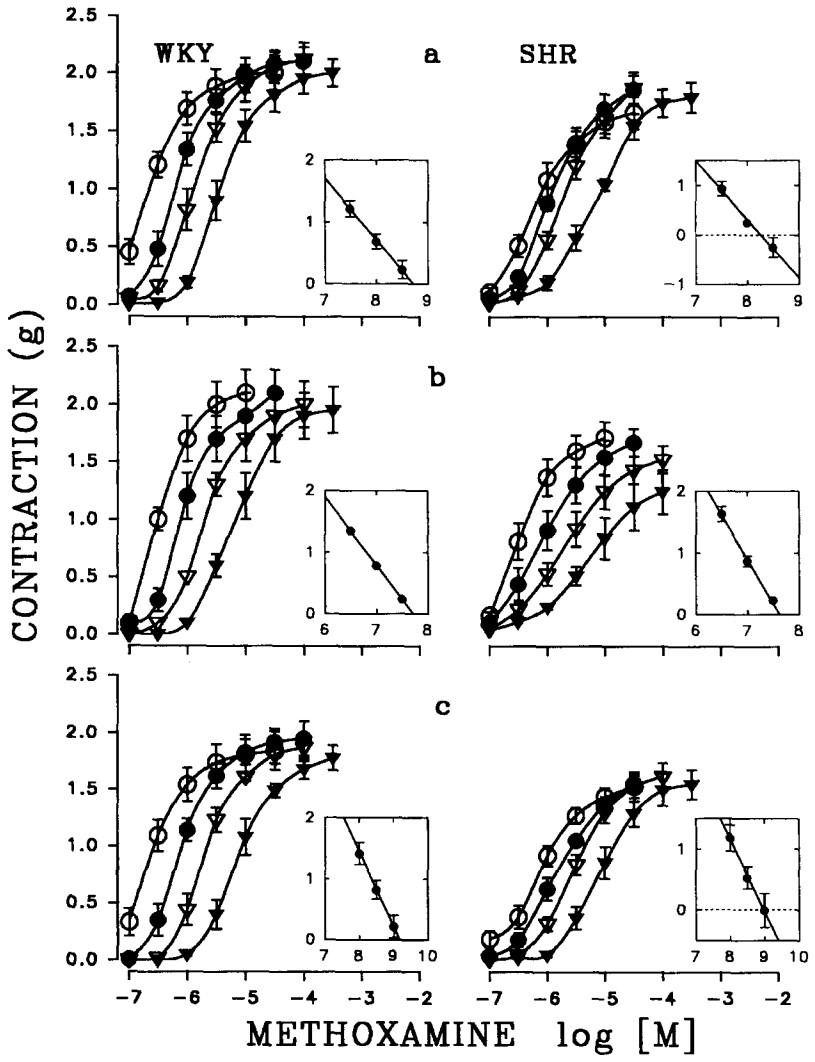

Fig. 5. Antagonism of methoxamine-induced contractions in aortae from WKY and SHR rats. (a) BMY 7378, as in Fig. 3. (b) 5-Methyl urapidil, Control (○); 3.1×10^{-8} M (●); 10^{-7} M (▽); 3.1×10^{-7} M (▼). (c) WB-4101 and insets as in Fig. 2. Data are the means ± S.E.M. of 4–7 different preparations.

partially supported by grant 5110-M9406 from CONACYT.

References

Abdul Sattar, M. and E.J. Johns, 1994a, Evidence for an α_1-adrenoceptor subtype mediating adrenergic vasoconstriction in Wistar normotensive and stroke-prone spontaneously hypertensive rat kidney, J. Cardiovasc. Pharmacol. 23, 232.

Abdul Sattar, M. and E.J. Johns, 1994b, α_1-Adrenoceptor subtypes mediating adrenergic vasoconstriction in kidney of two kidney, one clip Goldblatt and deoxycorticosterone acetate-salt hypertensive rats, J. Cardiovasc. Pharmacol. 24, 420.

Arunlakshana, O. and H.O. Schild, 1959, Some quantitative uses of drug antagonists, Br. J. Pharmacol. 14, 48.

Burt, R.P., C.R. Chapple and I. Marshall, 1995, Evidence for a functional α_{1A}-(α_{1C}-)adrenoceptor mediating contraction of rat epididymal vas deferens and an α_{1B}-adrenoceptor mediating contraction of the rat spleen, Br. J. Pharmacol. 115, 467.

Faure, C., C. Pimoule, S. Arbilla, S.Z. Langer and D. Graham, 1994, Expression of α_1-adrenoceptor subtype in rat tissues: implications for α_1-adrenoceptor classification, Eur. J. Pharmacol. Mol. Pharmacol. 268, 141.

Fujimoto, S., 1994, α_1-Adrenoceptor subtypes mediating contraction of the femoral artery in spontaneously hypertensive rats, Can. J. Physiol. Pharmacol. 72, 862.

Goetz, A.S., H.K. King, S.D.C. Ward, T.A. True, T.J. Rimele and D.L. Saussy Jr., 1995, BMY 7378 is a selective antagonist of the D subtype of α_1-adrenoceptors, Eur. J. Pharmacol. 272, R5.

Han, C., J. Li and K.P. Minneman, 1990, Subtypes of α_1-adrenoceptors in rat blood vessels, Eur. J. Pharmacol. 190, 97.

Hieble, J.P., D.B. Bylund, D.E. Clark, D.C. Eikenburg, S.Z. Langer, R.J. Lefkowitz, K.P. Minneman and R.R. Ruffolo Jr., 1995, International Union of Pharmacology X. Recommendation for nomenclature of α_1-adrenoceptors: consensus update, Pharmacol. Rev. 47, 267.

Laz, T.M., C. Forray, K. Smith, J.A. Bard, P.J.-J. Vaysse, T.A. Branchek and R.L. Weinshank, 1994, The rat homologue of the bovine α_{1c}-

adrenergic receptor shows the pharmacological properties of the classical α_{1A} subtype, Mol. Pharmacol. 46, 414.

Michel, M.C., P.A. Insel and O.E. Brodde, 1989, Renal α-adrenergic receptor alterations: a cause of essential hypertension?, FASEB J. 3, 139.

Minneman, K.P., 1988, α_1-Adrenergic receptor subtypes, inositol phosphates and sources of cell Ca^{2+}, Pharmacol. Rev. 40, 87.

Perez, D.M., M.T. Piascik and R.M. Graham, 1991, Solution-phase library screening for the identification of rare clones: isolation of and α_{1D}-adrenergic receptor cDNA, Mol. Pharmacol. 40, 876.

Perez, D.M., M.T. Piascik, N. Malik, R. Gaivin and R.M. Graham, 1994, Cloning, expression, and tissue distribution of the rat homolog of the bovine α_{1C}-adrenergic receptor provide evidence for its classification as the α_{1A} subtype, Mol. Pharmacol. 46, 823.

Piascik, M.T., M.S. Smith, E.E. Soltis and D.M. Perez, 1994, Identification of the mRNA for the novel α_{1D}-adrenoceptor and two other α_1-adrenoceptors in vascular smooth muscle, Mol. Pharmacol. 46, 30.

Schwinn, D.A. and J.W. Lomasney, 1992, Pharmacologic characterization of cloned α_1-adrenoceptor subtypes: selective antagonists suggests the existence of a fourth subtype, Eur. J. Pharmacol. 227, 433.

Silver, P.J., W.R. Cumiskey and A.L. Harris, 1992, Vascular protein kinase C in Wistar-Kyoto and spontaneously hypertensive rats, Eur. J. Pharmacol. 212, 143.

Suzuki, S., Y. Takata, S. Kubota, S. Ozaki and H. Kato, 1994, Characterization of the alpha-1 adrenoceptors in the mesenteric vasculature from deoxycorticosterone-salt hypertensive rats: studies on vasoconstriction, radioligand binding and postreceptor events, J. Pharmacol. Exp. Ther. 268, 576.

Thorin-Trescases, N., C.A. Hamilton, E. Jardine and J.L. Reid, 1994, Signal transduction mechanisms of the vasoconstriction in hypertension, Eur. J. Pharmacol. Mol. Pharmacol. 268, 199.

Tsujimoto, G., A. Hirasawa, K. Kimura, N. Ishizuka and K. Takeda, 1994, The α_{1C}-adrenoceptor mediates contraction in the rat tail artery, Can. J. Physiol. Pharmacol. 72 (Suppl. 1) P1.13.76.

Turla, M.B. and R.C. Webb, 1987, Enhanced vascular reactivity to protein kinase C activators in genetically hypertensive rats, Hypertension 9, III-150.

Turla, M.B. and R.C. Webb, 1990, Augmented phosphoinositide metabolism in aortas from genetically hypertensive rats, Am. J. Physiol. 258, H-173.

Watson, S. and D. Girdlstone, 1995, Receptor and ion channel nomenclature supplement, Trends Pharmacol. Sci. 1.