ELSEVIER

European Journal of Pharmacology 322 (1997) 225–227

ejp

Short communication

α_{1D}- and α_{1A}-adrenoceptors mediate contraction in rat renal artery

Rafael Villalobos-Molina *, J. Javier López-Guerrero, Maximiliano Ibarra

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

Received 20 December 1996; revised 27 January 1997; accepted 4 February 1997

Abstract

To investigate the α_1-adrenoceptor subtype(s) mediating contraction in rat renal artery, we have compared the effect of the α_1-adrenoceptor antagonists, 5-methylurapidil, BMY 7378 (8-(2-(4-(2-methoxyphenyl)-1-piperazinyl) ethyl) 8-azaspiro (4.5) decane-7,9-dione 2HCl) and chloroethylclonidine on functional responses to noradrenaline. A clear blockade by chloroethylclonidine (10^{-4} M) of noradrenaline-induced contraction was observed and, along with this effect, pK_B values of 9.12 and 8.40 for BMY 7378 and 9.75 and 10.06 for 5-methylurapidil were obtained, indicating that the renal artery expresses the α_{1D}-adrenoceptor subtype as the one involved in contraction and not only the α_{1A} subtype as has been reported. © 1997 Elsevier Science B.V.

Keywords: α_{1D}-Adrenoceptor; Renal artery; Contraction; BMY 7378; Chloroethylclonidine; 5-Methylurapidil

1. Introduction

α_1-Adrenoceptors have been classified into three subtypes named α_{1A}, α_{1B} and α_{1D}, based on pharmacological and molecular biology criteria (Hieble et al., 1995). These α_1-adrenoceptors are inactivated by chloroethylclonidine with the following sensitivity: $\alpha_{1B} > \alpha_{1D} \gg \alpha_{1A}$ (Perez et al., 1994). The recent discovery of BMY 7378 (8-(2-(4-(2-methoxyphenyl)-1-piperazinyl) ethyl)-8-azaspiro (4.5) decane-7,9-dione 2HCl) as a highly selective antagonist of the α_{1D}-adrenoceptor subtype (Goetz et al., 1995) has made it the pharmacological agent to use in order to discriminate among the α_1-adrenoceptor subtypes present in different tissues. In this regard, rat aorta is now known to express the α_{1D}-adrenoceptor subtype as the one responsible for regulation of contraction in this artery (Goetz et al., 1995; Kenny et al., 1995; Piascik et al., 1995; Villalobos-Molina and Ibarra, 1996) and not the α_{1B}-subtype as was originally reported (Han et al., 1990). In the same line of thought, the aim of the experiments described in this report was to re-evaluate the functional α_1-adrenoceptor subtype(s) present in renal artery since it has been reported to express the α_{1A} subtype, based on its insensitivity to the alkylating agent, chloroethylclonidine, its sensitivity to Ca^{2+} channel blockers and the antagonism of 5-methylurapidil (Han et al., 1990; Piascik et al., 1994, 1995). However, the presence of the mRNA for the α_{1D}-adrenoceptor subtype has also been reported for this artery (Piascik et al., 1994, 1995).

2. Materials and methods

2.1. Experimental procedure

Male Wistar rats 2–3 months of age and fed ad libitum were used. The general procedure has been described elsewhere (Villalobos-Molina and Ibarra, 1996). Renal arterial rings were subjected to an initial optimal tension of 2 g as done in preliminary experiments using increments in initial tension, to reach the optimal tension. The tissue was challenged with noradrenaline (10^{-7} M) in the presence of 10^{-7} M propranolol and 10^{-7} M rauwolscine to block β- and α_2-adrenoceptors, respectively, and washed every 30 min for 2 h. Reproducible cumulative concentration-response curves to noradrenaline (10^{-9} to 10^{-6} M) were then obtained. The arteries were incubated with chloroethylclonidine (10^{-4} M) for 30 min, then thoroughly washed. In the case of BMY 7378 or 5-methylurapidil, incubation was for 30 min prior to and during stimulation with the agonist. In α_1-adrenoceptor protection experiments, phentolamine (10^{-6} M) was present 15 min before and during chloroethylclonidine incubation, then extensively washed

* Corresponding author. Tel.: (52-5) 675-9341; Fax: (52-5) 675-9168.

PII S0014-2999(97)00095-2

out. In order to avoid fatigue of the arterial preparation, a 60-min recovery period was allowed between noradrenaline curves, and to rule out any change in tissue sensitivity to the agonist, due to the long duration of these experiments, parallel assays were done, i.e., with control arteries in the presence of the agonist and arteries in the presence of the agonist plus the antagonist.

Endothelium-denuded arteries were used in these experiments, in order to avoid possible involvement of endothelium-derived factors in the mechanical response. pA_2 and Schild slopes were obtained as described by Arunlakshana and Schild (1959); pK_B values were calculated as described by Furchgott (1972). Statistical analysis was done using the parallelism test (Tallarida and Murray, 1981) and Student's *t*-test.

2.2. Drugs

Noradrenaline, chloroethylclonidine, phentolamine, BMY 7378 and 5-methylurapidil were from Research Biochemicals International (Natick, MA, USA); all other reagents were of analytical grade from local sources.

3. Results

As can be observed in Fig. 1, noradrenaline elicited a concentration-dependent response in renal artery, with a maximal contraction (1.17 ± 0.08 g) at 10^{-5} M of the agonist with an apparent pD_2 ($-\log EC_{50}$) of 7.5. Incubation with chloroethylclonidine (10^{-4} M) produced a small increase in contraction (0.1 ± 0.06 g) that was persistent after washout, displaced to the right the contractile response to noradrenaline (apparent pD_2 of 5.8) and depressed the maximal effect (0.4 ± 0.05 g). α_1-Adrenoceptor protection experiments showed that phentolamine (10^{-6} M) prevented chloroethylclonidine-induced alkylation of the α_1-adrenoceptor(s) present in this artery; however, the noradrenaline-elicited contraction was shifted to the right, with an apparent pD_2 of 6.3 but the maximal effect (0.96 ± 0.22 g, Fig. 1) was reached. When the competitive antagonist, BMY 7378, was used rightward shifts were obtained, with changes in the pD_2 but not in the maximal contraction in response to noradrenaline; however, the parallelism test showed statistical significance ($P < 0.05$) when 3.1×10^{-8} M of the antagonist was used (Fig. 2). The same pattern was observed with 5-methylurapidil but in this case the parallelism test showed a significant difference ($P < 0.05$), when 10^{-8} M of the antagonist was used (not shown). Linear transformation of these curves gave pK_B values of 9.12 ± 0.13 at 3.1 nM, 8.40 ± 0.13 at 310 nM and a Schild slope of -0.55 (significantly different from 1, $P < 0.01$) for BMY 7378 and a pA_2 of 9.42 ± 0.22; pK_B of 9.75 ± 0.04 at 1 nM, 10.06 ± 0.25 at 10 nM, with a slope of -1.32 (not different from 1) for 5-methylurapidil. In a different set of experiments, BMY 7378 (3.1×10^{-9} to 10^{-6} M) antagonized methoxamine-elicited contraction in the renal artery, with a pK_B of 8.30 ± 0.13 at 3.1 nM, 7.07 ± 0.18 at 1 μM, and a slope of -0.53.

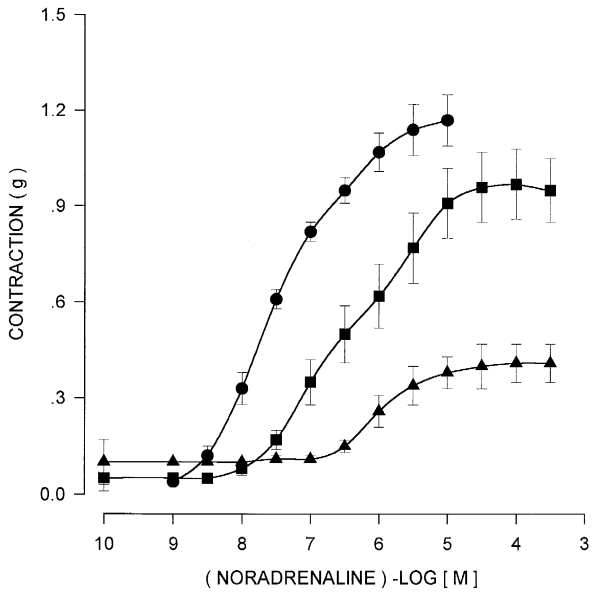

Fig. 1. Effect of chloroethylclonidine and phentolamine prior to chloroethylclonidine on noradrenaline-induced contraction in renal artery. Arterial rings were incubated for 30 min with chloroethylclonidine (10^{-4} M), or for 15 min with phentolamine (10^{-6} M) prior to chloroethylclonidine, then extensively washed out and exposed to noradrenaline. Results represent the means ± S.E.M. for 6 different preparations. Control (●), chloroethylclonidine (▲), phentolamine prior to chloroethylclonidine (■). Experiments were done in the presence of propranolol (10^{-7} M) and rauwolscine (10^{-7} M), β- and α_2-adrenoceptor antagonists, respectively.

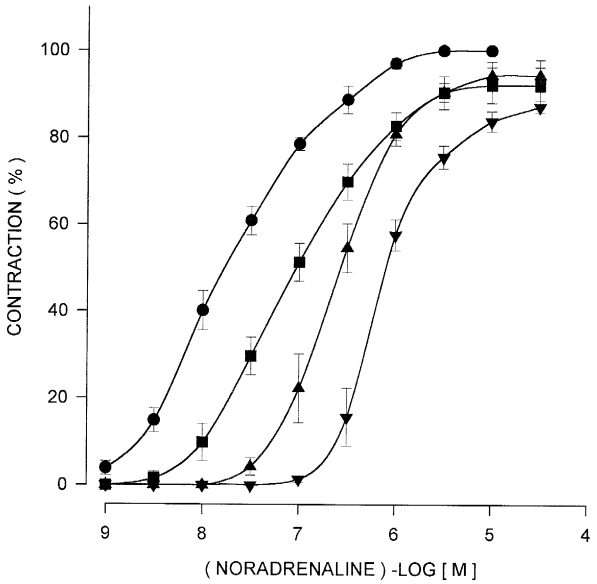

Fig. 2. Effect of BMY 7378 on noradrenaline-induced contraction in renal artery. Arterial rings were incubated in the absence (●), or the presence of 3.1×10^{-9} M (■); 3.1×10^{-8} M (▲) or 3.1×10^{-7} M (▼) of BMY 7378, then were exposed to noradrenaline. Results represent the means ± S.E.M. for 6 different preparations.

4. Discussion

Recent studies measuring mRNA demonstrated that rat renal artery expresses α_{1D}-adrenoceptors (Piascik et al., 1994, 1995; Guarino et al., 1996). The renal vessel contains an amount of mRNA (for the α_{1D}-adrenoceptor) comparable to that of the aorta (Piascik et al., 1995); however, these and other authors have reported that this artery expresses the α_{1A}-adrenoceptor subtype as the predominant regulatory adrenoceptor for contraction, based on its resistance to the alkylating effect of chloroethylclonidine (Han et al., 1990; Piascik et al., 1994), and to the weak antagonist effect of BMY 7378 (Piascik et al., 1995). In contrast with these reports, our results show clearly that the interaction of chloroethylclonidine with α_1-adrenoceptors, (a) produced a discrete contraction in the renal artery, probably due to a postreceptor effect (similar actions of chloroethylclonidine have been observed in rat aorta (Muramatsu et al., 1990; O'Rourke et al., 1995)); (b) shifted to the right the noradrenaline contractile effect, which was prevented in part by protecting the α_1-adrenoceptors with phentolamine (similar results have been reported by O'Rourke et al. (1995) who used noradrenaline or prazosin to protect the receptor); and (c) depressed the maximal effect. The contraction obtained after chloroethylclonidine treatment could be explained if the α_{1A}-adrenoceptors present in the tissue (Piascik et al., 1994; Guarino et al., 1996), but not inactivated by the alkylating agent, were responding to high noradrenaline concentrations or if there was incomplete inactivation of the α_{1D}-adrenoceptor population in the artery. This result indicates that the α_1-adrenoceptor present in the renal artery is of either the α_{1D} or the α_{1B} subtype; however, along with those effects, BMY 7378 at 3.1 nM showed a high pK_B value (9.12) which is similar to that reported in other arteries expressing the α_{1D}-adrenoceptor (Goetz et al., 1995; Piascik et al., 1995; Villalobos-Molina and Ibarra, 1996) as well as in human and rat cloned α_{1d}-adrenoceptor (Goetz et al., 1995), and a Schild slope of -0.55 which is similar to that of 0.65 found by Piascik et al. (1995). These findings are consistent with the suggestion that noradrenaline activates more than one α_1-adrenoceptor type present in this artery (Kenny et al., 1995). In addition, the pK_B values for 5-methylurapidil were also high (9.75–10.06) and the Schild slope was not different from 1 (-1.32), excluding the presence of the α_{1B}-adrenoceptor as a mediator and leaving the α_{1A} subtype as the other adrenoceptor involved, yet playing a minor role in contraction in the renal artery, even though the mRNA for this subtype is 30 times more abundant than that for the α_{1D} subtype (Guarino et al., 1996). In the study reported by Piascik et al. (1995), the authors found a weak BMY 7378 antagonism to phenylephrine-induced contraction in the renal artery. Furthermore, in the present study we found that another selective α_1-adrenoceptor agonist, methoxamine, was antagonized by BMY 7378 with lower affinity (pK_B, 8.30 at 3.1 nM and 7.07 at 1 μM of the antagonist and Schild slope of -0.53), suggesting that there is a heterogeneous receptor population. In support of this suggestion there appear to be subtypes of the α_{1D}-adrenoceptor (Hussain and Marshall, 1996). In conclusion, our results indicate that the α_{1D}-adrenoceptor plays a predominant role in contraction of the renal artery and suggest that rat vasculature expresses this subtype.

Acknowledgements

This work was supported in part by grant 5110-M9406 from CONACYT.

References

Arunlakshana, O., Schild, H.O., 1959. Some quantitative uses of drug antagonists. Br. J. Pharmacol. 14, 48–58.

Furchgott, R.F., 1972. The classification of adrenoceptors (adrenergic receptors). An evaluation from the standpoint of receptor theory. In: Blaschko, H., Muscholl, E. (Eds.), Catecholamines, Handbook of Experimental Pharmacology, Vol. 33. Springer, Berlin, pp. 283–335.

Goetz, A.S., King, H.K., Ward, S.D.C., True, T.A., Rimele, T.J., Saussy Jr., D.L., 1995. BMY 7378 is a selective antagonist of the D subtype of α_1-adrenoceptors. Eur. J. Pharmacol. 272, R5–R6.

Guarino, R.D., Perez, D.M., Piascik, M.T., 1996. Recent advances in the molecular pharmacology of the α_1-adrenergic receptors. Cell Signal. 8, 323–333.

Han, C., Li, J., Minneman, K.P., 1990. Subtypes of α_1-adrenoceptors in rat blood vessels. Eur. J. Pharmacol. 190, 97–104.

Hieble, J.P., Bylund, D.B., Clarke, D.E., Eikenburg, D.C., Langer, S.Z., Lefkowitz, R.J., Minneman, K.P., Ruffolo Jr., R.R., 1995. International Union of Pharmacology X. recommendation for nomenclature of α_1-adrenoceptors: consensus update. Pharmacol. Rev. 47, 267–270.

Hussain, M., Marshall, I., 1996. Subtypes of α_{1D}-adrenoceptors in rat blood vessels?, VIII International Catecholamine Symposium, Abstract, D V.

Kenny, B.A., Chalmers, D.H., Philpott, P.C., Naylor, A.M., 1995. Characterization of an α_{1D}-adrenoceptor mediating the contractile response of rat aorta to noradrenaline. Br. J. Pharmacol. 115, 981–986.

Muramatsu, I., Ohmura, T., Kigoshi, S., Hashimoto, S., Oshita, M., 1990. Pharmacological subclassification of α_1-adrenoceptors in vascular smooth muscle. Br. J. Pharmacol. 99, 197–201.

O'Rourke, M., Kearns, S., Docherty, J.R., 1995. Investigation of the actions of chloroethylclonidine in rat aorta. Br. J. Pharmacol. 115, 1399–1406.

Perez, D.M., Piascik, M.T., Malik, N., Gaivin, R., Graham, R.M., 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, 823–831.

Piascik, M.T., Smith, M.S., Soltis, E.E., Perez, D.M., 1994. Identification of the mRNA for the novel α_{1D}-adrenoceptor and two other α_1-adrenoceptors in vascular smooth muscle. Mol. Pharmacol. 46, 30–40.

Piascik, M.T., Guarino, R.D., Smith, M.S., Soltis, E.E., Saussy Jr., D.L., Perez, D.M., 1995. The specific contribution of the novel alpha-1D adrenoceptor to the contraction of vascular smooth muscle. J. Pharmacol. Exp. Ther. 275, 1583–1589.

Tallarida, R.J., Murray, R.B., 1981. Manual of Pharmacological Calculations with Computer Programs. Springer, New York, NY.

Villalobos-Molina, R., Ibarra, M., 1996. α_1-Adrenoceptors mediating contraction in arteries of normotensive and spontaneously hypertensive rats are of the α_{1D}- or α_{1A} subtypes. Eur. J. Pharmacol. 298, 257–263.