J. Pharm. Pharmacol. 1993, 45: 907–909
Communicated January 14, 1993

COMMUNICATIONS

Effects of age on α_1-adrenoceptor subtypes in the heart ventricular muscle of the rat

SILVIA GASCÓN, MARA DIERSSEN, *FREDERIC MÀRMOL, NURIA M. VIVAS, ALBERT BADIA, *Departament de Farmacologia i Psiquiatria, Divisió de Farmacologia, Universitat Autònoma de Barcelona, 08193 Bellaterra, Barcelona, Spain, and *Unitat de Farmacologia, Facultat de Medicina, Universitat de Barcelona, Spain*

Abstract—The effects of ageing on α_1-adrenoceptor subtypes have been examined in heart ventricular muscle of young (2–3 months) and middle-aged (18 months) Sprague-Dawley rats. Radioligand binding studies with [^{3}H]prazosin revealed an age-related loss of binding sites (B_{max} 56·7 ± 1·93 fmol (mg protein)$^{-1}$ age 2 months vs 31·7 ± 2·45 fmol (mg protein)$^{-1}$ age 18 months) not followed by changes in the dissociation constant value (K_d 0·16 ± 0·03 nM age 2 months and 0·10 ± 0·03 nM age 18 months). Competition curves with WB 4101 showed two distinct sites with different affinities, the proportion of sites with high affinity being similar for both age groups (22·2 ± 1·89% vs 17·8 ± 1·96% for animals aged 2 and 18 months, respectively). Agonist displacement curves of [^{3}H]prazosin indicate the existence of two different affinity sites for the agonist, that are maintained regardless of the ageing process (R_{high} = 16·2 ± 1·54% and R_{low} = 83·8 ± 1·89% in rats aged 2 months and R_{high} = 16·3 ± 3·23% and R_{low} = 83·7 ± 3·95% in rats aged 18 months). The fractional inactivation of α_1-adrenoceptors by chloroethylclonidine resulted in a loss of [^{3}H]prazosin specific binding, and a percentage of 22·5 ± 0·95 and 22·6 ± 4·2 of remaining binding sites for the groups of 2 and 18 months of age, respectively. The percentage of chloroethylclonidine-insensitive [^{3}H]prazosin binding sites was similar to those with high affinity for WB 4101. The present study confirms a decline of α_1-adrenoceptors with increasing age and reveals that the equilibrium of the expression of the two existing subpopulations of the receptor is maintained during ageing.

The age-related decline in the adrenergic nervous system control of cardiac function has been clearly established (Roberts & Tumer 1987). However, the mechanism responsible for this impairment is far from clear. Although an age-related impairment in α_1-adrenoceptor cardiac responsiveness has been reported to occur (Docherty & Hyland 1986), few studies have assessed the changes in α_1-adrenoceptors during maturation and senescence. There is some evidence for a decrease in ventricular α_1-adrenoceptor concentration measured by specific binding of [^{3}H]prazosin, during ageing (Partilla et al 1982; Schaffer & Williams 1986). However, no data show the pattern of loss of the different subtypes of α_1-adrenoceptors with ageing in the heart. Several lines of evidence from binding and functional studies suggest that α_1-adrenoceptors may be divided into at least two pharmacologically distinct subtypes (Hieble et al 1986; Han et al 1987a; Gross et al 1988a). Using [^{3}H]prazosin and [^{3}H]WB4101 as α_1-adrenoceptor ligands, Morrow & Creese (1986) could demonstrate two separate populations of α_1-adrenoceptor binding sites in the rat central nervous system. The binding sites with high affinity for WB4101 were designated α_{1A} and the low affinity site, preferentially blocked by chloroethylclonidine, an irreversible α_1-adrenoceptor antagonist, α_{1B} (Minneman et al 1988). These two subtypes could also be identified in the rat ventricular muscle (Han et al 1987b; Hanft & Gross 1989) and it has been demonstrated that thyroid hormone shows different effects on the α_1-adrenoceptor subtypes in the heart (Gross & Hanft 1988).

The aim of this study was firstly to determine the density of α_1-adrenoceptors present in membrane fractions of ventricular myocardium isolated from young and mature rats and secondly, to assess whether differences in the expression of the two distinct α_1-adrenoceptor subtypes are detectable with increasing age.

Correspondence: A. Badia, Departament de Farmacologia i Psiquiatria, Divisió de Farmacologia, Universitat Autònoma de Barcelona, 08193 Bellaterra, Barcelona, Spain.

Materials and methods

The hearts of the rats were removed and dissected free of pericardium, atria, fat and large vessels. Ventricles were weighed and placed in 10 vol ice-cold incubation buffer (50 mM Tris-HCl, pH 7·5). Crude membrane homogenates were prepared according to the method of Baker et al (1980) with minor modifications. Briefly, the hearts were minced and homogenized in a pre-cooled polytron tissue homogenizer (2 × 10 s bursts). The homogenate was diluted with an equal volume of ice-cold 1·2 M KCl solution and left on ice, with stirring for 15 min. The suspension was filtered through 4 layers of gauze before centrifugation at 50 000 *g* for 10 min at 4°C. Pellets were resuspended with a glass-Teflon homogenizer. This procedure was repeated twice. The final pellet was resuspended in 30 vol incubation buffer (to adjust the protein content to approximately 400 μg). Samples (0·8 mL) of the membrane preparation were incubated in a total volume of 1 mL with [^{3}H]prazosin (0·025–2 nM in saturation binding studies and 0·25 nM in competition experiments) for 45 min at 25°C. Competition curves with WB 4101 were carried out using 14 concentrations (range 0·01 nM–100 μM) of the antagonist, and displacement of specific [^{3}H]prazosin binding by noradrenaline was performed using 14 concentrations (range 1 nM–10 mM) of the agonist. In experiments involving the competition of noradrenaline for the specific [^{3}H]prazosin binding sites, 0·1% ascorbic acid was included in the incubation medium to prevent oxidation of the catecholamine. For the differential inactivation of α_1-adrenoceptors, membranes of rat ventricle muscle were incubated for 30 min at 37°C with 100 μM chloroethylclonidine, followed by extensive washing to remove any unbound chloroethylclonidine. Incubation was terminated by rapid vacuum filtration (Brandel M24R Cell Harvester) through Whatman GF/C filters. The tubes were rinsed four times with 5 mL ice-cold incubation buffer which was subsequently filtered. Phentolamine (10 μM) was used to define nonspecific binding which usually amounted to 20–30% of total binding (at [^{3}H]prazosin concentrations close to the K_d value). The protein content was determined by the method of Lowry et al (1951). The radioactivity retained on the filters was determined by liquid scintillation spectrometry with a counting efficiency of 60%. Data derived from radioligand studies were analysed by the weighted least-squares iterative curve fitting programme, LIGAND (Munson & Rodbard 1980). Competition data were first fitted to a one- and a two-site model. The statistical differences between one- or two-site models were determined by comparing the residual variance between the actual and predicted data points, and F-test analysis was used to decide whether a model of one or two binding site fit was more appropriate. When the *P* value was less than 0·05 the two-site model was considered a significantly better fit. Assays were carried out in duplicate and data are expressed as the means ± s.e.m. of at least five independent experiments. The statistical significance of differences between mean data was evaluated by Student's *t*-test. The level of significance was set at $P < 0.05$.

Results

Saturation experiments. Crude particulate ventricular membrane preparations prepared from six hearts of 2- and 18-month-old rats were incubated with [^{3}H]prazosin. The binding of [^{3}H]prazosin to rat ventricular homogenates was monophasic and clearly saturable, with an age-related loss of binding sites revealed by a decrease in the maximal number of binding sites (B_{max}) that was of 56·7 ± 1·93 fmol (mg protein)$^{-1}$ in 2-month-old animals in comparison with a binding capacity of 31·7 ± 2·45 fmol (mg protein)$^{-1}$ in the 18-month-old group ($P < 0·001$). The Scatchard plot of the data was linear in both groups with a K_d value of 0·16 ± 0·03 and 0·10 ± 0·03 nM in rats of 2- and 18-months of age, respectively, indicating that the loss of receptor density in the aged animals was not followed by changes in K_d for [^{3}H]prazosin.

Competition curves for WB4101. The competition curves of the antagonist WB4101 for the specific [^{3}H]prazosin binding to ventricle membranes prepared from young and aged animals are shown in Fig. 1. The pseudo-Hill coefficients were smaller than unity in both groups of animals. Computerized analysis of the competition curves revealed that WB 4101 binds to two distinct sites with different affinities in hearts of 2-month-old ($pK_{high} = 9·33 \pm 0·34$; $pK_{low} = 7·41 \pm 0·07$) and 18 month-old rats ($pK_{high} = 9·42 \pm 0·13$; $pK_{low} = 7·59 \pm 0·19$) (Fig. 1). The proportion of sites with high affinity for WB4101 was similar for both groups ($R_{high} = 22·2 \pm 1·89\%$ vs $R_{high} = 17·8 \pm 1·96\%$ for animals aged 2 and 18 months, respectively).

Competition curves for noradrenaline. The α-adrenoceptor agonist noradrenaline displayed a biphasic competition curve for [^{3}H]prazosin binding sites, with pseudo-Hill coefficients less than 1·0 in both young and mature rats (Fig. 2), indicating the existence of two different affinity sites for the agonist, that are maintained regardless of the ageing process. Noradrenaline bound to a small fraction of sites ($R_{high} = 16·2 \pm 1·54\%$) with high affinity ($pK_{high} = 6·9 \pm 0·08\%$) and to a greater fraction of sites ($R_{low} = 83·8 \pm 1·89\%$) with low affinity ($pK_{low} = 4·67 \pm 0·02\%$) in the 2 month-old animals. This was also the case for the 18 month-old group in which noradrenaline again bound to a small fraction of sites ($R_{high} = 16·3 \pm 3·23\%$) with high affinity ($pK_{high} = 6·79 \pm 0·14\%$) and to a greater fraction of sites ($R_{low} = 83·7 \pm 3·95\%$) with low affinity ($pK_{low} = 4·35 \pm 0·06\%$).

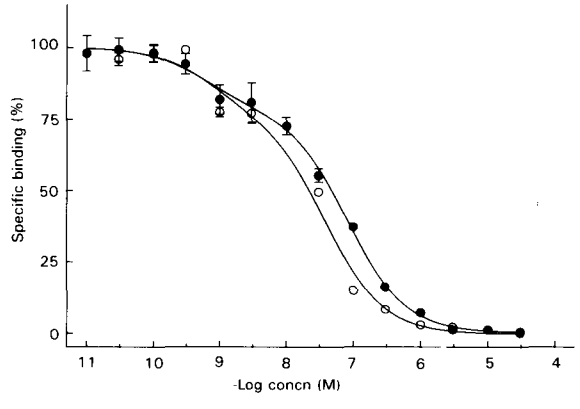

FIG. 1. Competition curves of the antagonist WB4101 for the specific [^{3}H]prazosin binding to heart ventricle membranes prepared from young (●) and middle-aged (○) rats. Vertical bars show s.e.m. Each point is the mean of at least five experiments in duplicate.

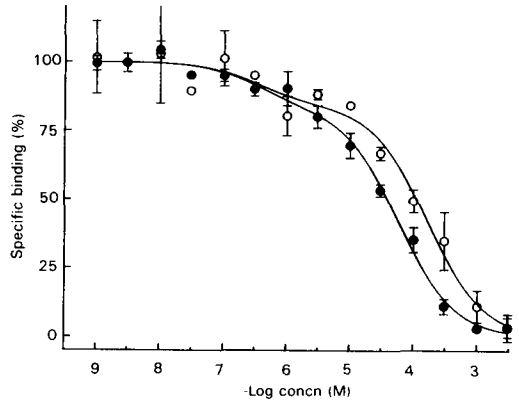

FIG. 2. Displacement of specific [^{3}H]prazosin binding to heart ventricle membranes prepared from young (●) and middle-aged (○) rats by noradrenaline. Vertical bars show s.e.m. Each point is at least the mean of five experiments in duplicate.

Table 1. Differential inactivation of α_1-adrenoceptors by means of 100 μM chloroethylclonidine in ventricle membranes of 2- and 18-month-old rats.

	B_{max} (fmol (mg protein)$^{-1}$)	
Rats	Control	With chloroethylclonidine
2 months	56·7 ± 1·33	11·8 ± 0·05***
18 months	131·7 ± 2·45	7·18 ± 1·34***

Each value is the mean of at least five experiments ± s.e.m.
*** $P < 0·001$.

Fractional inactivation of α_1-adrenoceptors by chloroethylclonidine. The heterogeneity of the α_1-adrenoceptor population in rat heart and its possible alteration with age was again analysed by studying the differential inactivation of the α_1-adrenoceptor population labelled with [^{3}H]prazosin by means of chloroethylclonidine. Preincubation of ventricle membranes with 100 μM chloroethylclonidine (Michel et al 1990) for 30 min resulted in a loss of [^{3}H]prazosin specific binding. The maximal number of binding sites remaining in ventricle membranes after treatment with chloroethylclonidine were 11·8 ± 0·5 and 7·18 ± 1·34 fmol (mg protein)$^{-1}$ in the groups of 2 and 18 months of age, respectively. These results reflect a proportional percentage of 22·5 ± 0·95% remaining binding sites for the 2-month-old rats and 22·6 ± 4·2% remaining binding sites for the 18-month-old rats, results that agree with the proportion of sites displaying high affinity for WB4101 in competition experiments (Table 1).

Discussion

The present experiments confirm and extend previous investigations suggesting a loss of cardiac α_1-adrenoceptors with the ageing process (Partilla et al 1982; Schaffer & Williams 1986). Functional and radioligand binding data have demonstrated that α_1-adrenoceptors can be subdivided into α_{1A}- and α_{1B}-subtypes in several tissues including cardiac muscle (Han et al 1987a; Minneman et al 1988; Hanft & Gross 1989). The α_{1A}-subtype has a relatively high affinity for the competitive α_1-adrenoceptor antagonists WB4101, 5-methyl- urapidil and phentolamine (Morrow & Creese 1986; Hanft & Gross 1989), is not inactivated by the alkylating agent chloroethylclonidine and is coupled to voltage-dependent Ca^{2+} channels (Han et al 1987a;

Minneman et al 1988). In contrast, the α_{1B}-receptor has a lower affinity for these antagonists, is inactivated by chloroethylclonidine and is coupled to inositol phosphate generation (Michel et al 1990). Our study reveals that the loss of [^{3}H]prazosin binding sites is not dependent on differential changes in the proportion of the two existing α_1-adrenoceptor subtypes. Thus, in the present study, competition of WB 4101 for [^{3}H]prazosin specific binding demonstrates that both the affinity and ratio of the two α_1-adrenoceptor subtypes are similar in young and middle-aged rats. In addition, pretreatment of membranes with 100 μM of the irreversible alkylating α_{1B}-adrenoceptor agent chloroethylclonidine revealed the same proportion of chloroethylclonidine-insensitive α_{1A}-adrenoceptors in both groups of animals. The age-related changes of α_1-adrenoceptors observed in the present experiments affect concentration of binding sites, with no changes in binding affinity for [^{3}H]prazosin, a pattern similar to that displayed by other neurotransmitter receptor systems with increasing age (Roth 1986).

The possible changes in α_1-adrenoceptor coupling to the G-protein were checked by means of noradrenaline. These experiments demonstrated the existence of two different affinity states for the agonist, that are maintained regardless of the ageing process. Noradrenaline bound to a similar fraction of high and low affinity sites both in young and middle-aged rats. Several observations suggest that the two affinity states for agonists are distinct from the α_{1A}/α_{1B}-adrenoceptor subtypes. Thus, it has been demonstrated that agonists, such as adrenaline and noradrenaline, can bind to α_1-adrenoceptors with two affinity constants in various tissues (Ernsberger & U'Prichard 1987; Gross et al 1988b). Moreover, the addition of guanosine 5'-triphosphate or its non-hydrolysable analogues can convert high-affinity binding sites of α_1-adrenoceptors for agonists into low affinity state without affecting the antagonist binding (Gross et al 1988b). Our displacement studies with noradrenaline in aged animals contrast with those obtained in bovine aorta where adrenaline displacement studies revealed both high- and low-affinity binding in membranes from young animals, whereas preparations from adult animals exhibited only a single class of low-affinity sites (Jagadeesh et al 1990).

The major finding in our study is that the loss of α_1-adrenoceptors with age does not involve differential losses of the two subpopulations of α_1-adrenoceptor binding sites present in cardiac membranes. To date there are no studies dealing with the pattern of loss of the two distinct α_1-adrenoceptor subtypes with the ageing process. The present study confirms the decline of α_1-adrenoceptors with age and reveals that the equilibrium in the expression of the two existing subpopulations of the receptor is maintained during ageing with a similar ratio of loss, demonstrating that the two subpopulations do not change differentially in the aged rats.

This study has been supported by grants from DGICYT PM88-0110 and CIRIT AR90-3.674. Mara Dierssen was a recipient of a grant from Fundación Rich, Spain.

References

Baker, S. P., Boyd, H. M., Potter, L. T. (1980) Effect of propranolol on β-adrenoceptors in rat hearts. Br. J. Pharmacol. 68: 8–10

Docherty, J. R., Hyland, L. (1986) α-Adrenoceptor responsiveness in the aged rat. Eur. J. Pharmacol. 126: 75–80

Ernsberger, P., U'Prichard, D. C. (1987) Modulation of agonist and antagonist interactions at kidney α_1-adrenoceptors by nucleotides and metal ions. Eur. J. Pharmacol. 133: 165–176

Gross, G., Hanft, G. (1988) α_1-Adrenoceptor subtypes in different rat tissues effect of hypo- and hyperthyroidism. Naunyn Schmiedebergs Arch. Pharmacol. 338: R64

Gross, G., Hanft, G., Rugevics, C. (1988a) 5-Methyl-urapidil discriminates between subtypes of the α_1 adrenoceptor. Eur. J. Pharmacol. 151: 333–335

Gross, G., Hanft, G., Rugevics, C. (1988b) α_1-Adrenoceptors of rat myocardium: comparison of agonist binding and positive inotropic response. Naunyn Schmiedebergs Arch. Pharmacol. 338: 582–588

Han, C., Abel, P. W., Minneman, K. P. (1987a) α_1-Adrenoceptor subtypes linked to different mechanisms for increasing intracellular Ca^{2+} in smooth muscle. Nature 329: 333–335

Han, C., Abel, P. W., Minneman, K. P. (1987b) Heterogeneity of α_1-adrenergic receptors revealed by chloroethylclonidine. Mol. Pharmacol. 32: 505–510

Hanft, G., Gross, G. (1989) Subclassification of α_1-adrenoceptor recognition sites by urapidil derivatives and other selective antagonists. Br. J. Pharmacol. 97: 691–700

Hieble, J. P., DeMarinis, R. M., Matthews, W. D. (1986) Evidence for and against heterogeneity of α_1 adrenoceptors. Life Sci. 38: 1339–1350

Jagadeesh, G., Tian, W. N., Gupta, S., Deth, R. C. (1990) Developmental changes in α_1-adrenoceptor coupling to G-protein in bovine aorta. Eur. J. Pharmacol. 189: 11–21

Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J. (1951) Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265–275

Michel, M. C., Hanft, G., Gross, G. (1990) α_{1B}- but not α_{1A}-adrenoceptors mediate inositol phosphate generation. Naunyn Schmiedebergs Arch. Pharmacol. 341: 385–387

Minneman, K. P., Han, C., Abel, P. W. (1988) Comparison of α_1-adrenergic receptor subtypes by chloroethylclonidine and WB 4101. J. Pharmacol. Exp. Ther. 33: 509–514

Morrow, A. L., Creese, I. (1986) Characterization of α_1 adrenergic receptor subtypes in rat brain: a revaluation of [^{3}H] WB 4104 and [^{3}H]prazosin binding. Mol. Pharmacol. 29: 321–330

Munson, P. V., Rodbard, D. (1980) A versatile computerized approach for characterization of ligand-binding systems. Anal. Biochem. 107: 220–228

Partilla, J. S., Hoopes, M. T., Ito, H., Dax, E. M., Roth, G. S. (1982) Loss of ventricular α_1-adrenergic receptors during aging. Life Sci. 31: 2507–2512

Roberts, J., Tumer, N. (1987) Age-related changes in autonomic function of catecholamines. In: Rothstein, M. (ed.) Review of Biological Research in Aging. Alan R. Liss, New York, pp 257–298

Roth, G. S. (1986) Effects of aging on mechanisms of α-adrenergic and dopaminergic action. Fed. Proc. 45: 60–64

Schaffer, W., Williams, R. S. (1986) Age-dependent changes in expression of alpha$_1$-adrenergic receptors in rat myocardium. Biochem. Biophys. Res. Commun. 138: 387–391