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α_1-Adrenoceptor responsiveness in the aging aorta

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Abstract

Previous studies from this laboratory have shown that aortic α_1-adrenoceptor-mediated responsiveness is altered during maturation and aging. This study examines the possibility that there is a change in the α_1-adrenoceptor subtypes in the aorta during maturation and aging. The apparent affinity of norepinephrine, as determined by partial receptor inactivation with the α_1-adrenoceptor antagonist phenoxybenzamine, was found to be higher in 1-month-old rats compared to 6- and 24-month-old rats. The α_{1B}-adrenoceptor subtype-selective antagonist chlorethylclonidine was used to examine possible heterogeneity in aortic α_1-adrenoceptors. The inhibitory effect of chlorethylclonidine on norepinephrine-stimulated contraction was greater in young animals compared to aged animals. Chlorethylclonidine blocked norepinephrine-stimulated inositol phosphate accumulation in 1-month-old aorta but it produced only partial inhibition in the 6- and 24-month-old aortas. The relatively non-selective α_1-adrenoceptor antagonists phenoxybenzamine (0.1 μM) and prazosin (0.1 μM) inhibited inositol phosphate accumulation and contractile responses in all ages. The complete block of α_1-adrenoceptor-mediated responses by chlorethylclonidine in younger animals shows that α_1-adrenoceptor-mediated responses are mediated by the chlorethylclonidine-sensitive α_1-adrenoceptor subtypes. The partial inhibition by chlorethylclonidine of α_1-adrenoceptor-mediated responses in 6- and 24-month-old animals indicates an increased role of an α_1-adrenoceptor subtype that is relatively insensitive to chlorethylclonidine.

Keywords: α_1-Adrenoceptor subtype; Aging; Aorta, rat

1. Introduction

Clinical and experimental evidence indicates a variety of alterations in cardiovascular function during maturation and aging (Docherty, 1990). Vascular responsiveness to several agonists, including noradrenaline, serotonin and angiotensin is reported to change during maturation or aging (Wanstall and O'Donnell, 1988, 1989; Docherty, 1988, 1990; Wakabayashi et al., 1990). Responses to α_1-adrenergic agonists have been reported to increase, decrease or not change with age (Carrier et al., 1979; Hynes and Duckles, 1987; Wanstall and O'Donnell, 1989; Docherty, 1990; Johnson and Wray, 1990). The discrepancies are probably attributable at least in part to differences in experimental design including the use of different animal models and different blood vessels. Previous studies from this laboratory showed a decreased potency of norepinephrine at eliciting a contractile response and an increased potency of norepinephrine at stimulating inositol phosphate accumulation in the rat aorta during maturation and aging (Gurdal et al., 1995).

Vascular α_1-adrenoceptors are not a homogenous population. Three subtypes have been cloned: α_{1B}, α_{1C} and α_{1D} (Cotecchia et al., 1988; Schwinn et al., 1990; Lomasney et al., 1991; Perez et al., 1991), and α_{1B}, α_{1C} and α_{1D}-adrenoceptor mRNAs have been detected in rat aorta (Ping and Faber, 1993; Piascik et al., 1994). Pharmacological studies indicate that there is a fourth subtype which has been designated the α_{1A}-adrenoceptor (Minneman, 1988), and it too is present in rat aorta (Han et al., 1990; Piascik et al., 1994).

α_{1A}-Adrenoceptors stimulate Ca^{2+} influx through voltage-dependent Ca^{2+} channels and α_{1A}-adrenoceptor-mediated stimulation of contraction and inositol phosphate accumulation are sensitive to Ca^{2+} channel blockers while α_{1B}-adrenoceptors increase intracellular Ca^{2+} through direct activation of phospholipase C and are less sensitive to Ca^{2+} channel blockers (Hanft and Gross, 1989; Han et al., 1987a, 1990; Wilson and Minneman, 1990). Previous results from this laboratory

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showed an increased inhibitory effect of the Ca^{2+} channel blocker nifedipine on α_1-adrenoceptor responsiveness during maturation and aging (Gurdal et al., 1995).

Differential sensitivity of α_1-adrenoceptor responses to the alkylating agent chlorethylclonidine has been used to distinguish the receptor subtypes. α_{1B}-Adrenoceptor-mediated responses are sensitive to chlorethylclonidine, while α_{1A}-adrenoceptor-mediated responses are resistant (Minneman, 1988; Han et al., 1987b, 1990; Hanft and Gross, 1989). The α_{1C}- and α_{1D}-adrenoceptors are intermediate in sensitivity to chlorethylclonidine (Schwinn et al., 1991; Minneman and Esbenshade, 1994). These studies examine the effect of chlorethylclonidine on norepinephrine-mediated contractile responses, and on norepinephrine-stimulated accumulation of inositol phosphates in the rat aorta during maturation and aging.

2. Materials and methods

2.1. Animals

Male Fischer 344 rats, 1, 6 and 24 months old, were obtained from Harlan Sprague Dawley, Indianapolis, IN, USA, where they are bred and maintained under the auspices of the National Institute on Aging. Upon receipt at this institution animals were maintained for 1–2 weeks under barrier conditions comparable to those under which they were raised.

2.2. Contraction

Rats were decapitated and the aorta was removed and placed in an ice-cold physiological salt solution (PSS) of the following composition (mM): NaCl, 120; KCl, 4.7; $MgCl_2$, 1.2; NaH_2PO_4, 1.0; $NaCO_3$, 25; $CaCl_2$, 1.8; glucose, 11; EDTA, 0.024. Vessels were cleaned of fat and connective tissue and cut into rings 3 mm wide. Aortic ring segments were mounted at 37°C in 15 ml organ baths using stainless steel hooks connected by fine gold chain at the bottom to a stationary glass rod attached to the bath and at the top to a Grass model FT.03 force-displacement transducer and bubbled continuously with 95% O_2/5% CO_2. Responses were recorded on a Grass model 7 polygraph. Rings were equilibrated for 1 h at an optimal resting tension of 1 g in 1-month-old aorta and 1.5 g in 6- and 24-month-old aorta. Concentration-response curves were determined by cumulatively increasing the concentration of agonist. Rings were then washed extensively by several changes of PSS over 1–2 h until tension stabilized at the precontraction level. In some experiments, concentration-response curves were determined before and after treatment of rings for 30 min with chlorethylclonidine (1–100 μM) or phenoxybenzamine (1–100 nM). After phenoxybenzamine or chlorethylclonidine treatment, aortic ring segments were washed for 60 min, then concentration-response curves for norepinephrine were repeated. Preliminary experiments have established that desmethylimipramine (0.1 μM) and propranolol (1 μM) have no effect on norepinephrine-stimulated concentration-response curves at any of the ages.

2.3. Calculation of receptor reserve and apparent affinity

To delineate the relationship between response and receptor occupation, the effects of phenoxybenzamine were examined. Cumulative concentration-response curves to norepinephrine were obtained before and after treatment of the rings for 30 min with phenoxybenzamine (1–100 nM). Data obtained from receptor inactivation studies were used to calculate apparent affinity and receptor reserve as described by Furchgott (1966). According to the law of mass action, the relationship between the agonist curves obtained before and after partial receptor inactivation is described by the equation

$$\frac{1}{[A]} = \frac{1}{q[A']} + \frac{1-q}{qK_d}$$

where $[A]$ and $[A']$ are equieffective concentrations of agonist before and after partial irreversible receptor inactivation, respectively, K_d is the equilibrium dissociation constant for the agonist and q represents the fraction of active receptors after partial receptor inactivation.

2.4. Inositol phosphate accumulation

The method used for measuring [^{3}H]inositol metabolism has been described previously (Kendall and Hill, 1990). Aortic rings were prepared as described above and then preincubated in oxygenated buffer containing (mM): NaCl, 122; KCl, 4.9; $MgCl_2$, 1.2; KH_2PO_4, 1.2; $NaCO_3$, 3.6; $CaCl_2$, 1.3; glucose, 11; Hepes, 30, (pH 7.4) at 37°C for 1 h. Subsequently, artery segments were incubated for 1.5 h in 20 μCi/ml of [^{3}H]*myo*-inositol (17 Ci/mmol, NEN) under the same conditions. Labeled artery segments were washed 4 times and placed in individual tubes containing buffer with 10 mM LiCl (total assay volume, 300 μl). In experiments using chlorethylclonidine and phenoxybenzamine, artery segments were exposed to chlorethylclonidine (100 μM) or phenoxybenzamine (0.1 μM) and aortic ring segments were washed for 60 min before the addition of agonists. In some experiments prazosin 0.1 μM was added 20 min before the addition of agonist. Incubation with agonist was for 60 min and

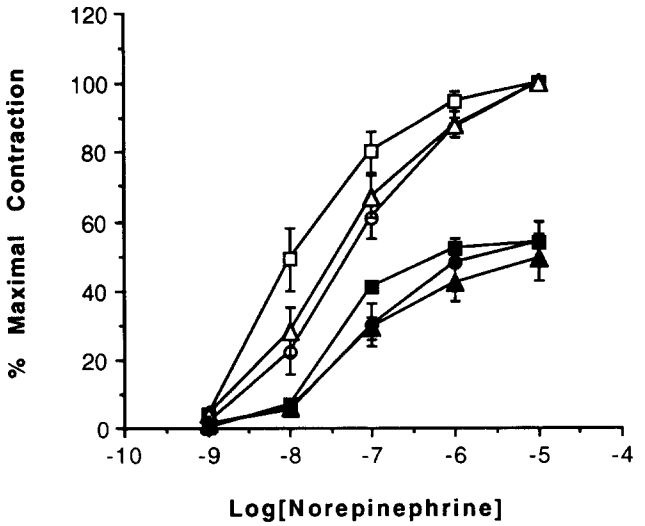

Fig. 1. Norepinephrine-stimulated contraction of aortic ring segments from 1- (□,■), 6- (○,●) and 24- (△,▲) month-old rats before and after phenoxybenzamine treatment (5 nM, 30 min), respectively. Data represent means and standard errors of determinations from 5–6 animals from each group. Two-way ANOVA (factors of treatment and concentration of agonist) indicates no significant difference in the effect of phenoxybenzamine on the concentration-response curves of the different ages.

was stopped by addition of 300 μl of ice-cold 15% trichloroacetic acid and then left on ice for 15 min. The tubes were centrifuged (1500 × *g*, 10 min) and aliquots (350 μl) of supernatant were then added to 125 μl of 10 mM EDTA in 1.5 ml microcentrifuge tubes, followed by 500 μl of 1:1 Freon tri-*n*-octylamine. The samples were then vortexed and left to stand for 10 min prior to centrifugation (12000 × *g*, 10 min) and 350 μl of upper aqueous phase was taken for analysis of inositol phosphates. Samples were loaded on Dowex-1(×8) ion exchange columns (formate form, 100–200 mesh, 1 ml). The columns were washed initially with 16 ml *myo*-inositol (5 mM). Then inositol phosphates were eluted with 4 ml of 0.1 M formic acid/1 M ammonium formate. Radioactivity was measured by liquid scintillation spectrometry.

2.5. Data analysis

Concentration-response curve parameters were estimated by means of nonlinear regression of a three-parameter logistic function (pD_2, slope and maximum response) (Kenakin, 1984). Differences between means were evaluated by ANOVA.

2.6. Drugs

The following drugs were used: chlorethylclonidine (Research Biochemicals International), phenoxybenzamine (SmithKline, Beecham), prazosin (Pfizer). Other drugs and reagents were obtained from Sigma Chemical.

3. Results

3.1. Phenoxybenzamine and chlorethylclonidine effects on contractile responses to norepinephrine

There was no difference in the inhibitory effect of phenoxybenzamine (5 nM) on norepinephrine-

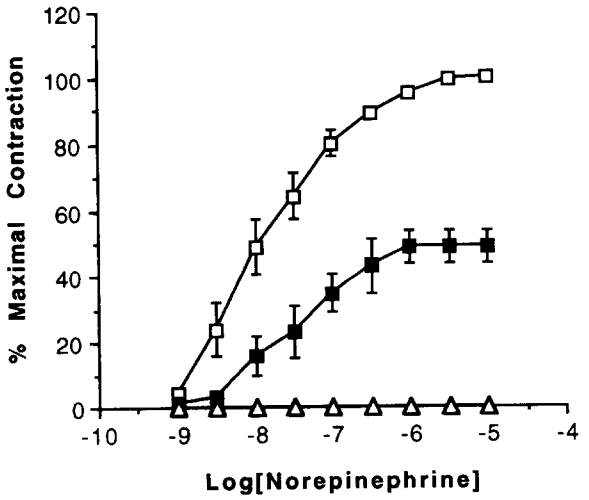

Fig. 2. Norepinephrine-stimulated contraction of aortic ring segments from 1-month-old rat before (□) and after chlorethylclonidine treatment (1 μM, ■; 10 μM, △, 30 min), respectively. Data represent means and standard errors of determinations from 5–6 animals from each group.

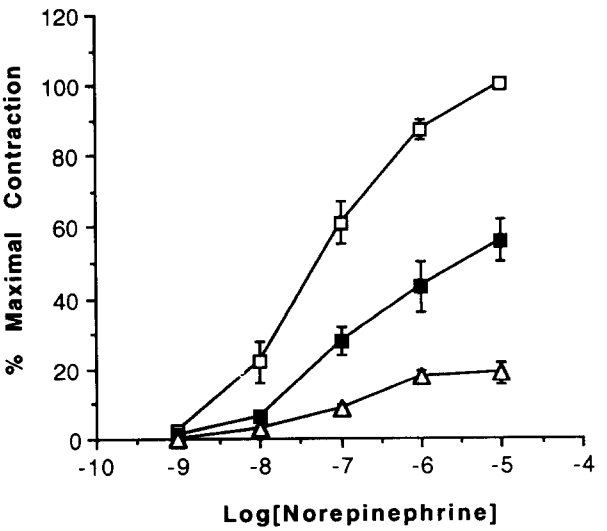

Fig. 3. Norepinephrine-stimulated contraction of aortic ring segments from 6-month-old rat before (□) and after chlorethylclonidine treatment (1 μM, ■; 10 μM, △, 30 min), respectively. Data represent means and standard errors of determinations from 5–6 animals from each group.

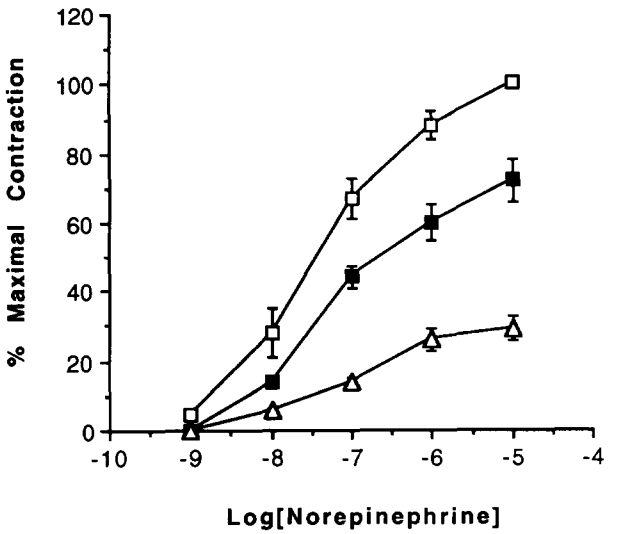

Fig. 4. Norepinephrine-stimulated contraction of aortic ring segments from 24-month-old rat before (□) and after chlorethylclonidine treatment (1 μM, ■; 10 μM, △, 30 min), respectively. Data represent means and standard errors of determinations from 5–6 animals from each group.

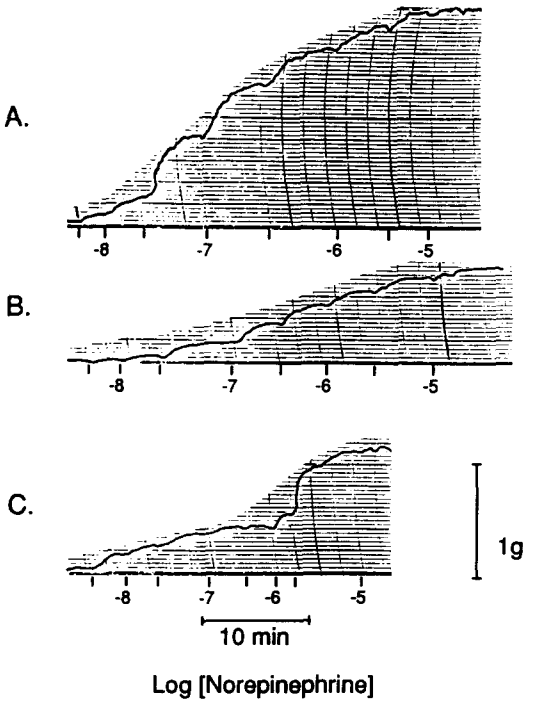

Fig. 5. Representative concentration-response curves for norepinephrine-stimulated contraction of aortic ring segments from 1-month-old rats before (A) and after treatment with phenoxybenzamine (5 nM, 30 min) (B) or chlorethylclonidine (1 μM, 30 min) (C).

stimulated contraction among the different ages (Fig. 1). Different concentrations of phenoxybenzamine (1, 5 and 10 nM) caused 25–70% inhibition of the contractile response without showing age differences. In contrast, inhibition by chlorethylclonidine (1 and 10 μM, 30 min) was greater in aortas from younger rats (Figs. 2, 3 and 4). The inhibition of the contractile response by 1 μM chlorethyclonidine treatment was 51 ± 5%, 46 ± 6% and 27 ± 5% in 1-, 6- and 24-month-old aortas, respectively, and was significantly different between 1 and 24 months. While 10 μM chlorethylclonidine caused complete inhibition of norepinephrine-stimulated contraction in 1-month-old aortas, it inhibited only partially in 6- and 24-month-old aortas. 100 μM chlorethylclonidine and 0.1 μM phenoxybenzamine completely blocked the contractile response in all ages (data not shown). After treatment with chlorethylclonidine (1 μM, 30 min), the norepinephrine concentration-response curve was biphasic in 1-month-old rat aortas (Figs. 2 and 5). The effect was more striking than it appears in the combined results because the inflection point varied somewhat between experiments, so a representative experiment is shown (Fig. 5). Biphasic concentration-response curves were not observed in 6- and 24-month-old aortas before and after chlorethylclonidine treatment, regardless of whether cumulative addition of agonist were in whole or half log increments.

The calculated apparent affinity of norepinephrine determined by partial inactivation of receptors with phenoxybenzamine was significantly higher in the 1-month-old aortas compared to 6- or 24-month-old aortas (Table 1). We could not compare receptor reserve between 1-month- and 6- or 24-month-old rats because the apparent affinity for norepinephrine was different. Apparent affinity and potency of norepinephrine were not different between 6- and 24-month-old rats and there was no difference in receptor reserve between these ages.

3.2. Inositol phosphate accumulation

Chlorethylclonidine (100 μM, 30 min) completely inhibited norepinephrine-stimulated (10 μM) inositol phosphate accumulation in 1-month-old aorta but resulted in only partial inhibition in 6- and 24-month-old aortas (Fig. 6). Phenoxybenzamine (0.1 μM) and pra-

Table 1
pD_2 ($-Log(EC_{50})$) and K_d values of norepinephrine-concentration response curves

	pD_2	$-Log[K_d]$
1 month old	8.02 ± 0.12 [a]	7.17 ± 0.10 [a]
6 months old	7.24 ± 0.03	6.67 ± 0.08
24 months old	7.41 ± 0.06	6.81 ± 0.09

[a] Indicates difference (n = 6–12) in pD_2 and K_d values compared to other ages.

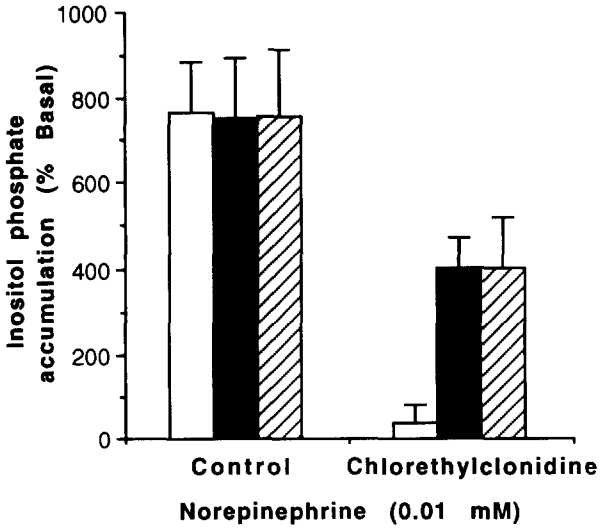

Fig. 6. The effect of chlorethylclonidine (100 μM, 30 min) treatment on norepinephrine-stimulated (10 μM) inositol phosphate accumulation of aortic ring segments from 1- (open columns), 6- (black columns) and 24- (hatched columns) month-old rats. Data represent means and standard errors of determinations from 5–6 animals from each group.

zosin (0.1 μM) completely inhibited the response in all ages (data not shown).

4. Discussion

Previous studies and the present results show that the aortic contractile response to norepinephrine changes during maturation or aging (Docherty, 1990; Gurdal et al., 1995). Norepinephrine is significantly more potent in aortas from 1-month-old rats compared to 6- or 24-month-old rats. Thus, the potency of norepinephrine for aortic contraction decreases during maturation but does not change further during aging. The present studies show that the apparent affinity of α_1-adrenoceptors for norepinephrine is significantly greater in aortas from 1-month-old rats compared to 6 or 24 months. The apparent affinity is not different between 6- and 24-month-old rats. This indicates that apparent affinity is reduced during maturation but does not change further during aging. The apparent affinity constants obtained for aortas from 6- and 24-month-old animals agree closely with those previously determined by other investigators (Ruffolo and Waddel, 1982; Digges and Summers, 1983). The estimated apparent affinity constants of norepinephrine in isolated aortic rings do not represent the actual affinity constants, especially in view of the multiple affinity states associated with coupling to G proteins and the possible presence of receptor subtypes. The differences in apparent affinities between the ages could indicate differences at both the receptor and postreceptor levels.

Reduced receptor number during maturation could cause the reduced potency of norepinephrine, but should not change the apparent affinity. The change in both apparent affinity and potency during maturation may be due to a change in receptor coupling or a change in the receptor subtype mediating the response to norepinephrine.

Norepinephrine binding affinity to α_1-adrenoceptors is affected by guanine nucleotides through alterations of coupling to G proteins (Colucci et al., 1984; Lynch et al., 1984). Agonists bind with high affinity to receptors that are coupled to G proteins. Conversely, receptors that are not coupled to G proteins have a lower affinity for agonists (Lynch et al., 1984; Lefkowitz et al., 1987). The maturational decrease in agonist affinity noted in the present studies may reflect a decrease in receptor coupling to G proteins.

Additional differences were also noted in α_1-adrenoceptor-mediated responses during maturation. The potency of norepinephrine at eliciting inositol phosphate accumulation was less and the magnitude of norepinephrine-stimulated ^{45}Ca influx and efflux were significantly greater in 1-month-old rats compared to 6- or 24-month-old rats (Gurdal et al., 1995). This suggests that norepinephrine stimulation more effectively activates Ca^{2+} fluxes in aortas from the younger animals. Nifedipine, a voltage-dependent Ca^{2+} channel blocker, inhibited both norepinephrine-stimulated contraction and inositol phosphate accumulation more effectively in aortas from older animals (Gurdal et al., 1995). It appears that activation of voltage-dependent Ca^{2+} channels becomes a more important component of α_1-adrenoceptor responses in the aorta as the animal ages.

To determine whether a change in the adrenoceptor subtypes could account for the differences in norepinephrine responses in 1-, 6- and 24-month-old aortas, the effects of the antagonist chlorethylclonidine were studied. Chlorethylclonidine was significantly more effective at inhibiting norepinephrine-stimulated aortic contraction in the younger rats. In contrast, a comparable treatment with the nonselective α_1-adrenoceptor antagonist phenoxybenzamine caused equivalent inhibition at each age. The subdivision of α_1-adrenoceptors pharmacologically into those that are sensitive to inhibition by Ca^{2+} channel blockers but insensitive to chlorethylclonidine and those with inverse properties was previously proposed (Minneman, 1988; Han et al., 1990). In old rats α_1-adrenoceptor-mediated aortic contraction was sensitive to inhibition by nifedipine but relatively insensitive to chlorethylclonidine, while the converse was true of the younger aortas.

The rat aorta has been shown to contain mRNA for the α_{1B}-, α_{1C}- and α_{1D}-adrenoceptor subtypes (Lomasney et al., 1991; Schwinn et al., 1990; Perez et al., 1991; Ping and Faber, 1993; Piascik et al., 1994). Radioligand binding studies indicate that the rat aorta also contains the pharmacologically defined α_{1A}-adrenoceptor (Piascik et al., 1991, 1994). Pharmacological studies have not yet established the role of the subtypes in mediating blood vessel contraction and regulating peripheral resistance. Several studies have concluded that the rat aorta contains predominantly α_{1B}-adrenoceptors because chlorethylclonidine effectively inhibits contraction to norepinephrine (Han et al., 1990) and we find that chlorethylclonidine can block norepinephrine-stimulated contraction in the Fisher 344 rat aorta. Our results indicate a predominance of chlorethylclonidine-sensitive adrenoceptor subtypes in the young rat aorta. However, the response is also inhibited by nifedipine (30%) (Gurdal et al., 1995), suggesting the possible presence of another chlorethylclonidine-sensitive subtype in young aorta, since α_{1B}-adrenoceptor-mediated responses are resistant to Ca^{2+} channel blockers. Furthermore, in 1-month-old rats chlorethylclonidine treatment (1 μM, 30 min) that partially inhibits norepinephrine-stimulated contraction converts an apparently monophasic concentration-response curve into one that is decidedly biphasic. This could indicate the possible role of a second chlorethylclonidine-sensitive α_1-adrenoceptor subtype in the contractile response (e.g. α_{1D}). In older aortas, the α_1-adrenoceptor-mediated contractile response was relatively insensitive to chlorethylclonidine treatment. Thus, while 10 μM chlorethylclonidine completely blocked contraction in 1-month-old aorta, 100 μM was required to block the response in the older ages. These results indicate an increased role of relatively chlorethylclonidine-insensitive subtypes (α_{1C}, α_{1D} or α_{1A}) in older ages. Effective inhibition of aortic contraction by chlorethylclonidine occurs despite the apparent presence of the α_{1A}-adrenoceptor, as evidenced in radioligand binding studies by high affinity for WB4101 and norepinephrine, and resistance to chlorethyclonidine (Piascik et al., 1994; Gurdal et al., unpublished results). This raises the question as to the function of the α_{1A}-adrenoceptor in rat aorta. The present results show that while 100 μM chlorethylclonidine completely blocked contractile responses at all ages and inositol phosphate accumulation in young aortas, it produced only partial inhibition of inositol phosphate accumulation in older aortas. This may be an indication of increased expression of a chlorethylclonidine-insensitive α_1-adrenoceptor subtype (α_{1A}) during maturation and aging and its function to stimulate formation of inositol phosphates.

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