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α_1-adrenoceptor subtypes mediating noradrenaline-induced contraction of pulmonary artery from pulmonary hypertensive rats

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Abstract

The effect of monocrotaline-induced pulmonary hypertension on α_1-adrenoceptor-mediated contractions of pulmonary artery segments was studied. In control and monocrotaline-treated rats, noradrenaline evoked concentration-dependent contractions of the pulmonary artery. There was no change in the potency and affinity of noradrenaline but the maximum response and receptor reserve were significantly reduced. Noradrenaline-induced contractions were competitively antagonized by prazosin, 2-(2-6dimethoxyphenoxyethyl)aminomethyl-1,4-benzodioxane hydrochloride (WB 4101) and 8-[2-[4-(2methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspiro[4,5]decane-7,9 dione dihydrochloride (BMY 7378) with pA_2 values of 9.64 ± 0.16, 9.45 ± 0.10 and 8.30 ± 0.14, respectively. These antagonists also competitively antagonized noradrenaline-induced contractions of pulmonary artery segments isolated from rats with monocrotaline-induced pulmonary hypertension. The pA_2 values were 9.66 ± 0.11 (prazosin), 9.62 ± 0.09 (WB 4101) and 8.47 ± 0.15 (BMY 7378). Chloroethylclonidine (CEC) shifted noradrenaline concentration–response curve to the right and depressed the maximum response. There was no difference between the effects of CEC in both groups. It was therefore concluded that pulmonary hypertension significantly reduced noradrenaline-induced contractions of the rat pulmonary artery without affecting the sensitivity. Studies with receptor-selective antagonists confirmed that α_{1D}-adrenoceptor subtype is the predominant receptor subtype in the pulmonary artery and this was maintained in this disease state.

Keywords: α_{1D}-Adrenoceptor subtype; WB 4101; Chloroethylclonidine; Pulmonary artery; Pulmonary hypertension

1. Introduction

Pulmonary hypertension is a chronic disabling disease that affects the pulmonary vasculature. It is associated with decreased vascular compliance, elevated pulmonary artery pressure, right heart failure and, eventually, death. Pulmonary hypertension is characterized by vasoconstriction of the pulmonary arteries, vascular remodeling and structural changes resulting from smooth muscle proliferation and migration. The primary mechanism responsible for the vasoconstriction is not fully understood. Recently, α_1-adrenoceptor-mediated events have been proposed as the predominant contributors to pulmonary hypertension (Salvi, 1999). This is consistent with the effectiveness of α_1-adrenoceptor antagonists in pulmonary hypertension (Kanemoto et al., 1984; Lewczuk et al., 1992; Alpert et al., 1994; Inoue et al., 1994; Salvi, 1999). Chemical sympathectomy has also been shown to reduce right ventricular hypertrophy induced by monocrotaline in rats (Tucker et al., 1983).

Previous studies have shown that responsiveness of the pulmonary artery to noradrenaline is reduced in monocrotaline-induced pulmonary hypertension in rats (Tabritzchi et al., 1995; Wanstall et al., 1990; Morita et al., 1996; Altiere et al., 1986; Miyauchi et al., 1993; Kanai et al., 1993; Dhein et al., 2002), a model widely used to study the pathogenesis of primary pulmonary hypertension (Kay et al., 1967). This reduced vascular reactivity is unlikely to be due to a nonspecific damage to the contractile apparatus since potassium chloride-induced contraction was not reduced while 5-hydroxytryptamine- and prostaglandin $F_{2\alpha}$-induced contractions were enhanced in pulmonary artery segments from rats treated with monocrotaline (Wanstall and O'Donnell, 1990; Kanai et al., 1993; Brown et al., 1998). The reason for the reduced vascular reactivity of the pulmonary artery to noradrenaline has not been investigated. Previous studies have shown that reactivity of vascular smooth muscles to contractile agonists could be modulated

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by affinity of the agonists for the receptor and receptor number (Oriowo et al., 1989; Laher and Bevan, 1985). We therefore hypothesized that altered vascular reactivity of the pulmonary artery to noradrenaline in pulmonary hypertension could possibly be due to alterations in receptor affinity and/or density. The main objective of the present study was therefore to compare receptor characteristics, dissociation constants and receptor reserves in pulmonary artery segments isolated from control and monocrotaline-treated rats in an attempt to determine the role, if any, of changes in these parameters in the altered reactivity of the pulmonary artery to noradrenaline.

Vascular smooth muscle contraction induced by α_1-adrenoceptor agonists is mediated by α_{1A}-, α_{1B}- and α_{1D}-subtypes. The distribution and contribution of these α_1-adrenoceptor subtypes to agonist-induced contraction varies significantly. Thus even though all three α_1-adrenoceptor subtypes are expressed in the pulmonary artery (Xu et al., 1997), contractions of the pulmonary artery induced by adrenoceptor agonists are mediated predominantly by α_{1D}-subtype (Hussain and Marshall, 1997; Dhein et al., 2002). This conclusion was based on the high potency of BMY 7378, a selective antagonist of α_{1D}-adrenoceptor subtype (Goetz et al., 1995; Kenny et al., 1995; Saussy et al., 1994) against noradrenaline-induced contractions and the ability of chloroethylclonidine (CEC), an irreversible alkylating antagonist of α_{1D}- and α_{1B}-adrenoceptor subtypes, to significantly depress noradrenaline-induced contractions. Monocrotaline-induced pulmonary hypertension is accompanied by increased plasma concentration of noradrenaline (Ishikawa et al., 1991; Vescovo et al., 1989). Therefore prolonged exposure of the receptors to elevated levels of noradrenaline could result in down-regulation of the receptors. However, since it has been established that the expression of α_1-adrenoceptor subtypes can be differentially regulated (Dong and Han, 1995; Rokosh et al., 1996; Lei et al., 2001, 2002; Yang et al., 1999) by prolonged exposure to adrenoceptor agonists, the possibility of a switch between α_1-adrenoceptor subtypes mediating noradrenaline-induced contractions of the pulmonary artery cannot be ruled out. The second objective of this study was therefore to characterize the α_1-adrenoceptor subtype mediating noradrenaline-induced contractions in pulmonary artery segments from rats with monocrotaline-induced pulmonary hypertension.

2. Materials and methods

Adult male rats (150–200 g) were used in this investigation. These rats were bred and maintained under internationally accepted conditions in the animal resource center of the Faculty of Medicine, Kuwait University. This study was approved by the Institution's research ethics committee. The rats had free access to food and water. Pulmonary hypertension was induced by treating the rats with monocrotaline (60 mg/kg) and used 3 weeks later. Age-matched littermates were used as controls. On the day of the experiments, each rat was anaesthetized with sodium pentobarbitone (35 mg/kg, i.v.). The abdominal cavity was opened up and the lungs together with the heart were removed en bloc and placed in a Petri dish containing cold Krebs solution. After carefully isolating the extralobar pulmonary arteries, the heart was weighed and the ratio of heart weight to body weight was used as an index of cardiac hypertrophy. The pulmonary artery segments were carefully cleared of any adhering connective tissues. The endothelium was removed by gently rubbing the artery lumen with fine wire. Endothelial denudation was confirmed by the inability of acetylcholine (10^{-6} M) to relax artery segments contracted with noradrenaline (10^{-7} M). Artery ring segments (3–4 mm in length) were set up in 25.0 ml tissue baths containing Krebs solution (NaCl, 119; KCl, 4.7; $NaHCO_3$, 25; KH_2PO_4, 1.2; $MgSO_4$, 1.2; $CaCl_2$, 2.5; and glucose, 11 mM) at 37 °C. The solution was continuously gassed with a 5% CO_2/95% O_2 mixture and the pH was approximately 7.4. Desipramine (10^{-7} M) and propranolol (10^{-6} M) were included in the Krebs solution to block neuronal uptake and β-adrenoceptors, respectively. The preparations were allowed to equilibrate under a resting tension of 1.0 g (control rats) or 2.0 g (monocrotaline-treated rats) for up to 60 min, during which the bath fluid was changed at least once. Isometric contractions were recorded through dynamometer UF1 transducers on a Lectromed four-channel polygraph (MultiTrace 4P). After the period of equilibration, KCl (80 mM) was added to the bath to test for tissue viability. This concentration of KCl was repeated after 30 min. The tissues were then washed repeatedly over the next 30-min period. Thereafter, agonist concentration–response curves were established by adding, cumulatively, increasing concentrations (0.5 log unit increments) of each agonist to the bath and allowing the response to each concentration reach a peak before adding the next concentration. After obtaining the maximum response, the preparations were washed several times and allowed to rest for 60 min before repeating agonist concentration–response curve. Agonist potencies were expressed as pD_2 values where pD_2 is the negative logarithm of the agonist concentration producing 50% of the maximum response.

At the end of the experiments, the artery segments were blotted and weighed. Maximum response was expressed as mg/mg tissue weight to normalize for changes in tissue weight as a result of tissue weight changes due to hypertrophy.

2.1. Determination of agonist dissociation constant

In this study, agonist dissociation constant (K_A) was determined according to the method described by Furchgott and Bursztyn (1967). This procedure involved partial receptor occlusion by treatment with an irreversible (alkylating) α_1-adrenoceptor antagonist. Briefly, after obtaining a control dose–response curve for noradrenaline, phenoxybenzamine (PBZ, 10^{-8}–3×10^{-8} M) was added to the bath

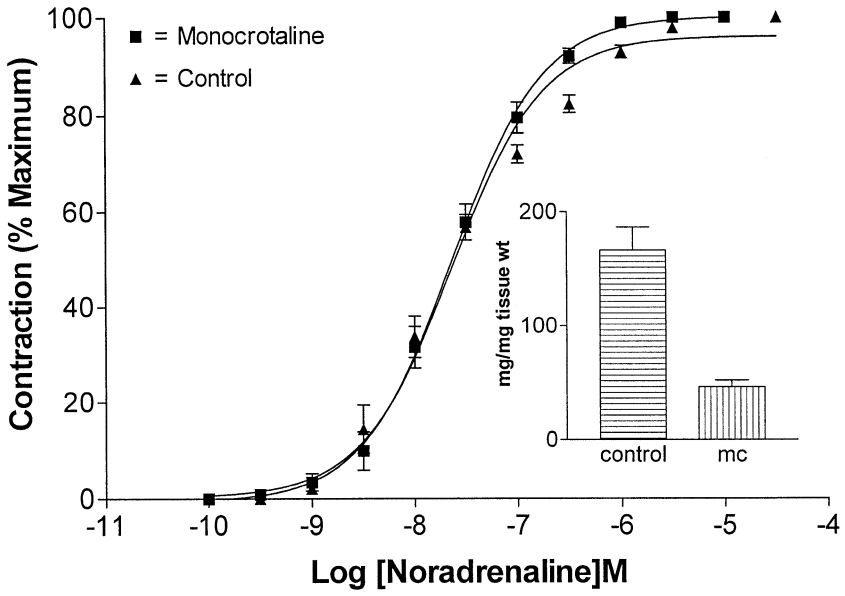

Fig. 1. Concentration–response curves to noradrenaline in pulmonary artery ring segments. The maximum response (mg/mg tissue weight) is shown in the inset. Each point on the graph is the mean ± S.E.M of 14–16 experiments.

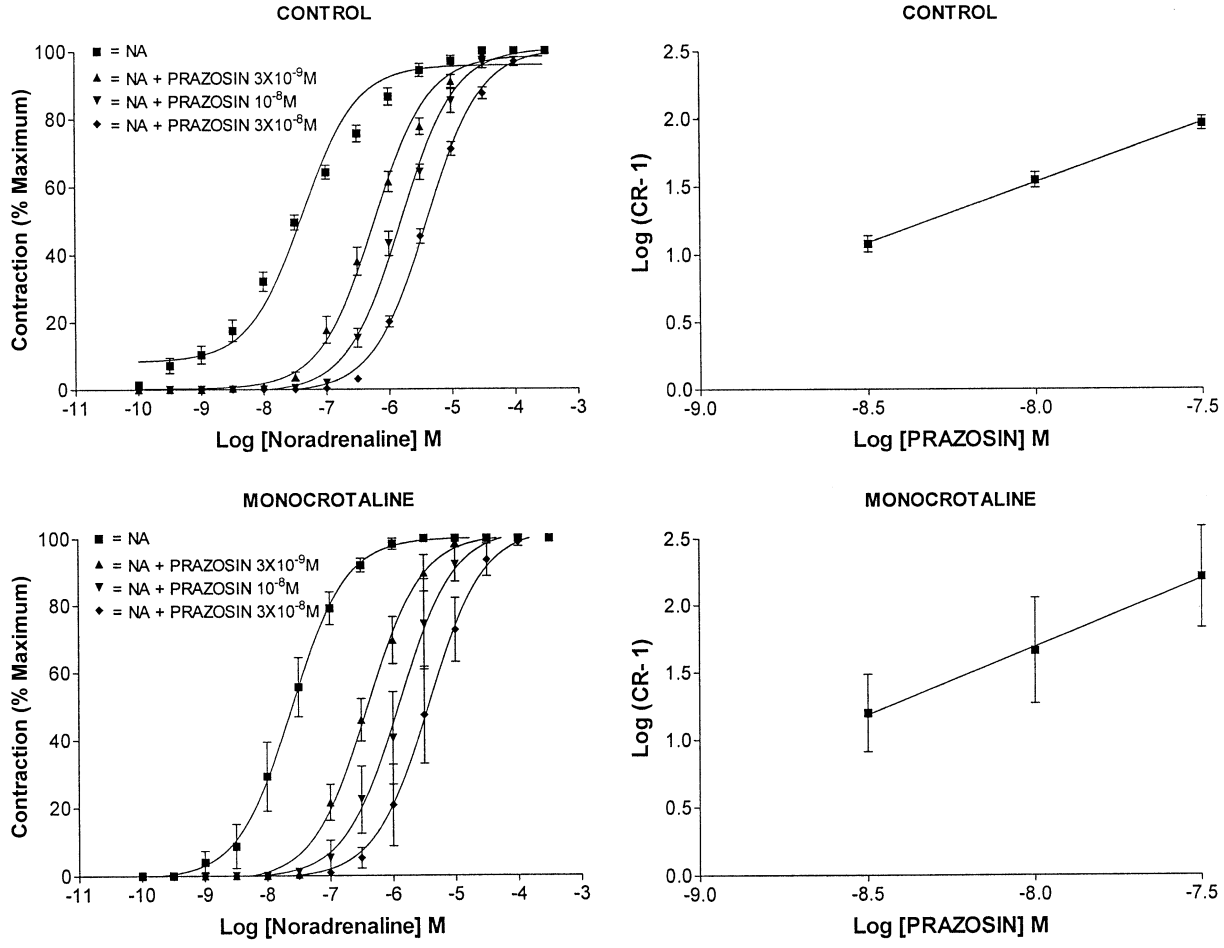

Fig. 2. Concentration–response curves to noradrenaline in pulmonary artery ring segments from control and monocrotaline-treated rats. Symbols represent responses to noradrenaline in the absence (■) and presence of prazosin 3×10^{-9} M (▲), 10^{-8} M (▼) and 3×10^{-8} M (♦). The Schild linear regression plot is shown on the right. Each point on the graph is the mean ± S.E.M of four experiments.

Table 1
Effect of some α_1-adrenergic receptor antagonists on noradrenaline-induced contractions of the rat pulmonary artery

Antagonists	Control		Antagonist	
	pA_2 value	Slope	pA_2 value	Slope
Prazosin	9.64 ± 0.16 (n=4)	0.99 ± 0.05 (n=4)	9.66 ± 0.10 (n=4)	1.01 ± 0.17 (n=4)
WB 4101	9.45 ± 0.10 (n=4)	0.97 ± 0.05 (n=4)	9.62 ± 0.09 (n=4)	1.01 ± 0.07 (n=4)
BMY 7378	8.30 ± 0.14 (n=4)	1.04 ± 0.08 (n=4)	8.47 ± 0.15 (n=4)	1.05 ± 0.10 (n=4)

Each antagonist was equilibrated with the tissue for 30 min before re-establishing agonist concentration–response curves. At least three concentrations of each antagonist were tested in all experiments. Antagonist potencies were expressed as pA_2 values.

and allowed to equilibrate with the tissue for 30 min. This was followed by washing with PBZ-free Krebs solution twice within 30 min before re-establishing noradrenaline concentration–response curve. Noradrenaline-induced contractions after treatment with PBZ were expressed as a percentage of the maximum response to noradrenaline before treatment. From the concentration–response curves, equi-effective concentrations of noradrenaline before (A) and after (A^1) treatment with PBZ were obtained and used to plot a graph of $1/A$ versus $1/A^1$.

The slope of the regression line and the Y-intercept were used to calculate agonist dissociation constant using the equation—K_A = slope − 1/intercept. K_A values are presented as pK_A (where $pK_A = -\log K_A$). Receptor reserve was calculated using the equation—receptor reserve = anti-log($pD_2 - pK_A$), while fractional receptor occupancy was calculated using the equation $A/K_A + A$ where A is the concentration of the agonist (Ruffolo, 1982).

2.2. Effect of α_1-adrenoceptor antagonists on agonist-induced contraction

After obtaining control concentration–response curves, appropriate concentrations of the antagonists were added to the bath and allowed to equilibrate with the tissue for 30 min before repeating agonist concentration–response curves. Contractions in the presence of the antagonists were

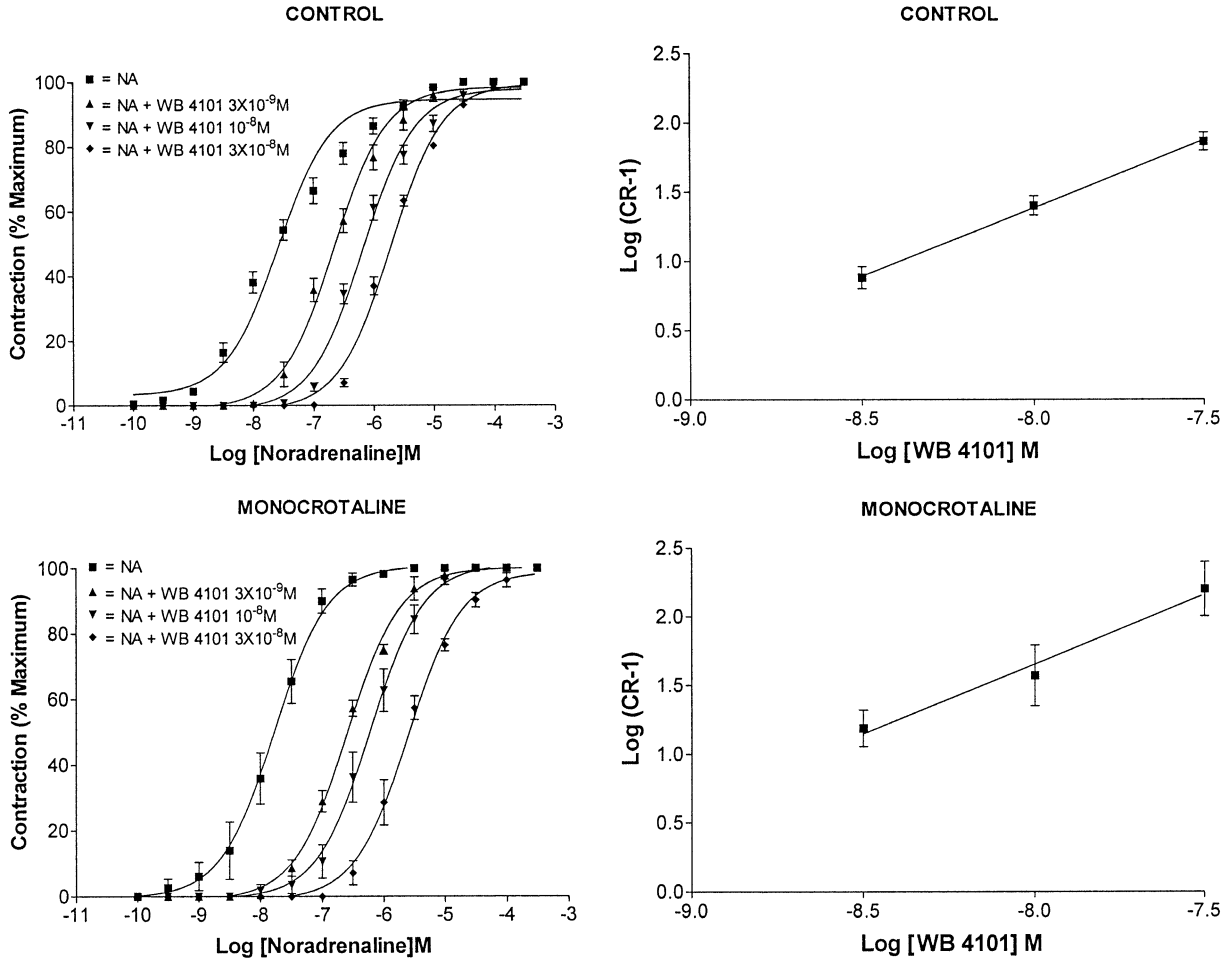

Fig. 3. Concentration–response curves to noradrenaline in pulmonary artery ring segments from control and monocrotaline-treated rats. Symbols represent responses to noradrenaline in the absence (■) and presence of WB 4101, 3×10^{-9} M (▲), 10^{-8} M (▼) and 3×10^{-8} M (◆). The Schild linear regression plot is shown on the right. Each point on the graph is the mean ± S.E.M of four experiments.

expressed as a percentage of the maximum response to noradrenaline in the absence of the antagonists. Antagonist potency was expressed as pA_2 values determined according to the method of Arunlakshana and Schild (1959). pA_2 is defined as the negative logarithm of the antagonist concentration which would require a doubling of the agonist concentration to produce the same response as in the absence of the antagonist. Antagonism was taken to be competitive when the slope of the Schild (linear regression) plot was not significantly different from 1. Only one agonist/antagonist pair was tested on a given preparation and at least three antagonist concentrations were tested on each preparation.

2.3. Effect of chloroethylclonidine (CEC) on noradrenaline-induced contraction

After obtaining control concentration–response curves to noradrenaline, CEC (10^{-4} M) was added to the bath and left to equilibrate with the tissue for 30 min before washing. The tissues were then allowed a 30-min period of equilibration in antagonist-free Krebs solution before re-establishing agonist concentration–response curves. Noradrenaline-induced contractions after treatment with CEC were expressed as a percentage of the control maximum response.

2.4. Drugs

The following compounds were used in this study: acetylcholine chloride, prazosin hydrochloride, chloroethylclonidine hydrochloride, desipramine hydrochloride, (−)-noradrenaline (arterenol) bitartrate, phenoxybenzamine hydrochloride, 2-(2-6dimethoxyphenoxyethyl)aminomethyl-1,4-benzodioxane hydrochloride (WB 4101), {8-[2-[4-(2methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspiro[4,5]decane-7,9 dione}dihydrochloride (BMY 7378), monocrotaline, (±)-propranolol hydrochloride (Sigma, St. Louis, MO). Phenoxybenzamine hydrochloride and prazosin were dissolved in absolute ethanol. All other compounds were dissolved in distilled water.

2.4.1. Dissolution of monocrotaline

Monocrotaline (150 mg) was dissolved in 1.0 ml of 1 N HCl. Distilled water (2.0 ml) was added and the pH was

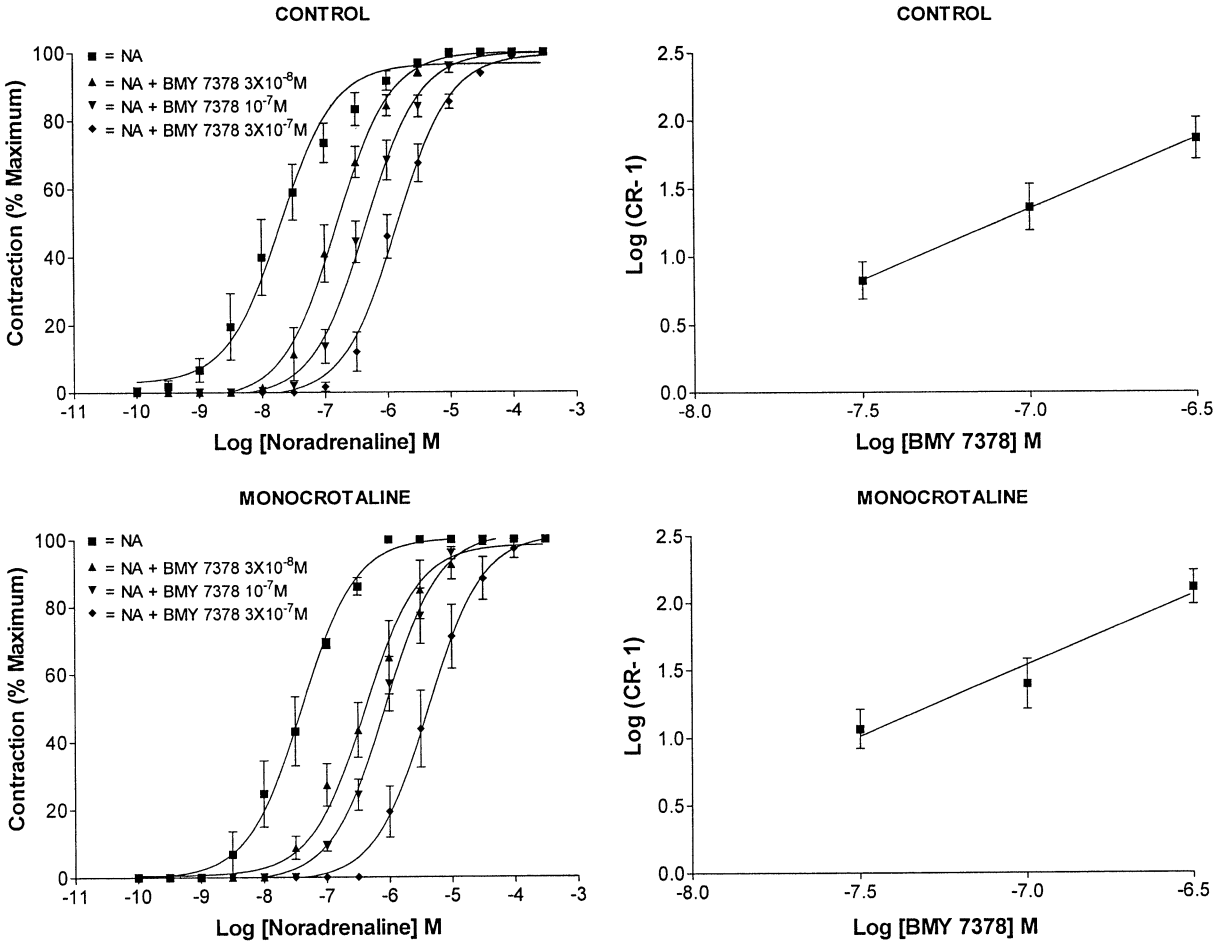

Fig. 4. Concentration–response curves to noradrenaline in pulmonary artery ring segments from control and monocrotaline-treated rats. Symbols represent responses to noradrenaline in the absence (■) and presence of BMY 7378, 3×10^{-8} M (▲), 10^{-7} M (▼) and 3×10^{-7} M (◆).The Schild linear regression plot is shown on the right. Each point on the graph is the mean ± S.E.M of four experiments.

adjusted to 7.0 with NaOH. Thereafter, the volume was made up to 5.0 ml with distilled water to give a concentration of 30 mg/ml.

2.5. Statistical analysis

All graphs were analyzed using GraphPad Prism software. Data are presented as mean ± S.E.M of n observations. Where necessary, differences between mean values were tested for significance using Student's t-test. Differences were assumed to be significant when $P<0.05$.

3. Results

The mean weight of monocrotaline-treated rats after 3 weeks was 293.0 ± 9.0 g while age-matched control rats weighed 327.0 ± 6.8 g. These values were significantly ($P<0.05$) different from each other. Three weeks after treatment of the rats with monocrotaline, the mean weights of the hearts were 1.03 ± 0.02 and 1.13 ± 0.05 in control and monocrotaline-treated rats, respectively. These values were significantly ($P<0.05$) different from each other. Heart weight to body weight ratio increased from 2.99 ± 0.10 ($n=13$) in control rats to 3.90 ± 0.20 ($n=18$) in rats treated with monocrotaline. These values were significantly ($P<0.05$) different from each other indicating cardiac hypertrophy.

3.1. Contractile response induced by noradrenaline

Noradrenaline (10^{-9}–10^{-6} M) evoked concentration-dependent contractions of the pulmonary artery segments (Fig. 1). The contractions were reproducible as up to five consecutive concentration–response curves to the agonists could be constructed with no significant loss in sensitivity or maximum response. The pD_2 values were 7.64 ± 0.04 ($n=15$), and 7.64 ± 0.04 ($n=16$) in control and monocrotaline-treated rats, respectively. These values were not significantly ($p>0.05$) different from each other. However, as shown in Fig. 1, the maximum response to noradrenaline was significantly ($P<0.05$) less in monocrotaline-treated rats.

3.2. Agonist dissociation constants and receptor reserves

Phenoxybenzamine (10^{-8}–3×10^{-8} M) displaced noradrenaline concentration–response curves to the right and depressed the maximum responses. The pK_A values were 6.58 ± 0.09 ($n=7$) and 6.72 ± 0.09 ($n=4$) for noradrenaline in control and monocrotaline-treated rats, respectively. These values were not significantly different ($P>0.05$) from each other. The receptor reserves were calculated to be 11.0 ± 1.7 and 7.9 ± 1.2 in control and monocrotaline-treated rats, respectively. These values were significantly ($P<0.05$) different from each other. Noradrenaline produced 50% of the maximum response while occupying 9.5% and 13.3% of the receptors in artery segments from control and monocrotaline-treated rats, respectively.

3.3. Effect of prazosin, WB 4101 and BMY 7378 on noradrenaline-induced contraction

Prazosin (3×10^{-9}–3×10^{-8} M) produced parallel rightward shift of the concentration–response curves to noradrenaline (Fig. 2) in pulmonary artery segments from control and monocrotaline-treated rats. The pA_2 values (and slopes) are shown in Table 1. These values were not significantly different from each other. As shown in Figs. 3 and 4, WB 4101 (3×10^{-9}–3×10^{-8} M) and BMY 7378 (3×10^{-8}–3×10^{-7} M) also produced concentration-dependent antagonism of noradrenaline-induced contractions in pulmonary artery segments from control and monocrotaline-treated rats. The pA_2 values (and slopes) are shown in Table 1. For each antagonist, there was no significant difference ($P>0.05$) between the potencies of the antagonists in control and monocrotaline-treated rats. The slopes of the Schild regression lines were not significantly different ($P>0.05$) from 1 indicating competitive antagonism.

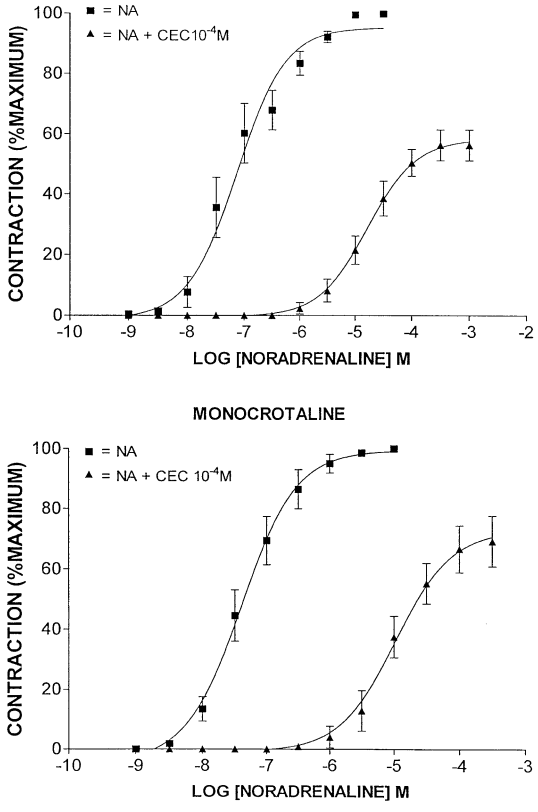

Fig. 5. Effect of CEC (10^{-4} M) on noradrenaline-induced contractions of pulmonary artery ring segments from control and monocrotaline-treated rats. The tissues were treated with CEC for 30 min and then washed twice with antagonist-free Krebs solution. Each point on the graph is the mean ± S.E.M of five to six experiments.

3.4. Effect of CEC on noradrenaline-induced contraction

CEC (10^{-4} M), alone, did not contract the pulmonary artery segments from control or monocrotaline-treated rats. However, it significantly and irreversibly reduced noradrenaline-induced contractions in pulmonary artery segments from control and monocrotaline-treated rats (Fig. 5). The potency of noradrenaline was reduced by more than 200-fold in control and monocrotaline-treated rats. The maximum response was reduced by $43.8 \pm 5.1\%$ and $31.0 \pm 8.3\%$ in control and monocrotaline-treated rats, respectively. These values were not significantly different from each other.

4. Discussion

The results described above showed that α_1-adrenoceptor responsiveness to noradrenaline was attenuated in rats with monocrotaline-induced pulmonary hypertension confirming previous reports in the literature (Altiere et al., 1986; Wanstall et al., 1990; Kanai et al., 1993; Miyauchi et al., 1993; Morita et al., 1996; Tabritzchi et al., 1995; Dhein et al., 2002). The reduced responsiveness was characterized as a reduction in the maximum response with no change in sensitivity to noradrenaline. This would probably suggest that the attenuated response could not be due to a change in agonist affinity but most likely to a change in post-receptor events. This is supported by our observation, in this study, that dissociation constants determined for noradrenaline in artery segments from control (6.58) and monocrotaline-treated (6.72) rats were not significantly different from each other. Values of dissociation constant observed in this study are similar to values previously reported in the literature (Wanstall and O'Donnell, 1988). The receptor reserve in pulmonary arteries from monocrotaline-treated rats was however significantly lower than in control rats. In addition, noradrenaline occupied 9.5% and 13.3% of the receptors to produce 50% of the maximum response in control and monocrotaline-treated rats, respectively. Reduced receptor reserve could result from down-regulation of the receptors, inefficient stimulus–response coupling and/or changes in signaling mechanisms. Elevated plasma level of noradrenaline is a hallmark of right ventricular and other forms of cardiac failure. Prolonged exposure to noradrenaline has been shown to down-regulate cardiac β-adrenoceptors (Seyfarth et al., 2000; Brown et al., 1998). It is therefore conceivable that prolonged exposure of α_1-adrenoceptors to raised levels of noradrenaline could result in down-regulation of these receptors. However, it is unlikely that this alone could account for the reduction in the maximum response to noradrenaline considering the fact that nordarenaline was a full agonist in the pulmonary artery occupying only approximately 10% of the receptors to produce 50% of the maximum response (this study). The reduced receptor reserve may most likely be due to changes in the efficiency of stimulus–receptor coupling since, relative to control rats, noradrenaline occupied more receptors in artery segments from monocrotaline-treated rats to produce 50% of the maximum response. The observation that ischemia–reperfusion increased reactivity of the rat tail artery to noradrenaline was associated with increased efficiency of stimulus–receptor coupling with no change in receptor density (Seasholtz et al., 2001) is in agreement with this suggestion. The possibility of a change in signaling mechanism contributing to the change in receptor reserve cannot be ruled out since noradrenaline-induced contractions of pulmonary arteries from rats with monocrotaline-induced pulmonary hypertension have been shown to be more sensitive to inhibition by calcium channel blockers (Tabritzchi et al., 1995). Several studies have shown that a reduction in receptor reserve increases the susceptibility of noradrenaline-induced contractions to calcium channel inhibitors (Ruffolo and Nichols, 1988; Bognar and Enero, 1988).

Vascular smooth muscle contraction induced by α_1-adrenoceptor agonists is mediated by α_{1A}-, α_{1B}- and α_{1D}-subtypes. The distribution and function of these subtypes varies significantly. In the rat aorta, noradrenaline and other α_1-adrenergic agonists activate α_{1D}-adrenoceptors to evoke a contraction (Saussy et al., 1994; Testa et al., 1995; Dhein et al., 2002) even though all three subtypes, α_{1A}, α_{1B} and α_{1D}, are expressed (Piascik et al., 1994; Xu and Han, 1996; Xu et al., 1997, 1998). Later studies have shown that the expression and function of α_1-adrenoceptor subtypes are subjected to modulation by disease states. For example, α_{1D}-subtype in the aorta is increased while that of α_{1A}-subtype is decreased in 12-month-old SHRs compared with age-matched Wistar Kyoto rats (Xu et al., 1998). Similarly, hypothyroidism is associated with increased expression of α_{1A}- and α_{1B}- but reduced expression of α_{1D}-subtype in cardiac muscles (Zhang et al., 1997). Despite the change in the levels of expression, there was no change in receptor affinity (Villalobos-Molina and Ibarra, 1996). α_{1A}-, α_{1B}- and α_{1D}-adrenoceptor subtypes are expressed in the pulmonary artery (Xu et al., 1997). However, contractions of the pulmonary artery induced by adrenergic agonists are mediated predominantly by α_{1D}-subtype (Hussain and Marshall, 1997; Dhein et al., 2002). Monocrotaline-induced pulmonary hypertension is accompanied by increased plasma concentration of noradrenaline (Ishikawa et al., 1991; Vescovo et al., 1989) which could result in downregulation of vascular α_1-adrenoceptors. However, since it has been established that the expression of α_1-adrenoceptor subtypes can be differentially regulated (Dong and Han, 1995; Rokosh et al., 1996; Lei et al., 2001, 2002; Yang et al., 1999) by prolonged exposure to adrenergic agonists, the possibility of a switch between α_1-adrenoceptor subtypes mediating NA-induced contractions of the pulmonary artery cannot be ruled out. We explored the possibility that other receptor subtypes could become functionally relevant in monocrotaline-induced pulmonary hypertension.

Basically, we studied the effect of prazosin on noradrenaline-induced contractions in order to confirm that noradrenaline-induced contractions were mediated via α_1-adrenoceptors. Reduced potency of prazosin would indicate a reduced role for α_1-adrenoceptors. We also studied the effect of WB 4101 and BMY 7378 on noradrenaline-induced contractions. WB 4101 is a potent α_{1A}- and α_{1D}-adrenoceptor antagonist while BMY 7378 is selective for α_{1D}-subtype. Our results showed that prazosin potently and competitively antagonized noradrenaline in the pulmonary artery segments. The pA_2 values are similar to values previously in the literature and there was no difference in the potencies of prazosin between the two groups, confirming that α_1-adrenoceptors mediated noradrenaline-induced contractions in both groups. WB 4101 and BMY 7378 also potently and competitively antagonized noradrenaline-induced contractions of the rat pulmonary artery. The pA_2 values are similar to values previously reported in the literature for an action on α_{1D}-adrenoceptors. This would confirm mediation of noradrenaline-induced contractions via α_{1D}-subtype. In addition, there was no difference in the potencies of WB 4101 and BMY 7378 in artery segments from control and monocrotaline-treated rats, indicating that pulmonary hypertension was not accompanied by a change in the contribution of α_{1D}-subtype to noradrenaline-induced contractions of the pulmonary artery.

CEC is an alkylating analog of clonidine. It differentiates between α_1-adrenoceptor subtypes inactivating α_{1B}- and α_{1D}-adrenoceptor subtypes (Han et al., 1987; Hieble et al., 1995) with little or no effect on α_{1A}-adrenoceptors. In this study, CEC (10^{-4} M) remarkably attenuated noradrenaline-induced contraction of the rat pulmonary artery. This is in agreement with previous observations of Dhein et al., (2002). Taken together with the potencies of WB 4101 and BMY 7378, this would confirm mediation of noradrenaline-induced contractions via α_{1D}-adrenoceptor subtype. The fact that the efficacy of CEC was similar in artery segments from control and monocrotaline-treated rats would confirm that monocrotaline-induced pulmonary hypertension in rats was not associated with a change in the contribution of α_{1D}-adrenoceptor subtype to noradrenaline-induced contraction of the rat pulmonary artery. Martinez et al., (1999) have also concluded that vascular α_{1D}-adrenoceptor function is maintained in aorta and carotid arteries of rats with congestive heart failure after myocardial infarction.

We concluded that monocrotaline-induced pulmonary hypertension in rats is associated with attenuated reactivity of pulmonary artery segments to noradrenaline characterized by a reduction in the maximum response but no change in agonist or antagonist affinities for the receptors. Based on antagonist affinities, we confirmed that α_{1D}-adrenoceptor subtype mediated noradrenaline-induced contractions in control and monocrotaline-treated rats. There was a reduced receptor reserve for noradrenaline in arteries from rats with monocrotaline-induced pulmonary hypertension.

Acknowledgements

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