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Chloride channels and α_1-adrenoceptor-mediated pulmonary artery smooth muscle contraction: effect of pulmonary hypertension

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Abstract

Noradrenaline induced concentration-dependent contractions of pulmonary artery segments from control and monocrotaline-treated rats. There was a significant decrease in the maximum response but not sensitivity in artery segments from monocrotaline-treated rats. At a concentration (10^{-6} M) that abolished KCl-induced contraction, nifedipine attenuated but did not abolish, noradrenaline-induced contraction in both groups. However, noradrenaline-induced contraction in artery segments from pulmonary hypertensive rats was more susceptible to inhibition by nifedipine. Bumetanide (10^{-4} M), a chloride transport inhibitor and niflumic acid, a chloride channel inhibitor, reduced noradrenaline-induced contraction of the pulmonary artery in control and pulmonary hypertensive groups. These compounds were more effective in ring segments from pulmonary hypertensive rats. It was concluded that activation of chloride channels was involved in noradrenaline-induced contraction and that the contribution of chloride channels was enhanced in pulmonary hypertensive rats.

Keywords: Chloride channel; Niflumic acid; Bumetanide; Nifedipine; Pulmonary artery, rat

1. Introduction

Calcium-activated chloride channels have been identified in vascular smooth muscle. Activation of these channels leads to membrane depolarization accompanied by influx of Ca^{2+} through voltage-gated Ca^{2+} channels and contraction. The role of chloride (Cl^-) channels in vascular smooth muscle contraction has received a lot of attention in recent years. Inhibitors of Cl^- channels or Cl^- transport have been shown to reduce agonist-induced vascular smooth muscle contraction in the rat aorta (Criddle et al., 1996; Lamb and Barna, 1998; Hyvelin et al., 1998), pulmonary artery (Yuan, 1997; Bieger et al., 2004) and rabbit basilar artery (Dai and Zhang, 2001a, b) confirming a role for activation of Cl^- channels in these contractile responses.

There is evidence that the role of Cl^- channel activation and/or Cl^- transport in vascular smooth muscle contraction can contribute to and be altered in pathological states. Passmore et al. (1985) showed that uninephrectomized rats fed a high sodium chloride diet developed higher blood pressures compared with uninephrectomized rats on sodium (without the chloride) diet suggesting that Cl^- is required for the blood pressure to be elevated. Davis et al. (1993) reported increased activity of the (Na–K–Cl) cotransporter (and increased intracellular accumulation of chloride) in the femoral artery from DOCA/salt hypertensive rats. These authors also observed enhanced inhibition of this process by bumetanide, an inhibitor of (Na–K–Cl) cotransporter, in the hypertensive rats. Bieger et al. (2004) have also shown that α_1-adrenoceptor-mediated cirazoline-induced contraction of the pulmonary artery from Dahl hypertensive rats was significantly reduced by removal of Cl^-, whereas no reduction was observed in Dahl salt-resistant normotensive rats indicating enhanced role of Cl^- in hypertension. However, the role of chloride channel activation is not

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enhanced in all forms of hypertension. It has been reported that Na–K–Cl cotransport was reduced in vascular smooth muscle cells from spontaneously hypertensive rats (O'Donnell and Owen, 1988). Also in the 2K-1C model of hypertension, the contribution of Cl^- to α_1-adrenoceptor-mediated vasoconstriction in the perfused rat mesenteric artery is reduced compared to similar preparations from normotensive rats (He and Tabrizchi, 1997). Finally, chloride channel blockade has been shown to attenuate the effect of angiotensin II on tuboglomerular feedback in normotensive WKY rats but not in spontaneously hypertensive rats (Hashimoto et al., 2004).

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. Basal vascular smooth muscle tone is elevated in pulmonary arteries from rats with monocrotaline-induced pulmonary hypertension (Wanstall et al., 1994; Nakazawa et al., 2001) compared with similar preparations from control rats. 4,4′ -Diisothiocyanato-stilbene-2,2′ -disulfonic acid (DIDS), a Cl^- channel blocker, inhibited the spontaneous tone (Nakazawa et al., 2001) suggesting that activation of Cl^- channels was involved in this spontaneous tone. However, the role of Cl^- channels in agonist-induced contractions of pulmonary artery segments from rats with monocrotaline-induced pulmonary hypertension has not been investigated. The present study was therefore designed to examine the effect of monocrotaline-induced pulmonary hypertension on the contribution of Cl^- channels to α_1-adrenoceptor-mediated noradrenaline-induced contractions of the pulmonary artery. Specifically, we studied the effect of Cl^- channel blocker (niflumic acid) and inhibitors of Cl^- transport, bumetanide (Na–K–Cl cotransport) and bicarbonate-free Krebs' solution (Cl^-/HCO_3 exchanger) on noradrenaline-induced contraction of the pulmonary artery from control and monocrotaline-treated rats.

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.

2.1. Induction of pulmonary hypertension

Pulmonary hypertension was induced by treating the rats with monocrotaline (60 mg/kg, intraperitoneally) 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 thoracic cavity was opened up and a 22-gauge needle connected to a pressure transducer was inserted into the right ventricle to record ventricular pressure. Thereafter, 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 lungs were weighed. An increase in right ventricular pressure and lung weight/body ratio were used as indices of pulmonary hypertension.

2.2. Preparation of pulmonary artery segments for isometric tension recording

The pulmonary artery segments were cleaned of any adhering connective tissues. Care was taken to preserve the integrity of the endothelium. Endothelial function was confirmed by the ability 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 4-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. In all cases, two consecutive concentration–response curves were separated by a rest period of 60 min. 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.

2.3. Effect of nifedipine on noradrenaline-induced contraction

After obtaining control concentration–response curve to noradrenaline in artery segments from control and monocrotaline-treated rats, nifedipine (10^{-6} M) was added to the bath and allowed to equilibrate with the tissue for 30 min before re-establishing concentration–

response curve to noradrenaline. Contractile response to noradrenaline in the presence of nifedipine was expressed as a percentage of the maximum response to noradrenaline obtained in the absence of nifedipine. The effect of nifedipine on KCl (60 mM)-induced contraction was also examined for comparison.

2.4. Effect of niflumic acid and bumetanide on noradrenaline-induced contraction

After establishing control concentration–response curves to noradrenaline in artery segments from control and monocrotaline-treated rats, niflumic acid (10^{-4} M) was added to the bath and allowed to equilibrate with the tissue for 30 min before reestablishing concentration–response curve to noradrenaline. The effect of niflumic acid on KCl (80 mM)-induced contraction was also examined for comparison. Similar experiments were carried out with bumetanide (10^{-4} M), a chloride transport inhibitor. The contractile response to noradrenaline in the presence of the inhibitors was expressed as a percentage of the maximum response to noradrenaline obtained in the absence of the inhibitors. The concentrations of niflumic acid and bumetanide used in this study were selected based on concentrations used by previous workers as reported in the literature.

2.5. Noradrenaline-induced contraction in low Cl^- medium

After obtaining control contractile response to noradrenaline (10^{-6} M) in normal Krebs' solution, the bath fluid was replaced with Krebs' solution containing low concentration of Cl^- (5.0 mM) and allowed to be in contact with the artery segment for 20 min. This solution was prepared by substituting sodium and potassium gluconate for sodium and potassium chloride, respectively. At the end of the equilibration period, noradrenaline was added to the bath and the contraction was recorded. The contractile response to noradrenaline in the modified Krebs' solution was expressed as a percentage of the response to noradrenaline obtained in regular Krebs' solution.

2.6. Noradrenaline-induced contraction in bicarbonate-free medium

After obtaining control contractile response to noradrenaline (10^{-6} M) in normal Krebs' solution, the bath fluid was replaced with bicarbonate-free Krebs' solution. This was prepared by replacing $NaHCO_3$ (25 mM) with HEPES (20 mM) and adjusting the pH to 7.4. This solution was allowed to be in contact with the artery segment for 20 min before noradrenaline was added to the bath. The contractile response to noradrenaline in the modified Krebs' solution was expressed as a percentage of the response to the same concentration of noradrenaline obtained in regular Krebs' solution.

2.7. Drugs

The following compounds were used in this investigation: niflumic acid and bumetanide were purchased from Tocris International, while monocrotaline and noradrenaline bitartrate were obtained from Sigma (St. Louis, MO, USA). Noradrenaline was dissolved in distilled water while niflumic acid and bumetanide were dissolved in dimethylsulphoxide (DMSO) and ethanol, respectively. The concentration of ethanol and DMSO in the bath did not exceed 0.1% and did not affect noradrenaline-induced responses. Stock solution of monocrotaline was prepared as follows: 150 mg of monocrotaline was dissolved in 1.0 ml of 1 N HCl. A total of 2.0 ml of distilled water was added and the pH was 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.

2.8. Statistical analysis

Results are presented as mean±S.E. of n experiments. Where necessary, differences between mean values were compared using Student's t-test. The difference was assumed to be significant when $P<0.05$.

3. Results

No rat died during the course of this investigation. The mean final body weight in monocrotaline-treated rats was 266.8±12.8 g (n=12), while the mean final body weight in age-matched control rats was 345.8±7.9 g (n=13). These values were significantly ($P<0.05$) different from each other. Three weeks after treatment with monocrotaline, the lung weight to body weight ratio increased significantly ($P<0.05$) from 4.1±0.2 (n=8) in control rats to 6.3±0.5 (n=8) in monocrotaline-treated rats. The right ventricular pressure also increased significantly ($P<0.05$) from 30.4±3.5 mm Hg (n=5) in control rats to 52.8±5.4 mm Hg (n=5) in rats treated with monocrotaline.

3.1. Noradrenaline-induced contraction of the rat pulmonary artery

Noradrenaline (10^{-10}–10^{-5} M) evoked concentration-dependent contractions of pulmonary artery segments from control and monocrotaline-treated rats. The contractions were reproducible as at least three to four consecutive concentration–response curves could be established without any significant change in E_{max} or sensitivity. The pD_2 values were 8.0±0.1 (n=12) and 7.9±0.1 (n=12), respectively, in control and monocrotaline-treated rats. These values were not significantly ($P>0.05$) different from each other. The maximum response to noradrenaline was however significantly ($P<0.05$) reduced in monocrotaline-treated rats (Fig. 1).

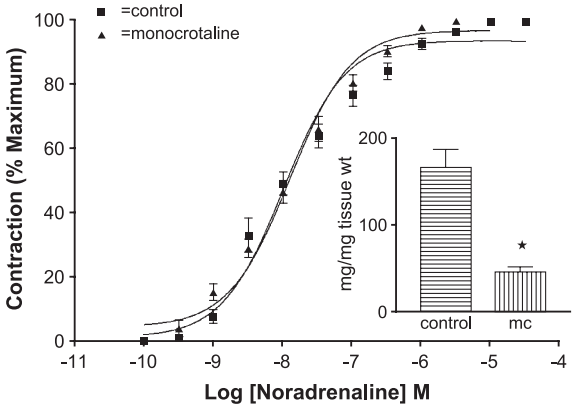

Fig. 1. Contractile effect of noradrenaline in pulmonary artery segments from control and monocrotaline-treated rats. Inset shows maximum responses to noradrenaline in artery segments from control and monocrotaline-treated rats. Each point on the graph is the mean±S.E. of 12 experiments. *P<0.05.

3.2. Effects of nifedipine on noradrenaline-induced contraction

Nifedipine (10^{-6} M) produced a rightward shift of the concentration–response curve to noradrenaline and reduced the maximal response (Fig. 2) in artery rings from control and

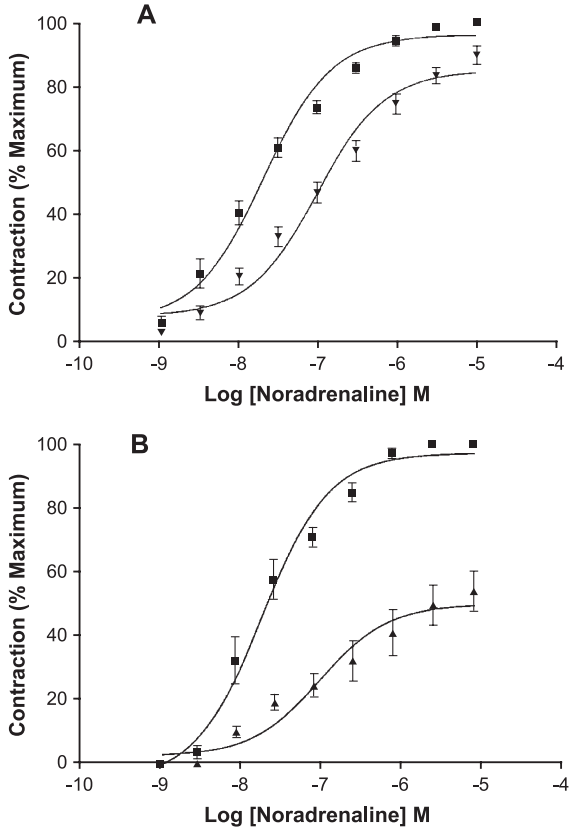

Fig. 2. Effect of nifedipine, 10^{-6} M (▲), on noradrenaline-induced (■) vasoconstrictor responses in the rat pulmonary artery from control (A) and monocrotaline-treated (B) rats (n=5). Nifedipine was allowed to equilibrate with the artery segment for 30 min before re-establishing noradrenaline concentration–response curve.

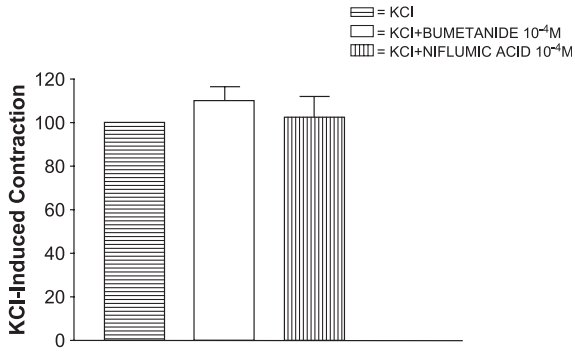

Fig. 3. Effect of bumetanide (10^{-4} M) and niflumic acid (10^{-4} M) on basal tone of pulmonary artery segments from control and monocrotaline-treated rats. (n=8 for control and n=4 for monocrotaline-treated rats).

pulmonary hypertensive rats. The pD_2 value was reduced from 7.7±0.1 to 7.0±0.1, while the maximum response was reduced by approximately 15%. Corresponding pD_2 values in pulmonary hypertensive rats were 7.7±0.1 before and 6.9±0.2 in the presence of nifedipine, respectively. The maximum response was reduced by approximately 50%. The reduction in maximum response to noradrenaline was greater in pulmonary hypertensive ring segments.

3.3. Effect of niflumic acid on noradrenaline-induced contraction

Niflumic acid (10^{-4} M) reduced basal tone in some but not all the preparations from control rats. The mean reduction in basal tone (Fig. 3) was 6.2±2.2% (expressed relative to noradrenaline-induced tone). The same concentration of niflumic acid (10^{-4} M) reduced basal tone in all preparations from monocrotaline-treated rats. The mean reduction of 28.8±2.2% was significantly (P<0.05) greater than in control artery segments. Niflumic acid (10^{-4} M) had no effect on KCl (80 mM)-induced contraction of the rat pulmonary artery (Fig. 4) but shifted noradrenaline concentration response curve to the right and reduced the maximum response (Fig. 5). The pD_2 value was reduced from 7.2±0.1

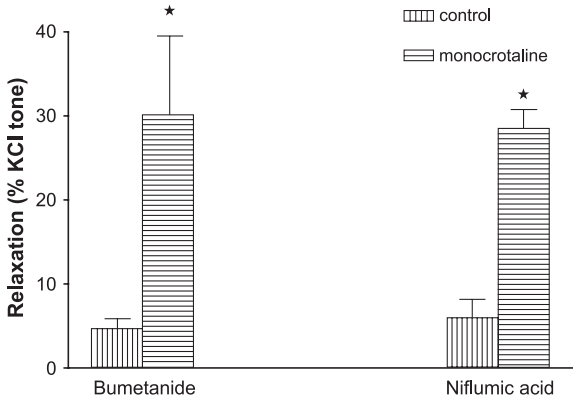

Fig. 4. Effect of bumetanide (10^{-4} M) and niflumic acid (10^{-4} M) on KCl-induced contraction of the rat pulmonary artery. Each point on the graph is the mean±S.E. of four experiments. *P<0.05.

to 6.6±0.1 (n=4), while the maximum response was reduced by approximately 30%. Niflumic acid (10^{-4} M) also inhibited noradrenaline-induced contraction in artery segments from monocrotaline-treated rats shifting the concentration response curve to the right and significantly reducing the maximum response by approximately 90%.

3.4. Effect of bumetanide on noradrenaline-induced contraction

Bumetanide (10^{-4} M) reduced basal tone in some but not all the preparations from control rats. The mean reduction in basal tone (Fig. 3) was 4.7±1.2% (expressed relative to noradrenaline-induced tone). The same concentration of bumetanide (10^{-4} M) reduced basal tone in all preparations from monocrotaline-treated rats. The mean reduction of 30.2±9.3% was significantly (P<0.05) greater than in control artery segments. Bumetanide had no effect on KCl (80 mM)-induced contraction of the rat pulmonary artery (Fig. 4) but shifted noradrenaline concentration response curve to the right and reduced the maximum response (Fig. 6). The pD_2 value was reduced from 7.8±0.1 to 7.6±0.1 (n=4), while the maximum response was reduced by approximately 18%. In monocrotaline-treated rats, bumetanide shifted noradrenaline concentration response curve to the right and reduced the maximum response. The pD_2 value was reduced from 7.1±0.1 to 6.6±0.3 (n=4), while the maximum response was reduced by approximately 50%.

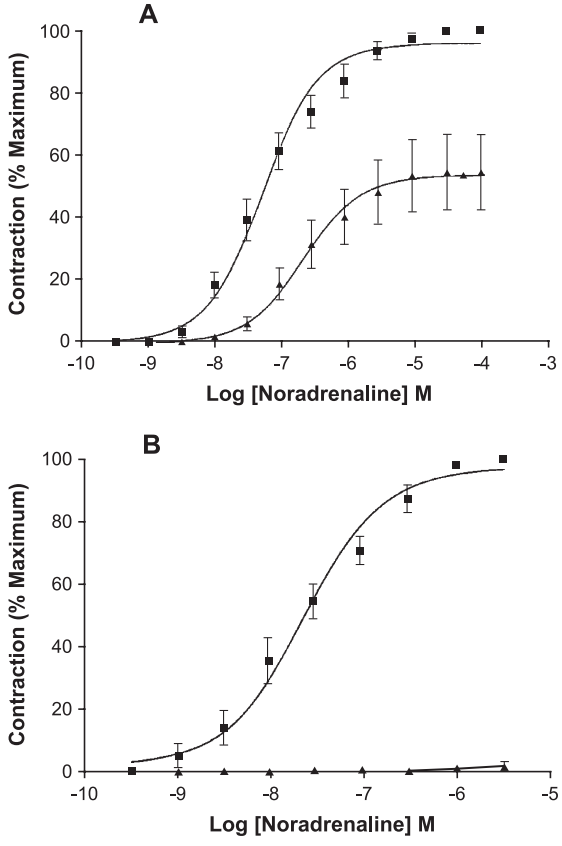

Fig. 5. Effect of niflumic acid (10^{-4} M) (▲) on noradrenaline-induced (■) contraction of pulmonary artery segments from control (A) and monocrotaline-treated (B) rats. Niflumic acid was added to the bath and allowed to equilibrate with the tissue for 30 min before re-establishing noradrenaline concentration–response curve. Each point on the graph is the mean±S.E. of five experiments.

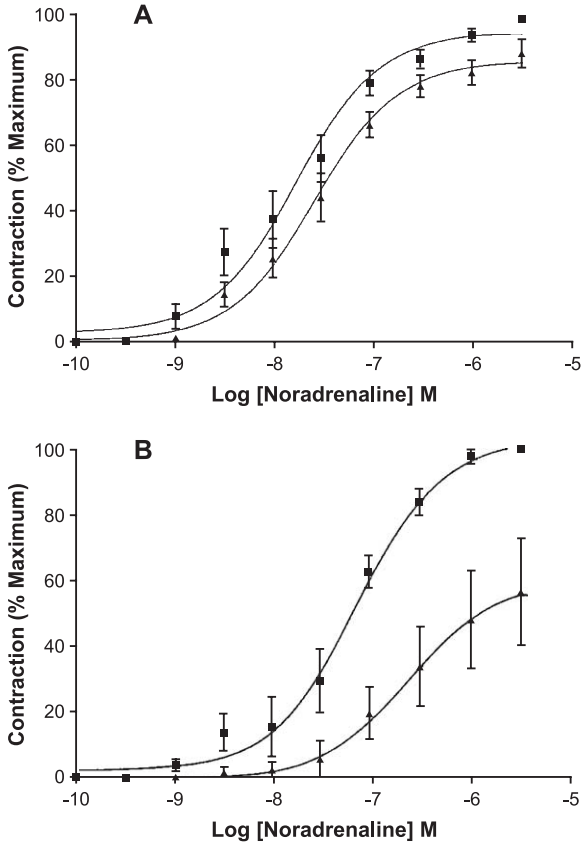

Fig. 6. Effect of bumetanide (10^{-4} M) (▲) on noradrenaline-induced (■) contraction of pulmonary artery segments from control (A) and monocrotaline-treated (B) rats. Bumetanide was added to the bath and allowed to equilibrate with the tissue for 30 min before re-establishing noradrenaline concentration–response curve. Each point on the graph is the mean±S.E. of five experiments.

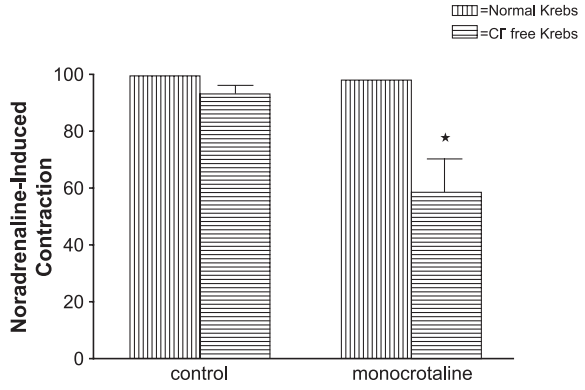

Fig. 7. Effect of a low Cl^- (5 mM) solution on noradrenaline (10^{-6} M)-induced contraction of the rat pulmonary artery isolated from control and monocrotaline-treated rats. Each point on the graph is the mean±S.E. of four experiments. *P<0.05.

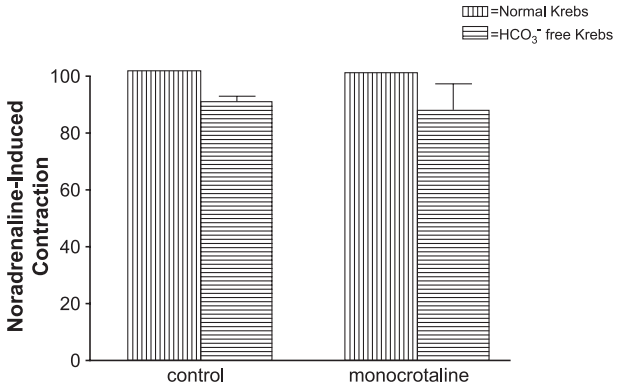

Fig. 8. Effect of bicarbonate-free solution on noradrenaline (10^{-6} M)-induced contraction of the rat pulmonary artery isolated from control and monocrotaline-treated rats. Each point on the graph is the mean±S.E. of four experiments.

3.5. Effect of low Cl^- concentration on noradrenaline-induced contraction

Replacement of the normal Krebs' solution with a low Cl^- (5.0 mM) solution produced contractions in some but not all preparations (control and monocrotaline-treated). In artery segments from control rats, this procedure did not significantly ($P>0.05$) reduce noradrenaline-induced contraction (Fig. 7). However, noradrenaline-induced contraction of pulmonary artery segments from monocrotaline-treated rats was significantly ($P<0.05$) reduced (Fig. 7).

3.6. Effect of Cl^-/HCO_3 exchanger blockade on noradrenaline-induced contraction

Inhibition of Cl^-/HCO_3 exchanger with bicarbonate-free Krebs' solution slightly but not significantly ($P>0.05$) reduced noradrenaline-induced contraction of pulmonary artery segments from control and monocrotaline-treated rats (Fig. 8). There was no significant difference ($P>0.05$) in the effectiveness of bicarbonate-free Krebs' solution between the two groups.

4. Discussion

Calcium-activated chloride channels have been identified in vascular smooth muscle. These channels are blocked by compounds like niflumic acid and 4,4′ -diisothiocyanatostilbene-2,2′ -disulfonic acid (DIDS). These compounds have been used to functionally determine the role of Cl^- channel activation in vascular smooth muscle contraction produced by a variety of agonists including noradrenaline, histamine and endothelin-1 (Criddle et al., 1996; Lamb and Barna, 1998; Hyvelin et al., 1998; Yuan, 1997; Bieger et al., 2004; Dai and Zhang, 2001a,b). Elevated pulmonary artery vascular tone is a well-documented feature of monocrotaline-induced pulmonary hypertension (Wanstall et al., 1994; Nakazawa et al., 2001). According to Nakazawa et al. (2001), Cl^- channel activation contributed significantly to the elevated tone. This was based on the greater relaxant effect DIDS in artery segments from monocrotaline-induced pulmonary hypertensive rats. This has been confirmed in the present study where we have observed a greater relaxation of basal smooth muscle tone, by niflumic acid, a chloride channel inhibitor and bumetanide, a Na–K–Cl cotransport inhibitor, in pulmonary artery segments from monocrotaline-induced pulmonary hypertensive rats.

4.1. Effect of pulmonary hypertension on the role of Cl^- channel activation in noradrenaline-induced contraction

Activation of α_1-adrenoceptors in vascular smooth muscles is associated with increased chloride conductance subsequent to Ca^{2+} release from intracellular stores. This is followed by opening of voltage-dependent Ca^{2+} channels, influx of extracellular Ca^{2+} and contraction. Intracellular accumulation of Cl^- involves two separate inward transport mechanisms, Na–K–Cl cotransport and Cl^-/HCO_3-exchanger operating in parallel. A third transport system, pump III, has also been identified but so far, in a few arteries including femoral (Chipperfield et al., 1993), human umbilical and placental arteries (Davis et al., 2000). In this study, it was observed that inhibiting Na–K–Cl cotransport with bumetanide reduced noradrenaline-induced contraction of the pulmonary artery segments consistent with a role for Cl^- transport through this pathway in these contractions. The concentration of bumetanide used in this study had no effect on KCl-induced contraction of the rat pulmonary artery (this study) possibly ruling out any direct influence of bumetanide on Ca^{2+} influx in this arterial smooth muscle. The inhibitory effect of bumetanide was enhanced in pulmonary hypertensive rats suggesting increased intracellular accumulation of Cl^- through increased activity of Na–K–Cl cotransport in pulmonary hypertension. Increased activity of the Na–K–Cl cotransport has been reported in arterial smooth muscles from DOCA-salt hypertensive rats (Davis et al., 1994; Brown et al., 1999). On the other hand, inhibition of Cl^-/HCO3 exchanger using bicarbonate-free Krebs' solution did not significantly affect noradrenaline-induced contraction in pulmonary artery segments from control and monocrotaline-treated rats. This would suggest that Cl^-/HCO_3 exchanger is not involved to any significant extent in intracellular accumulation of Cl^- in this arterial smooth muscle.

Results obtained from the few studies that have investigated the effect of Cl^- channel blockers on α_1-adrenoceptor-mediated contraction in pathological states vary with the model of hypertension. While niflumic acid was equally effective in reducing cirazoline-induced vasoconstriction in the perfused mesenteric artery of sham operated and 2K-1C rats (He and Tabrizchi, 1997), indanyloxyacetic acid, another Cl^- channel blocker was more effective in reducing cirazoline-induced contraction in aortic rings from Dahl salt-sensitive hypertensive rats compared with Dahl salt-resistant normotensive controls (Tabrizchi and Duggan, 2000). This

latter result would suggest a greater role for Cl^- channels in vessels from hypertensive rats. This is supported by the observation that Cl^- substitution was more effective in reducing cirazoline-induced contractions in pulmonary artery segments from Dahl salt-sensitive hypertensive rats (Bieger et al., 2004). We have compared the effect of niflumic acid on noradrenaline-induced contraction of pulmonary artery segments from control and monocrotaline-treated rats. The concentration of niflumic acid used in this study had no effect on KCl-induced contraction of the rat pulmonary artery (this study) indicating that niflumic acid was not directly influencing Ca^{2+} influx in this arterial smooth muscle. Our results showed that niflumic acid inhibited noradrenaline-induced contraction in pulmonary artery segments from both groups confirming a role for Cl^- channels in noradrenaline-induced contractions of this vessel. The results also showed that niflumic acid was significantly more effective in artery segments from rats treated with monocrotaline suggesting a greater role for Cl^- channels in monocrotaline-induced pulmonary hypertension. The fact that Cl^- substitution was more effective in reducing noradrenaline-induced contraction in artery segments from monocrotaline-treated rats is consistent with this observation. Nakazawa et al. (2001) studied the expression of Cl^- channels in the pulmonary artery and concluded that there was no difference between control and monocrotaline-treated rats and therefore that the increased role of Cl^- channels was due to increased activity and not increased expression of Cl^- channel mRNA.

Our results have also shown that sensitivity of noradrenaline-induced contraction of the pulmonary artery to nifedipine, an L-type voltage operated Ca^{2+} channel inhibitor, was enhanced in pulmonary hypertension confirming previous reports by Tabritzchi et al. (1995). We are therefore proposing that the increased Cl^- channel activity in the pulmonary artery during pulmonary hypertension leads to increased membrane depolarization and increased influx of extracellular Ca^{2+} through L-type Ca^{2+} channels. This could explain the increased efficacy of Ca^{2+} channel blockers in pulmonary hypertension.

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