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The effect of the endothelin ET_A receptor antagonist CI-1020 on hypoxic pulmonary vasoconstriction

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Abstract

The mechanism of Hypoxic Pulmonary Vasoconstriction is unknown. The role of endothelin-1 in hypoxic pulmonary vasoconstriction was studied in precontracted small and large pulmonary arteries using the endothelin ET_A receptor antagonist sodium-2-benzol [1,3]dioxol-5-yl-4-(4-methoxyphenyl)-4-oxo-3-(3,4,5-trimethoxy-benzyl)-but-2-enoate (CI-1020). Small rat pulmonary arteries exhibit a mixed endothelin ET_A receptor and endothelin ET_{B2} receptor population whereas large rat pulmonary arteries contain only endothelin ET_A receptors. CI-1020 inhibited endothelin-1 in small vessels via endothelin ET_A receptor blockade (1 and 10 μM) and at high concentrations via endothelin ET_A receptor and endothelin ET_{B2} receptor blockade (100 μM). CI-1020 (0.01, 0.1 and 1 μM) inhibited endothelin-1 in large vessels via endothelin ET_A receptor blockade alone. CI-1020 (1, 10 and 100 μM) significantly reduced hypoxic pulmonary vasoconstriction in small vessels, by -9.8 ± 1.4, -9.2 ± 2.3 and -8.0 ± 1.7% 80 mM K^+, respectively, compared to $+2.5 \pm 4.2$% with vehicle ($P < 0.05$). CI-1020 (0.01, 0.1 and 1 μM) had no significant effect upon hypoxic pulmonary vasoconstriction in large vessels. In small, but not large, pulmonary arteries hypoxic pulmonary vasoconstriction is due in part to the action of endothelin-1 at the endothelin ET_A receptor. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Hypoxic pulmonary vasoconstriction; Pulmonary artery; CI-1020; Endothelin-1; Endothelin ET_A receptor; Endothelin ET_{B2} receptor

1. Introduction

The response of pulmonary arteries to hypoxia was first described by Von Euler and Liljestrand (1946), who noted that the pulmonary arterial blood pressure of the cat was raised when exposed to hypoxia. Vasoconstriction occurs as an essential regulatory mechanism for the lung, whereby ventilation is matched with perfusion, resulting in the optimisation of gas exchange. Under pathophysiological conditions hypoxic pulmonary vasoconstriction may also be one of the underlying causes of numerous disease states including pulmonary hypertension. The precise mechanism of hypoxic pulmonary vasoconstriction has yet to be fully characterised, despite intensive research. Hypoxia is known to elicit closure of potassium channels (Post et al., 1992; Robertson et al., 1992; Turner and Kozlowski, 1997; Walker et al., 1998) with subsequent membrane depolarisation and activation of L-type calcium channels, although how these channel effects are triggered remains obscure.

Initial research into hypoxic pulmonary vasoconstriction relied heavily on the hypothesis that hypoxia triggered the release of an endogenous vasoconstrictive agent. Numerous potential mediators were studied but none was shown to be a prerequisite for hypoxic pulmonary vasoconstriction. A relatively recent discovery is the vasoconstrictor endothelin-1 (Yanagisawa et al., 1988). Evidence of endothelin-1 production in the lung (Kitamura et al., 1989), and the presence of endothelin receptors in the lung (Koseki et al., 1989) coupled with the observation that endothelin-1 produces prolonged pulmonary vasoconstriction similar to that due to hypoxia in vivo, raised the question of the potential role of this peptide in hypoxic pulmonary vasoconstriction. Endothelin receptors are classified into two main families; endothelin ET_A receptors and endothelin ET_B receptors. Endothelin ET_B receptors can be subdivided as follows; endothelin ET_{B1} receptors are located on

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endothelial cells and mediate vasodilatation via endothelial derived relaxing factor (nitric oxide) release, endothelin ET_{B2} receptors are located on smooth muscle cells and mediate constriction (MacLean and McCulloch, 1998).

Involvement of endothelin-1 in hypoxic pulmonary vasoconstriction is also implied by observations of elevated endothelin-1 plasma levels during hypoxia in the rat (Shirakami et al., 1991; Helset et al., 1995) and in humans at altitude (Goerre et al., 1995) or with pulmonary hypertension (Stewart et al., 1991; Giaid et al., 1993). In addition endothelin-1 has also been shown to cause membrane depolarisation of rat pulmonary arterial myocytes via inhibition of voltage sensitive potassium channels (Salter and Kozlowski, 1998; Shimoda et al., 1998a) via endothelin ET_A receptors and also endothelin ET_{B2} receptors (Salter and Kozlowski, 1998). These observations provide strong circumstantial evidence for the involvement of endothelin-1 in hypoxic pulmonary vasoconstriction.

Despite evidence of endothelin ET_A receptor antagonism (Chen et al., 1993; Bonvallet et al., 1994; DiCarlo et al., 1995; Oparil et al., 1995; Chen et al., 1997) and mixed endothelin ET_A receptor and endothelin ET_B receptor antagonism (Chen et al., 1995) preventing the development of pulmonary hypertension in the rat, to date only one report exists of endothelin-1 mediating hypoxic pulmonary vasoconstriction in isolated vessels from adult animals: Shimoda et al. (1998b) showed endothelin ET_A receptor antagonism to abolish hypoxic pulmonary vasoconstriction in isolated pig pulmonary arterial rings. In contrast neither endothelin ET_A receptor (Douglas et al., 1993; Ishizaki et al., 1995) nor combined endothelin ET_A receptor and endothelin ET_{B2} receptor (Lazor et al., 1996) blockade significantly attenuated hypoxic pulmonary vasoconstriction in isolated rat or canine pulmonary arteries.

We have previously shown that hypoxic pulmonary vasoconstriction of rat pulmonary arteries in vitro occurs as a four phase response (Rogers and Morice, 1993; Woodmansey et al., 1993; Wanstall and O'Brien, 1996) which is greatly increased in magnitude once the vessels have been primed with an agonist that elevates the level of intracellular free calcium (Hoshino et al., 1988; Teng and Barer, 1994). Following constriction to the agonist, a hypoxic oxygen tension at 37°C results initially in vasodilation (Phase 1), followed by a large hypoxic contraction (Phase 2), and further vasodilation (Phase 3) before the eventual development of a second sustained hypoxic contraction (Phase 4) (Fig. 1). Using the novel endothelin ET_A receptor selective antagonist, sodium-2-benzol [1,3]dioxol-5-yl-4-(4-methoxyphenyl)-4-oxo-3-(3,4,5-trimethoxy-benzyl)-but-2-enoate (CI-1020), we have studied the role of endothelin-1 in all four of the phases of the response of isolated rat pulmonary arteries to hypoxia, and also studied the effect of CI-1020 upon the endothelin-1 concentration–response curve. The effect of endothelin ET_A receptor antagonism via CI-1020 upon hypoxic pulmonary vasoconstriction has yet to be studied, and inhibition of the endothelin-1 concentration–response curve with CI-1020 in isolated pulmonary arteries has only been undertaken in rabbit vessels (Walker et al., 1995).

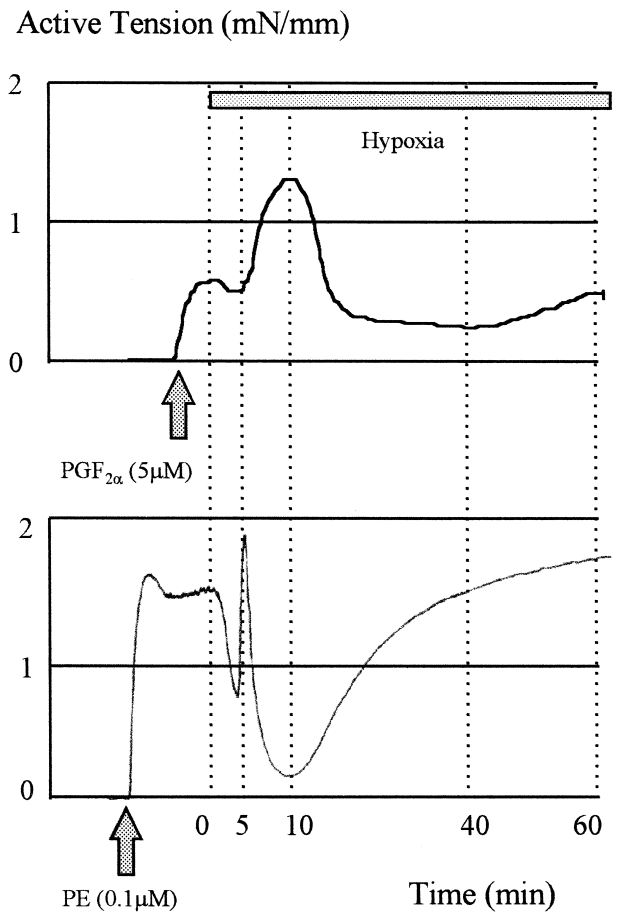

Fig. 1. The four phase response to hypoxia of small (top) and large (bottom) isolated rat pulmonary arteries following preconstriction with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$: 5 μM) and phenylephrine (PE: 0.1 μM), respectively.

2. Materials and methods

2.1. Vessel preparation

Male Wistar rats ($n = 55$, mean body weight = 290 ± 4 g) were killed by intra-peritoneal injection of sodium pentobarbitone (15 mg/100 g body weight) and either the main (large) pulmonary artery ($n = 33$, mean internal diameter = 2.27 ± 0.05 mm) or the lungs were removed and placed in chilled physiological saline solution. The lungs were pinned on an agarose plate, the main bronchus opened longitudinally and small side branch pulmonary arteries ($n = 59$, mean internal diameter = 478 ± 27 μm) were carefully dissected. Large and small vessels of similar diameter were used in all sets of experiments.

Small pulmonary arteries were mounted on two 40 μm stainless steel wires in a small vessel myograph (Cambus-

tion, UK), as described by Rogers et al. (1992). The myograph baths contained physiological saline (120 mM NaCl, 4.7 mM KCl, 1.17 mM $MgSO_4$, 25 mM $NaHCO_3$, 1.18 mM KH_2PO_4, 5.5 mM glucose, 2.5 mM $CaCl_2$ and 26.9 μM EDTA) warmed to 37°C and bubbled with 95% O_2/5% CO_2. Length–tension curves were plotted for each vessel before loading to a tension equivalent to a trans-mural pressure of 17.5 mm Hg, the normal in vivo resting tension.

Large pulmonary arteries were placed in vertical jacketed 25 ml organ baths filled with physiological saline

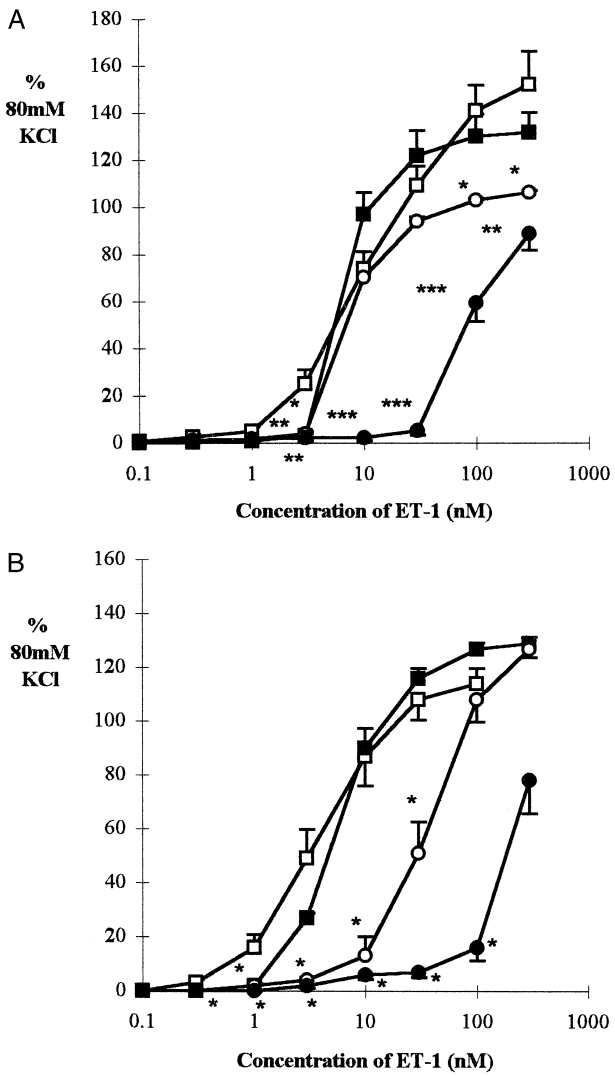

Fig. 2. (a) Concentration–response curves for endothelin-1 in the presence of 1 μM CI-1020 (■), 10 μM CI-1020 (○), 100 μM CI-1020 (●) or distilled water (□) in small isolated rat pulmonary arteries. Values are expressed as mean ± S.E.M. * Represents significance of $P < 0.05$. ** Represents significance of $P < 0.01$. *** Represents significance of $P < 0.001$ from vehicle via Mann–Whitney–Wilcoxon rank test. (b) Concentration–response curves for endothelin-1 in the presence of 0.01 μM CI-1020 (■), 0.1 μM CI-1020 (○), 1 μM CI-1020 (●) or PSS (□) in large isolated rat pulmonary arteries. Values are expressed as mean ± S.E.M. * Represents significance of $P < 0.05$ from vehicle via Mann–Whitney–Wilcoxon rank test.

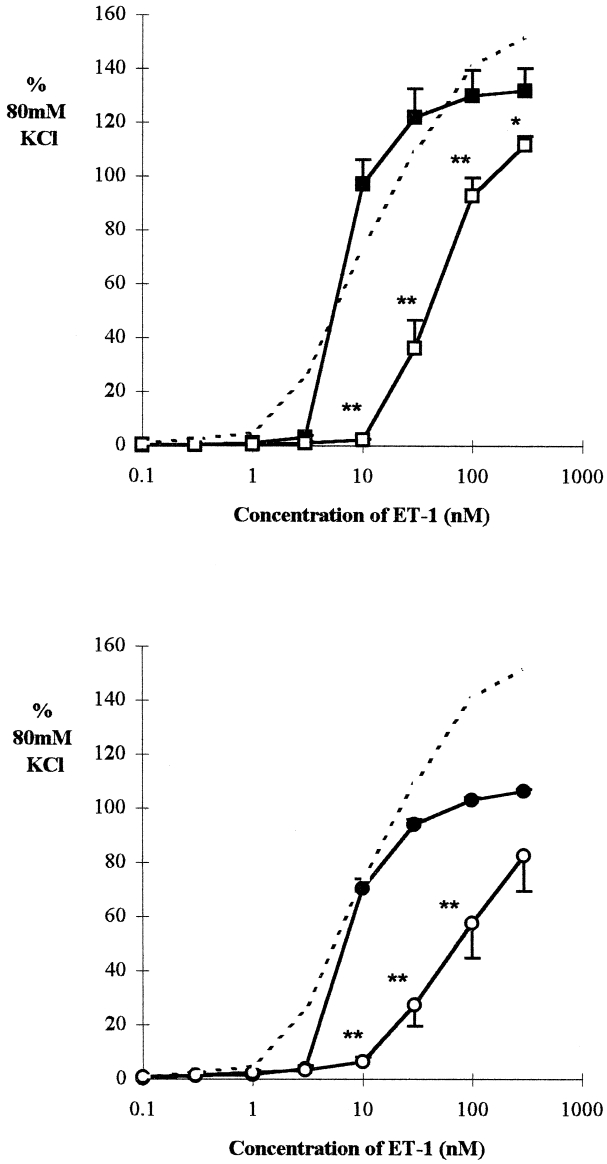

Fig. 3. Concentration–response curves for endothelin-1 in the presence of 1 μM CI-1020 (■), compared to 1 μM CI-1020 + 10 μM BQ788 (□) (top panel), and 10 μM CI-1020 (●), compared to 10 μM CI-1020 + 10 μM BQ788 (○) (bottom panel), in small isolated rat pulmonary arteries. Control endothelin-1 curves are shown for reference with a dotted line. Values are expressed as mean ± S.E.M. * Represents significance of $P < 0.05$. ** Represents significance of $P < 0.01$ via Mann–Whitney–Wilcoxon rank test.

(118 mM NaCl, 5.9 mM KCl, 0.72 mM $MgSO_4$, 25 mM $NaHCO_3$, 11.7 mM glucose, 1.5 mM $CaCl_2$ and 24 μM NaEDTA) at 37°C and bubbled with 95% O_2/5% CO_2. Vessels were loaded to a resting tension of 10 mN, representative of the in vivo trans-mural pressure (Wanstall et al., 1995).

All vessels were left to equilibrate at their resting tension for 1 h prior to double exposure to 80 mM potassium chloride to confirm that the vessels were reacting consistently to a set concentration of agonist. Subsequent responses were standardised via expression as a percentage

of the mean response to 80 mM potassium chloride. Mean 80 mM potassium chloride contractions in small and large pulmonary arteries were 3.41 ± 0.11 mN/mm ($n = 59$) and 4.39 ± 0.11 mN/mm ($n = 33$), respectively. Reproducibility and sensitivity to potassium chloride in all sets of small and large vessels were comparable throughout.

2.2. The effect of CI-1020 on the endothelin-1 concentration–response curve

Small ($n = 39$) and large ($n = 15$) pulmonary arteries were exposed to either CI-1020 (0.01, 0.1, 1, 10 or 100 μM) or distilled water or physiological saline in the dark (due to the light sensitivity of CI-1020) for 30 min before exposure to cumulative concentrations of endothelin-1 (0.1–300 nM). Concentration–response curves to endothelin-1 (0.1–300 nM) were also produced in small pulmonary arteries following 30 min incubation in the dark with the endothelin ET_B receptor antagonist, [*N*-[(2*R*,6*S*)-2,6-dimethyl-piperidino-carbonyl]-4-methyl-D-leucyl]-[*N*-omega (methoxycarbonyl)-D-tryptophanyl]-D-Nle-Ona (BQ788) (10 μM) in the presence of CI-1020 (1 or 10 μM).

Based on the results of these experiments, concentrations of CI-1020 of 1, 10 and 100 μM were used in small pulmonary arteries, and 0.1, 1 and 10 μM in large pul-

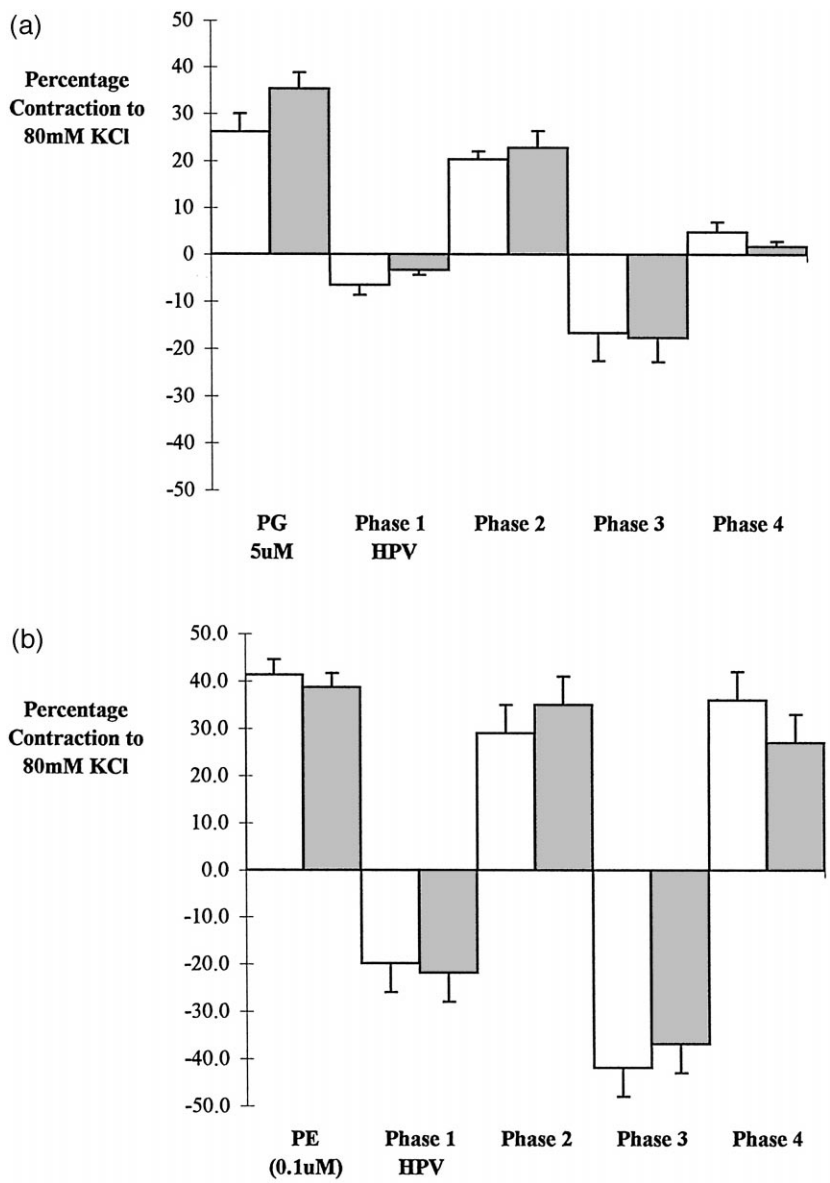

Fig. 4. (a) Bar chart showing the repeatability of responses to prostaglandin $F_{2\alpha}$ (5 μM) and hypoxia (four phases) in small isolated rat pulmonary arteries pre- (white bars) and post-vehicle-distilled water (grey bars). No significant differences are seen. Differences between pre- and post-vehicle responses are used as control values and compared to differences in pre- and post-CI-1020 values. (b) Bar chart showing the repeatability of responses to phenylephrine (0.1 μM) and hypoxia (four phases) in large isolated rat pulmonary arteries pre- (white bars) and post-vehicle-PSS (grey bars). No significant differences are seen. Differences between pre- and post-vehicle responses are used as control values and compared to differences in pre- and post-CI-1020 values.

monary arteries for the study of hypoxic pulmonary vasoconstriction.

2.3. The effect of CI-1020 on hypoxic pulmonary vasoconstriction

Small ($n = 32$) and large ($n = 18$) pulmonary arteries were preconstricted with prostaglandin $F_{2\alpha}$ (5 μM) and phenylephrine (0.1 μM), respectively. Phenylephrine was used in large pulmonary arteries since contractions to prostaglandin $F_{2\alpha}$ tended to fluctuate. The 'control' response was produced by changing the gas from 95% O_2/5% CO_2 to 95% N_2/5% CO_2 until the fourth phase of the hypoxic response had reached equilibrium, or for a total of 60 min if no Phase 4 was seen. Each phase was recorded as the change in tension from the previous peak or trough.

Following washout, replacing the original gas and re-establishment of the initial baseline, the vessels were exposed to either CI-1020 (0.1, 1, 10 or 100 μM) or distilled water or physiological saline in the dark for 30 min. Vessels were then preconstricted with prostaglandin $F_{2\alpha}$ or phenylephrine before the 'test' hypoxic response was obtained. Differences between test and control responses from vessels exposed to CI-1020 were compared with differences between test and control responses in vessels exposed to vehicle. The contractions to prostaglandin $F_{2\alpha}$ and phenylephrine, and each of the four phases of the control response to hypoxia were not significantly different in the various series of experiments ($P > 0.05$; Kruskal–Wallis non-parametric analysis of variance, ANOVA). Hence, the values cited in Section 3 represent the pooled data.

2.4. Solutions and drugs

Prostaglandin $F_{2\alpha}$ was obtained from the Royal Hallamshire Hospital pharmacy, Sheffield. BQ788 was obtained from Sigma, Poole, UK or Sigma, Australia. Endothelin-1 was obtained from Sigma, Poole, UK or Auspep, Australia. CI-1020 was a gift from Parke-Davis, Ann Arbor, MI, USA. Phenylephrine was obtained from Sigma, Australia and dissolved in 10 mM hydrochloric acid to give a 10 mM stock solution. All other drugs were dissolved in distilled water and diluted in either distilled water or physiological saline.

2.5. Statistical analysis

Values are expressed as mean ± S.E.M. and analysed via the Mann–Whitney–Wilcoxon rank test, Kruskal–Wallis non-parametric analysis of variance ANOVA, or Students paired or unpaired *t*-test where appropriate. Significance was assumed with values of $P < 0.05$.

3. Results

3.1. The effect of CI-1020 on the endothelin-1 concentration–response curve

Inhibition of the endothelin-1 concentration–response curves by CI-1020 in small and large pulmonary arteries are shown in Fig. 2a and b, respectively. In small pulmonary arteries a marked rightward shift of the endothelin-1 curve was produced by 100 μM CI-1020, with a reduction in E_{max} occurring in the presence of 10 μM CI-1020. Slight inhibition of the response to 3 nM endothelin-1 curve was also produced by 1 μM CI-1020. In large pulmonary arteries a parallel rightward shift of the endothelin-1 curve was produced by 0.1 and 1 μM CI-1020. Slight inhibition of the response to 1 nM endothelin-1 curve was produced by 0.01 μM CI-1020.

Inhibition of the endothelin-1 concentration–response curves by CI-1020 in the absence and presence of BQ788 (10 μM) in small pulmonary arteries are shown in Fig. 3. The shift in the endothelin-1 curve by a combination of 1 or 10 μM CI-1020 together with 10 μM BQ788 was greater than with CI-1020 alone.

Table 1
Small pulmonary artery: pre-treatment response to hypoxia (four phases) and changes in response induced by Cl-1020 or vehicle

	Treatment	Pre-treatment response[a] or change in response[b] (% of K^+)			
		Phase 1, relaxation	Phase 2, contraction	Phase 3, relaxation	Phase 4, contraction
Pre-treatment response[a]	None ($n = 32$)	5.2 ± 0.7	21.3 ± 1.4	21.7 ± 2.8	4.8 ± 0.9
Change in response[b]	Vehicle ($n = 8$)	−3.2 ± 1.9	+2.5 ± 4.2	+1.0 ± 6.7	−3.1 ± 2.0
	Cl-1020 1 μM ($n = 8$)	−2.9 ± 0.8	−9.8 ± 1.4[c]	−11.2 ± 5.0	−5.7 ± 2.0
	Cl-1020 10 μM ($n = 8$)	−1.5 ± 1.5	−9.2 ± 2.3[c]	−7.9 ± 4.2	−6.2 ± 2.1
	Cl-1020 100 μM ($n = 8$)	−2.5 ± 0.9	−8.0 ± 1.7[c]	−9.6 ± 3.2	−2.5 ± 1.3

Mean values ± S.E.M. are shown. n = number of vessels.
Data are expressed as a percentage of the reference contraction to 80 mM KCl.
[a] Pre-treatment responses in the four different series of experiments (corresponding to vehicle and three concentrations of CI-1020) were not significantly different ($P > 0.05$; Kruskal–Wallis non-parametric ANOVA) and have therefore been pooled.
[b] Changes in response are in the same unit as the pre-treatment response: + indicates an increase and − a decrease in the size of the response.
[c] Value significantly different from the corresponding post-vehicle value ($P < 0.05$; Mann–Whitney–Wilcoxon rank test).

Table 2
Large pulmonary artery: pre-treatment response to hypoxia (four phases) and changes in response induced by Cl-1020 or vehicle

	Treatment	Pre-treatment response[a] or change in response[b] (% of K^+)			
		Phase 1, relaxation	Phase 2, contraction	Phase 3, relaxation	Phase 4, contraction
Pre-treatment response[a]	None ($n = 18$)	17.0 ± 0.8	26.9 ± 1.7	44.6 ± 2.1	35.5 ± 1.2
Change in response[b]	Vehicle ($n = 6$)	$+3.0 \pm 1.2$	$+9.3 \pm 2.0$	-5.7 ± 4.1	-10.9 ± 3.1
	Cl-1020 0.1 μM ($n = 4$)	$+0.2 \pm 0.5$	$+1.5 \pm 2.2$	-6.9 ± 3.3	-10.3 ± 2.6
	Cl-1020 1 μM ($n = 4$)	$+3.3 \pm 0.8$	$+7.6 \pm 3.4$	-6.6 ± 3.4	-10.2 ± 0.8
	Cl-1020 10 μM ($n = 8$)	$+3.1 \pm 0.8$	$+0.1 \pm 2.4$	-13.9 ± 4.4	-8.3 ± 3.3

Mean values ± S.E.M. are shown. n = number of vessels.
Data are expressed as a percentage of the reference contraction to 80 mM KCl.
[a]Pre-treatment responses in the four different series of experiments (corresponding to vehicle and three concentrations of CI-1020) were not significantly different ($P > 0.05$; Kruskal–Wallis non-parametric ANOVA) and have therefore been pooled.
[b]Changes in response are in the same unit as the pre-treatment response: + indicates an increase and − a decrease in the size of the response. None of the post CI-1020 values were significantly different from the corresponding post vehicle value ($P > 0.05$; Mann–Whitney–Wilcoxon rank test).

3.2. The effect of CI-1020 on hypoxic pulmonary vasoconstriction

The pre-contractions did not alter significantly in magnitude in the two sizes of vessel small pulmonary artery ($n = 32$) $38.3 \pm 3.2\%$ $n = 32$; large pulmonary artery ($n = 18$) $41.6 \pm 2.1\%$ ($P < 0.05$). The four phases of the response to hypoxia had a markedly different time-course in the different size vessels (Fig. 1) with Phase 2 being far more transient in the large pulmonary arteries.

CI-1020 had no significant effect on baseline tension at any of the concentrations studied. It also had no effect on the pre-contractions to prostaglandin $F_{2\alpha}$ or phenylephrine, except at a concentration of 100 μM in small pulmonary arteries, where a significant decrease in the prostaglandin $F_{2\alpha}$ contraction occurred of $18.0 \pm 4.5\%$ ($P < 0.01$). However, the initial contraction to prostaglandin $F_{2\alpha}$ in this group of vessels was significantly higher than the other groups $50.4 \pm 6.1\%$ compared to $34.2 \pm 5.7\%$ ($P < 0.05$) whereas post drug responses are not significantly different $32.4 \pm 5.1\%$ compared to $37.7 \pm 4.1\%$ ($P > 0.1$). Consequently this may not represent inhibition of prostaglandin $F_{2\alpha}$ by CI-1020, but a different initial prostaglandin $F_{2\alpha}$ responsiveness in this set of vessels.

The four phase response to hypoxia was reproducible following the initial hypoxic exposure. In both small and large pulmonary arteries the vehicle (distilled water or PSS) had no effect (Fig. 4a and b). In small pulmonary arteries CI-1020 (1, 10 and 100 μM) significantly reduced, but did not abolish Phase 2 of hypoxic pulmonary vasoconstriction and had no effect upon any other phase (Table 1). The degree of inhibition was comparable for all three concentrations studied. In contrast, in large pulmonary arteries CI-1020 (0.1, 1 or 10 μM) had no significant effect on any phase of the hypoxic response (Table 2).

4. Discussion

The results of this study highlight the involvement of endothelin-1 in the response of small isolated rat pulmonary arteries to hypoxia. We have shown consistent inhibition of the first contractile phase (Phase 2) of hypoxic pulmonary vasoconstriction in these vessels, by the novel endothelin ET_A receptor antagonist CI-1020. In contrast, in large isolated rat pulmonary arteries CI-1020 had no significant effect.

CI-1020 (previously known as PD156707) is one of a newly developed series of non-peptide, γ-hydroxy butenolide endothelin ET_A receptor antagonists (Patt et al., 1997). Three studies previously report antagonism of endothelin-1 by CI-1020 in isolated vessels: the endothelin-1 concentration–response curve was shifted to a higher concentration range by CI-1020 (0.03 and 0.1 μM—30 min exposure) in human, isolated coronary arteries, a preparation which has no identifiable endothelin ET_{B2} receptors (Maguire et al., 1996). Further work with CI-1020 in the human saphenous vein and left internal mammary artery demonstrated similar antagonism (Maguire et al., 1997). Walker et al. (1995) showed similar concentrations of CI-1020 to be effective in the rabbit femoral artery and rat aorta, both preparations having a receptor population consisting predominantly of endothelin ET_A receptors. In preparations with a mixed endothelin ET_A receptor and endothelin ET_{B2} receptor population such as the rabbit pulmonary artery and rat trachea, higher concentrations of CI-1020 (10 and 100 μM) are required for endothelin-1 antagonism (Walker et al., 1995).

Our isolated vessel results agree with these observations. CI-1020 (0.1 and 1 μM) was sufficient to produce a rightwards shift in the endothelin-1 concentration–response curve in large rat pulmonary arteries, previously shown to contain only endothelin ET_A receptors (O'Donnell and Kay, 1995). In the small vessels, a preparation exhibiting both endothelin ET_A receptors and endothelin ET_{B2} receptors (MacLean et al., 1994; Bialecki et al., 1997; Higashi et al., 1997), slight inhibition was observed in the presence of 1 and 10 μM CI-1020, whereas a marked rightwards shift in the endothelin-1 concentration–response curve was produced only by the high concentration of CI-1020 (100 μM). The inhibition of the

endothelin-1 curve at this concentration of CI-1020 results in a pA2 value of approximately 5 (4.72 assuming the last contraction is maximal) which corresponds to endothelin ET_{B2} receptor inhibition (Walker et al., 1995). Consequently it would appear that in small rat pulmonary arteries inhibition at the lower concentrations of CI-1020 (1 and 10 μM) corresponds to ET_A antagonism; (similar dose ratios are seen at these concentrations), whereas at the highest concentration (100 μM) both endothelin ET_A receptor and endothelin ET_{B2} receptors are blocked. This data would also suggest that in this preparation endothelin ET_{B2} receptors predominate, which agrees with the report of MacLean et al. (1994).

In the small pulmonary arteries additional experiments were undertaken with CI-1020 (1 and 10 μM) in the presence of the endothelin ET_{B2} receptor antagonist BQ788 (10 μM). One and 10 μM CI-1020 together with BQ788 (10 μM) caused a marked rightward shift in the endothelin-1 curve (Fig. 3) which approached that produced with 100 μM CI-1020 alone (Fig. 5). These results strongly support the previous conclusion; that in small pulmonary arteries marked inhibition of endothelin-1 only occurs when both endothelin ET_A receptor and endothelin ET_{B2} receptors are blocked, and that the effect of 100 μM CI-1020 represents antagonism of both these receptors. At concentrations of 1 and 10 μM CI-1020 is acting only on endothelin ET_A receptors.

The effect of CI-1020 on hypoxic pulmonary vasoconstriction was subsequently studied. Hypoxic pulmonary vasoconstriction of rat pulmonary arteries in vitro is consistently represented by four phases in both small and large vessels (Fig. 1). Phase 2 is considered to be the physiologically relevant phase since it occurs over the range of oxygen tensions known to stimulate hypoxic pulmonary vasoconstriction in vivo and in isolated lungs (Teng and Barer, 1995).

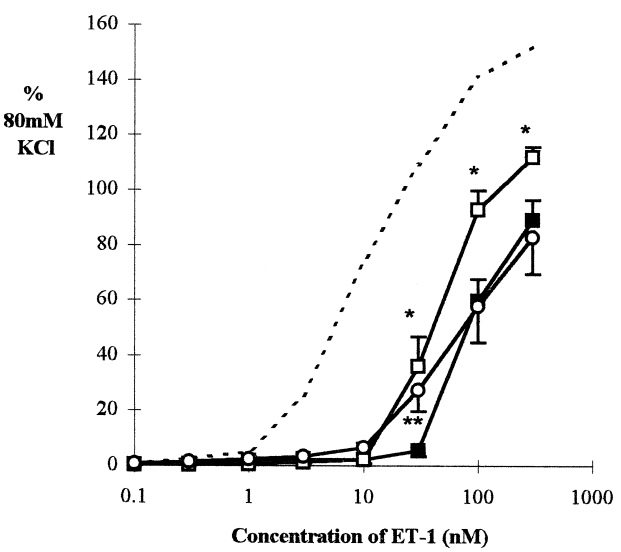

Fig. 5. Concentration–response curves for endothelin-1 in the presence of 1 μM CI-1020 + 10 μM BQ788 (□), and 10 μM CI-1020 + 10 μM BQ788 (○) compared to 100 μM CI-1020 (■), in small isolated rat pulmonary arteries. The control endothelin-1 curve is shown for reference with a dotted line. Values are expressed as mean ± S.E.M. * Represents significance of $P < 0.05$. ** Represents significance of $P < 0.01$ from CI-1020 (100 μM) via Mann–Whitney–Wilcoxon rank test

Our results show consistent inhibition of Phase 2 of hypoxic pulmonary vasoconstriction in small rat pulmonary arteries, at all three concentrations of CI-1020 studied (Table 1), irrespective of the degree of endothelin ET_{B2} receptor antagonism. This suggests that in these vessels CI-1020 inhibits hypoxic pulmonary vasoconstriction via its action at the endothelin ET_A receptor since the co-inhibition of endothelin ET_{B2} receptor at the highest concentration of CI-1020 (100 μM) has no greater inhibitory effect. These findings support the involvement of endothelin-1 in Phase 2 of hypoxic pulmonary vasoconstriction in small rat pulmonary arteries via the endothelin ET_A receptor. Absence of complete inhibition, even at the relatively high concentration of 100 μM would suggest that endothelin-1 either has a modulatory role, or is only one of the factors contributing to the contraction. The difference between hypoxia and endothelin-1 mediated contractions is also highlighted by these results. In small rat pulmonary arteries hypoxia-mediated contractions involve endothelin ET_A receptors alone, whereas endothelin-1 mediated contraction is via both endothelin ET_A receptors and endothelin ET_{B2} receptors.

The findings in small pulmonary arteries are in contrast to the observations in larger vessels where CI-1020 had no significant effect upon any phase of hypoxic pulmonary vasoconstriction (Table 2). In large rat pulmonary arteries, which have an endothelin-1 receptor population consisting entirely of endothelin ET_A receptors (O'Donnell and Kay, 1995), it would appear that there is no involvement of endothelin-1 in the response to hypoxia. Hence, there at least two differences between Phase 2 of hypoxic pulmonary vasoconstriction in large and small pulmonary arteries, namely the involvement of endothelin-1, and the time course of the response (Fig. 2). Whether or not these two differences are linked is purely speculative.

It would appear that the involvement of endothelin-1 in the pressor response to hypoxia is dependent upon the species involved and the preparation used. The reports of Holm (1997) and Shimoda et al. (1998b) show that in the pig, both in vivo and in vitro, the action of endothelin-1 at the endothelin ET_A receptor appears to play a key role in hypoxic pulmonary vasoconstriction. However, in the dog endothelin-1 would appear to have no role in hypoxic pulmonary vasoconstriction (Douglas et al., 1993). In the rat the situation is more complex: in the anaesthetised animal, endothelin ET_A receptor antagonism has been shown to significantly reduce hypoxic pulmonary vasoconstriction (Chen et al., 1997), as is seen in isolated small pulmonary arteries in the present study. In contrast, in the isolated perfused lung endothelin-1 appears not to be involved (Ishizaki et al., 1995; Takeoka et al., 1995).

Similarly, this study and the work of Ishizaki et al. (1995) have shown three separate endothelin ET_A receptor antagonists to have no effect upon hypoxic pulmonary vasoconstriction in large isolated rat pulmonary arteries. The reason for the difference between these different preparations in the rat remain to be elucidated. Another as yet unexplained discrepancy is that in small isolated pulmonary arteries the endothelin ET_A receptor and endothelin ET_B receptor antagonist bosentan had no effect on hypoxic pulmonary vasoconstriction (Lazor et al., 1996).

In the rat the size of artery is clearly an important factor. A recent report of Sasaki et al. (1995) characterised rat pulmonary arteries in detail using perfusion fixation and electroscopic microscopy. The small and large pulmonary arteries used in this study were predominantly from the 'thick muscular segment' and 'classical elastic segment,' respectively, the thick muscular segment being shown to contain a greater proportion of smooth muscle cells than the classical elastic segment. Since endothelin ET_A receptors are well recognised as being located upon smooth muscle, this may explain the different levels of involvement of this receptor in hypoxic pulmonary vasoconstriction in these vessels.

In summary, Phase 2 of hypoxic pulmonary vasoconstriction would seem to be due, in part, to the action of endothelin-1 at the endothelin ET_A receptor in small isolated rat pulmonary arteries, implying a significant role for endothelin-1 in hypoxic pulmonary vasoconstriction in rat resistance vessels. In larger pulmonary arteries CI-1020 had no effect upon any phase of the hypoxic response. Hence, although responses to hypoxia consist of four phases in both sets of vessel, the underlying mechanism, at least for Phase 2, is not identical. The literature does not consistently support the endothelin ET_A receptor as an obligatory common pathway for hypoxic pulmonary vasoconstriction. We suggest that endothelin ET_A receptor blockade may have a variety of effects on the pulmonary vascular response, pulmonary hypertension and pulmonary vascular remodelling, dependent on species and model chosen.

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