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In vitro hypoxia on rat pulmonary artery: effects on contractions to spasmogens and role of K_{ATP} channels

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Abstract

The effect of in vitro hypoxia for 1 h on concentration-response curves to vasoconstrictor spasmogens was examined in rat isolated pulmonary arteries. Hypoxia, like levcromakalim (K_{ATP} channel opener), did not affect contractions to endothelin-1 but attenuated contractions to U46619 ((1,5,5,)-hydroxy-11α,9α-epoxymethano) prosta 5Z, 13E-dienoic acid; thromboxane-mimetic), angiotensin II, noradrenaline and 5-hydroxytryptamine. The attenuation was prevented by glybenclamide. In pre-contracted arteries, subsequent exposure to hypoxia caused a response consisting of four phases (transient relaxation due to endothelium-derived nitric oxide; transient contraction; slow relaxation; sustained contraction). Glybenclamide, if added before hypoxia, did not eliminate either of the relaxant phases but, if added during the sustained contractile phase, caused further contraction. We conclude that exposure of pulmonary arteries to prolonged hypoxia causes K_{ATP} channels to open, as in systemic arteries; this diminishes contractions to some, but not all, vasoconstrictor spasmogens. The data suggest that endothelin-1, unlike other vasoconstrictors, would remain a highly effective pulmonary vasoconstrictor under severe hypoxic conditions.

Keywords: Hypoxia; K_{ATP} channel; Pulmonary artery; Pulmonary vasoconstrictor; Hypoxic pulmonary vasoconstriction

1. Introduction

One of the features that distinguish pulmonary blood vessels from systemic blood vessels is their response to hypoxia, i.e. hypoxia induces vasoconstriction in the pulmonary circulation but causes vasodilatation in the systemic circulation. The mechanism underlying hypoxic pulmonary vasoconstriction involves closure of K^+ channels in the vascular smooth muscle cell membrane (Post et al., 1992; Yuan et al., 1993) followed by depolarisation (Harder et al., 1985) and influx of extracellular Ca^{2+} (McMurtry et al., 1976). The particular type of K^+ channel involved is not glibenclamide-sensitive and is reported to be either a voltage-gated, Ca^{2+}-insensitive K^+ channel (Yuan et al., 1993) or a Ca^{2+}-activated K^+ channel (Post et al., 1992). Several mechanisms have been proposed to explain hypoxic vasodilatation in systemic vessels, including the release of vasodilator metabolites, such as adenosine, from hypoxic tissue and/or the opening of glibenclamide-sensitive K_{ATP} channels leading to K^+ efflux and hyperpolarisation (Berne, 1980; Daut et al., 1990; Bonnet et al., 1991; Von Beckerath et al., 1991; Mellemkjaer and Nielsen-Kudsk, 1994).

Hypoxic pulmonary vasoconstriction is physiologically important in that it diverts blood away from poorly ventilated regions of the lung. Nevertheless, constriction is not the only response of pulmonary blood vessels to hypoxia. If isolated pre-contracted pulmonary arteries (conduit or resistance) are exposed to in vitro hypoxia for extended periods e.g. 20–60 min, the response comprises relaxant as well as contractile phases (Bennie et al., 1991; Jin et al., 1992; Greenberg and Kishiyama, 1993; Teng and Barer, 1995). One of the possible mechanisms involved in the relaxation phase(s) could be the opening of K_{ATP} channels, as occurs in systemic vessels. If prolonged hypoxia does open K_{ATP} channels then, in addition to producing relaxation in pre-contracted pulmonary arteries as described above, it should also modulate concentration-response curves to at least some vasoconstrictor spasmogens.

In this study on rat pulmonary artery we have (a) examined the effects of in vitro hypoxia for 1 h on concentration-response (contraction) curves to spasmogens

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of possible physiological importance in the pulmonary circulation, (b) compared the effects of in vitro hypoxia with those of the K_{ATP} channel opening drug, levcromakalim, and (c) investigated whether the effects of in vitro hypoxia can be prevented by the K_{ATP} channel blocking drug, glybenclamide. The specific aims of these experiments were to identify which vasoconstrictor spasmogens might be attenuated under hypoxic conditions and, at the same time, test the hypothesis that prolonged hypoxia opens K_{ATP} channels in pulmonary arteries. We have also investigated whether the contraction induced by hypoxia in pre-contracted arteries (as opposed to the contraction induced by a spasmogen) might also be attenuated by the concurrent opening of K_{ATP} channels.

A preliminary account of these data was presented at the First European Congress of Pharmacology, Milan, Italy, July 1995 (Wanstall and O'Brien, 1995).

2. Materials and methods

2.1. Pulmonary artery preparations

Male Wistar rats (age 8–9 weeks; weight 270–390 g) were used. The rats were killed (stunned and exsanguinated) and the main pulmonary artery was removed and cleared of connective tissue. Ring preparations of the pulmonary artery (3 mm in length) were set up around two horizontal stainless steel wires (one fixed and one linked to a Statham Universal transducer, UC3 + UCL) in a 25 ml organ bath containing physiological salt solution (PSS) at 37°C. The endothelium was left intact except in a few of the preparations used for protocol B (Section 2.3.3.), when it was removed by gently rubbing the lumen of the vessel with a small pair of forceps. The preparations were set at a resting force of 10 mN; in these preparations this force corresponds to approximately 10 mmHg and was selected, as in previous studies, to reflect in vivo pulmonary artery pressure (Wanstall and O'Donnell, 1990; Wanstall et al., 1995). The preparations were then allowed to equilibrate for 1 h. During this time the PSS was changed every 15 min and the resting force readjusted if necessary. The composition of the PSS was (in mM): NaCl 118; KCl 5.9; $CaCl_2$ 1.5; $MgSO_4$ 0.72; $NaHCO_3$ 25; glucose 11.7; ascorbic acid 1.14 (95% O_2/5% CO_2, $P_{O_2} > 600$ mmHg, pH 7.4, 37°C).

2.2. P_{O_2} in the PSS

The P_{O_2} of the PSS was altered by changing the gas mixture that bubbled the PSS in the organ baths from 95% O_2/5% CO_2 (control conditions) to 95% N_2/5% CO_2 (hypoxic conditions). P_{O_2} was monitored throughout each experiment in a separate organ bath that contained no tissue preparation (Clark-type oxygen electrode, Strathkelvin, Model 1302 and oxygen meter, Strathkelvin, Model 781). In some experiments P_{O_2} was also measured, at the completion of the experiment, in the organ baths that contained the tissues. This procedure verified that the P_{O_2} value in the bath without any tissue was an accurate reflection of the P_{O_2} in the baths that contained the tissues. In six experiments, readings on the oxygen meter were recorded every few minutes, and in all experiments readings were recorded after 1 h in hypoxic conditions. The P_{O_2} of the PSS was reduced from > 600 mmHg (control conditions) to 23 ± 4.5 mmHg ($n = 6$) after 5 min of bubbling with 95% N_2/5% CO_2 and to 9 ± 1.7 mmHg ($n = 6$) after 8 min of bubbling. After 1 h the P_{O_2} was 5 ± 0.6 mmHg ($n = 27$).

2.3. Experimental protocols

2.3.1. Preliminary procedure

The preliminary procedure was carried out under control conditions in all experiments. The preparations were contracted with 0.1 μM noradrenaline and, when the contraction was stable, 1 μM acetylcholine was added. A relaxant response (52 ± 1.4% reversal of the noradrenaline contraction, $n = 88$) confirmed the presence of a functional endothelium in all preparations where the endothelium was not removed; endothelium-denuded preparations did not relax in response to acetylcholine. The tissues were washed and a reference contraction to 80 mM K^+ was obtained. The tissues were then washed again. In preliminary experiments it was established that the K^+ contraction was reproducible and hence it was not necessary to repeat it.

Following the preliminary procedure either protocol A or protocol B (detailed below) was carried out:

2.3.2. Protocol A

The preparations were equilibrated for 1 h either (a) under control conditions, (b) under hypoxic conditions or (c) in the presence of levcromakalim (1 μM, a maximal relaxant concentration of this K_{ATP} channel opening drug). A cumulative concentration-response (contraction) curve was then obtained (in the continued presence of conditions (a), (b) or (c) above), to one of the following spasmogens: U46619 (thromboxane-mimetic), angiotensin II, endothelin-1, noradrenaline, 5-hydroxytryptamine (5-HT). Only one concentration-response curve was obtained on each preparation. In one series of experiments glybenclamide (1 μM) was present throughout the protocol. Exposure to hypoxic conditions for 1 h either had no effect on resting tone (15 out of 19 preparations) or caused small increases in tone (5–10% of the reference contraction to K^+; four out of 19 preparations). In the presence of glybenclamide, hypoxic conditions for 1 h caused increases in tone in seven out of ten preparations, and in two of these the contractions were > 10% of the K^+ contraction (i.e. 13 and 20%). The latter two preparations were not included in the series of experiments in which spasmogen concentra-

tion-response curves were obtained. Levcromakalim had no effect on resting tone in any of the preparations.

2.3.3. Protocol B

The preparations were pre-contracted submaximally with noradrenaline (0.1 μM). When the contraction was stable, the gas mixture bubbling the PSS in the organ bath was changed to 95% N_2/5% CO_2 (hypoxic conditions) for 1 h; relaxations and contractions occurring during this period were recorded. In some experiments glybenclamide (1 μM or 10 μM) was added to the PSS in the tissue bath either (a) 15 min before pre-contraction or (b) 30–60 min after hypoxic conditions were commenced, i.e. once the multiphasic response to hypoxia had reached a stable plateau. In some experiments the contribution of endothelium-derived nitric oxide (NO) to hypoxia-induced vasorelaxation was determined either by using endothelium-denuded preparations or by the addition of the NO synthase inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME; 10 μM), 15 min before the spasmogen.

2.4. Analysis of data

Reference contractions to 80 mM K^+ were determined as force (mN). These contractions did not differ in magnitude in the different groups of experiments ($P > 0.05$; one-way analysis of variance (ANOVA); see Figs. 1 and 2). Contractile responses to U46619, angiotensin II, noradrenaline, endothelin-1 and 5-HT were expressed as a percentage of the reference contraction to K^+ and then

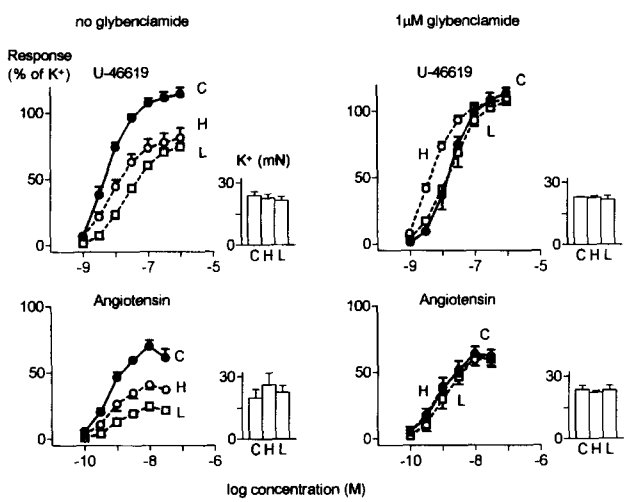

Fig. 1. Mean concentration-response curves to U46619 (top graphs) and angiotensin II (bottom graphs) on rat pulmonary artery preparations under control conditions (C ●——●) and in the presence of in vitro hypoxia (H ○---○) or 1 μM levcromakalim (L □---□). Data in the absence (left hand graphs) and presence (right hand graphs) of 1 μM glybenclamide are shown. Responses are expressed as a percentage of the contraction to 80 mM K^+. The K^+ contractions (in mN) for each group of experiments are shown by the histograms. The S.E.M. of the mean responses are shown by the vertical lines. For values of n refer to Table 1.

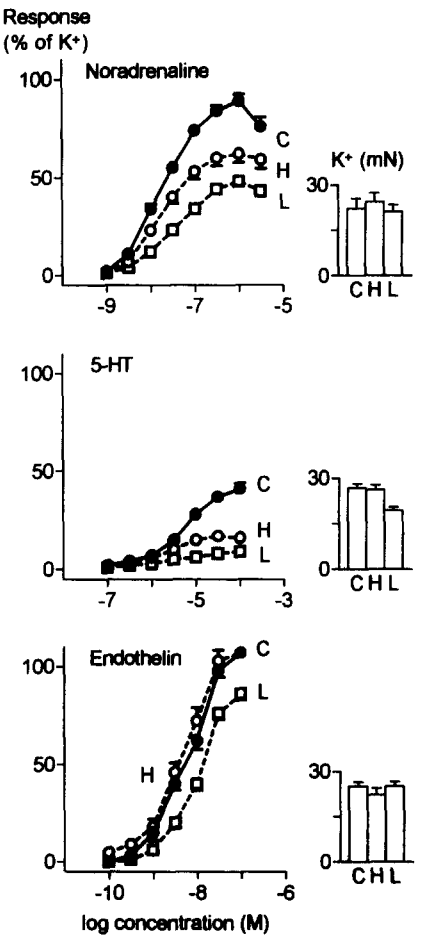

Fig. 2. Mean concentration-response curves to noradrenaline (top graph), 5-hydroxytryptamine (5-HT, centre graph) and endothelin-1 (bottom graph) on rat pulmonary artery preparations under control conditions (C ●——●) and in the presence of in vitro hypoxia (H ○---○) or 1 μM levcromakalim (L □---□). Responses are expressed as a percentage of the contraction to 80 mM K^+. The K^+ contractions (in mN) for each group of experiments are shown in the histograms. The S.E.M. of the mean responses are shown by the vertical lines. For values of n refer to Table 1.

plotted against spasmogen concentration on a logarithmic scale. For the purposes of determining EC_{50} values, responses were also calculated as a percentage of the maximum response to the particular spasmogen. EC_{50} values (concentration giving 50% of the maximum contraction to the spasmogen in each individual concentration-response curve) were interpolated from plots of response, as percent maximum, against log concentration. The potency of each spasmogen was taken as the negative log of the EC_{50}.

2.5. Drugs and solutions

The following drugs were used: acetylcholine chloride (Sigma), angiotensin II (Sigma), endothelin-1 (Peptide Institute), forskolin (Calbiochem-Behring), glybenclamide (Sigma), 5-hydroxytryptamine creatinine sulphate (Sigma), levcromakalim (gift from SmithKline Beecham), linsid-

Table 1
Maximum contractile responses (expressed as a percentage of the contraction to 80 mM K^+) for spasmogens on rat pulmonary artery under control conditions and in the presence of hypoxia or levcromakalim

	Mean maximum contraction ± S.E.M. (% of K^+ contraction)		
	Control	Hypoxia [b]	Levcromakalim [c]
Glybenclamide absent			
U46619	114 ± 4.9 (5)	81 ± 7.4 [a] (4)	74 ± 2.6 [a] (5)
Angiotensin II	70 ± 4.5 (4)	41 ± 3.4 [a] (4)	24 ± 2.8 [a] (4)
Noradrenaline	89 ± 3.5 (3)	62 ± 4.3 [a] (3)	48 ± 2.6 [a] (3)
5-Hydroxytryptamine	41 ± 3.0 (4)	17 ± 1.5 [a] (4)	9 ± 1.8 [a] (5)
Endothelin-1	107 ± 1.4 (4)	107 ± 4.6 (4)	86 ± 3.3 [a] (5)
Glybenclamide present [d]			
U46619	114 ± 4.1 (4)	109 ± 2.8 (4)	108 ± 1.3 (4)
Angiotensin II	64 ± 5.3 (4)	60 ± 5.1 (4)	61 ± 6.6 (4)

Values are means ± S.E.M. (numbers of preparations in parentheses). [a] Value significantly less than control value $P < 0.05$ (Mann Whitney U test). [b] Preparations exposed to in vitro hypoxia for 1 h. [c] Preparations exposed to levcromakalim (1 μM) for 1 h. [d] Glybenclamide (1 μM) present.

omine (SIN-1; 3-morpholino-sydnonimine; Sapphire Bioscience), N^G-nitro-L-arginine methyl ester (L-NAME; Sigma), (−)-noradrenaline acid tartrate (Sigma), U46619 ((1,5,5,)-hydroxy-11α,9α-epoxymethano) prosta 5Z, 13E-dienoic acid; Upjohn). The solutions of drugs were prepared as follows: acetylcholine (10 mM), endothelin-1 (10 μM), 5-hydroxytryptamine (25 mM), L-NAME (10 mM) and linsidomine (100 mM) in deionised water, noradrenaline (100 mM) in 10 mM HCl, forskolin (10 mM) and U46619 (10 mM) in absolute ethanol, levcromakalim (10 mM) in 70% ethanol, glybenclamide (1 mM) in dimethyl sulfoxide. Dilutions were prepared in PSS and kept on ice during the course of an experiment. None of the solvents, in the concentrations used, affected blood vessel tone in the preparations.

2.6. Statistical analyses

Mean values were calculated from data obtained in preparations from a number (n) of different animals and are quoted together with S.E.M. The statistical significance of differences between mean values of K^+ contraction or spasmogen negative log EC_{50} were assessed by one-way ANOVA followed, when appropriate, by Dunnett's post hoc t test, and between maximum spasmogen responses (expressed as percentage of the K^+ contraction; values not necessarily normally distributed) by Mann Whitney U test.

3. Results

3.1. The effects of in vitro hypoxia and levcromakalim on concentration-response curves to spasmogens

The spasmogens examined in this study differed markedly not only in contractile potency (neg log EC_{50}) but also in maximum contraction (Tables 1 and 2).

After exposure of the tissues to in vitro hypoxia or 1 μM levcromakalim, contractile responses to U46619, angiotensin II, noradrenaline and 5-HT were attenuated when compared with data obtained under control conditions (Figs. 1 and 2). This attenuation was seen chiefly as a

Table 2
Potency (neg. log EC_{50}) values for spasmogens on rat pulmonary artery under control conditions and in the presence of hypoxia or levcromakalim

	Mean neg. log EC_{50} ± S.E.M.		
	Control	Hypoxia [c]	Levcromakalim [d]
Glybenclamide absent			
U46619	8.25 ± 0.07 (5)	8.08 ± 0.03 (4)	7.66 ± 0.06 [a] (5)
Angiotensin II	9.22 ± 0.07 (4)	9.15 ± 0.09 (4)	8.98 ± 0.11 (4)
Noradrenaline	7.77 ± 0.07 (3)	7.76 ± 0.03 (3)	7.44 ± 0.05 [a] (3)
5-Hydroxytryptamine	5.29 ± 0.10 (4)	ND	ND
Endothelin-1	8.19 ± 0.08 (4)	8.37 ± 0.07 (4)	7.97 ± 0.05 (5)
Glybenclamide present [e]			
U46619	7.70 ± 0.06 (4)	8.30 ± 0.03 [b] (4)	7.82 ± 0.20 (4)
Angiotensin II	9.11 ± 0.14 (4)	9.20 ± 0.13 (4)	8.96 ± 0.11 (4)

Values are means ± S.E.M. (numbers of preparations in parentheses). [a] Value significantly less than control value $P < 0.05$. [b] Value significantly greater than control value $P < 0.05$ (one-way ANOVA and Dunnet's post hoc t test). [c] Preparations exposed to in vitro hypoxia for 1 h. [d] Preparations exposed to levcromakalim (1 μM) for 1 h. [e] Glybenclamide (1 μM) present. ND, not determined (responses to 5-HT too small to determine negative log EC_{50}).

significant depression in maximum contraction for each spasmogen (Table 1); a reduction in potency (negative log EC_{50}) was seen only with levcromakalim and only for U46619 and noradrenaline (Table 2). U46619, but not the other spasmogens, was further examined in the presence of in vitro hypoxia plus levcromakalin together. The effect of this combined treatment was no greater than the attenuation caused by either of these treatments alone (U46619 maximum contraction $82 \pm 2.4\%$ of K^+, neg. log EC_{50} 7.80 ± 0.03, $n = 4$; compare with values in Tables 1 and 2).

In contrast to the other spasmogens, contractile responses to endothelin-1 were not affected at all by in vitro hypoxia (no change in maximum response or potency) and were only slightly attenuated by levcromakalim (a small reduction in maximum response but no change in potency) (Fig. 2, Tables 1 and 2).

Glybenclamide (1 μM) completely prevented the attenuation by hypoxia or levcromakalim of responses to U46619 and angiotensin II, i.e. in the presence of glybenclamide there was no reduction in maximum contraction (Fig. 1, Table 1) or potency (Table 2). On the contrary, the potency of U46619 was increased under hypoxic conditions when glybenclamide was present (Table 2). Endothelin-1, noradrenaline and 5-HT were not studied in the presence of glybenclamide.

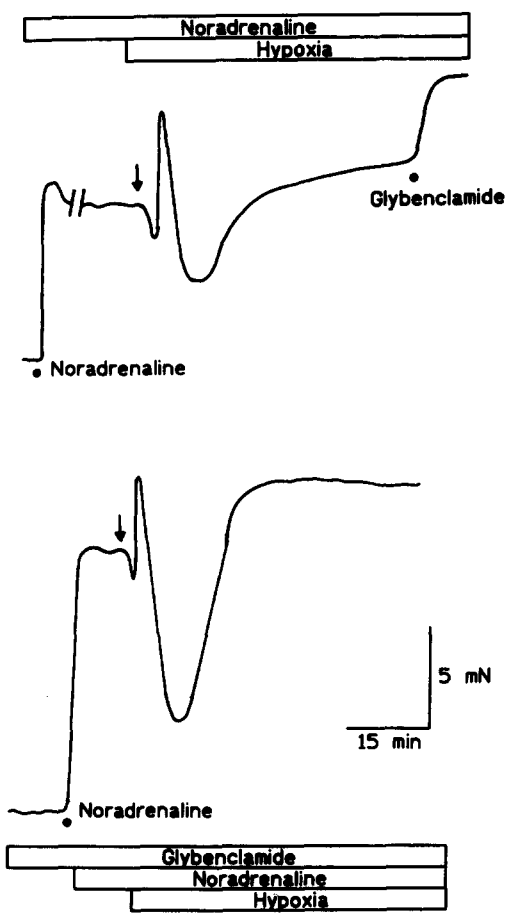

Fig. 3. Experimental records for two pulmonary artery preparations pre-contracted with noradrenaline (0.1 μM) and then exposed to hypoxic conditions (at ↓). The traces show the four phases of the response to hypoxia, viz. transient relaxation, transient contraction, second relaxation, sustained contraction. Glybenclamide (1 μM), added once the response to hypoxia had stabilised, caused a contraction (top trace). Glybenclamide (1 μM) added before exposure to hypoxia did not eliminate either of the relaxant phases (bottom trace).

3.2. *The effects of in vitro hypoxia on pre-contracted preparations*

On preparations pre-contracted submaximally with noradrenaline the response to in vitro hypoxia for 1 h consisted of four phases, viz. (i) a transient relaxation, (ii) a transient contraction, (iii) a second relaxation and (iv) a slow, sustained contraction (Fig. 3). The first two phases occurred within 5 min of exposure to hypoxia, i.e. when the P_{O_2}, although markedly reduced, was still > 23 mmHg. The second relaxation and the sustained contraction reached peaks when the P_{O_2} was < 10 mmHg. The same sequence of relaxant and contractile responses to hypoxia was seen if preparations were pre-contracted with U46619 (20 nM) or endothelin-1 (10 nM).

If glybenclamide (1 μM) was added to the tissue once the sustained contraction to hypoxia had stabilised (i.e. 30–60 min after the commencement of in vitro hypoxia) it caused the tissue to contract further (Fig. 3). The contraction induced by glibenclamide was 3.6 ± 0.7 mN ($n = 3$), i.e. about half the size of the sustained contraction to hypoxia in the same preparations (6.9 ± 1.0 mN). Control experiments showed that, in the absence of hypoxia, glybenclamide caused no contraction whether the tissue was (a) under conditions of resting tone, (b) contracted with 0.1 μM noradrenaline or (c) contracted with noradrenaline and then partially relaxed with either the nitric oxide donor drug, linsidomine (0.3 μM) or the adenylate cyclase activator, forskolin (0.1 μM).

Neither of the relaxant phases was abolished by the prior addition of glybenclamide (1 μM, Fig. 3, or 10 μM). The initial transient relaxation (1.5 ± 0.3 mN, $n = 8$) was not seen in the absence of the endothelium (6 preparations) or in the presence of L-NAME (10 μM; three preparations), but the second relaxation was still present under each of these conditions.

4. Discussion

Data obtained in this study demonstrate that in vitro hypoxia can cause vasorelaxation in rat isolated pulmonary arteries, and support the hypothesis that this relaxation involves the opening of K_{ATP} channels. The hypoxia-induced vasorelaxation is sufficient (a) to attenuate contractile responses to a number of physiologically important vasoconstrictor spasmogens, viz. U46619 (thromboxane-mimetic), angiotensin II, 5-HT and noradrenaline and (b)

to modulate the sustained contractile component of the multiphasic response to hypoxia seen in pre-contracted pulmonary arteries. The one spasmogen that was not affected by hypoxia was endothelin-1.

4.1. Involvement of K_{ATP} channels in hypoxia-induced vasorelaxation

The effects of hypoxia were comparable to the effects of the K_{ATP} channel opening drug, levcromakalim, in that both treatments markedly attenuated responses to each of the spasmogens studied except endothelin-1. When experiments with U46619 and angiotensin were repeated in the presence of glybenclamide, it was found that the effects of both hypoxia and levcromakalim were completely prevented by this K_{ATP} channel blocking drug. Hence it was concluded that the attenuation of spasmogen-induced contractions seen under hypoxic conditions probably involved opening of K_{ATP} channels. In light of this conclusion it was not surprising that hypoxia had no effect on responses to endothelin-1. Contractions to this vasoconstrictor peptide, in contrast to other spasmogens, are resistant to the vasorelaxant effects of K_{ATP} channel openers, not only in rat pulmonary artery (O'Donnell et al., 1991) but also in aorta (Lawson et al., 1992). The absence of any effect of hypoxia on contractions to endothelin-1 has also been noted by MacLean et al. (1994).

Although the attenuating effect of hypoxia on spasmogen-induced contractions resembled the effect of levcromakalim, the attenuation produced by levcromakalim was always slightly greater than that induced by hypoxia. Hypoxia depressed the maxima of each of the spasmogens (except endothelin-1) by an amount that corresponded to 24–33% of the reference contraction to K^+ (the differences between values in columns 1 and 2, Table 1), whereas the depression by levcromakalim was 32–40% of the K^+ contraction (the differences between values in columns 1 and 3, Table 1). It is possible that the vasorelaxant effect of hypoxia, but not levcromakalim, is partially offset by some additional, but opposing, action of hypoxia. This possibility is supported by the finding that when the vasorelaxant effect of hypoxia was blocked by glybenclamide, hypoxia slightly potentiated, rather than attenuated, responses to U46619.

The results of the experiments on pre-contracted pulmonary arteries supported the conclusion that prolonged hypoxia causes K_{ATP} channels to open. The response of pre-contracted preparations to hypoxia consisted of two relaxant and two contractile phases; some, or all, of these phases have been reported previously (Bennie et al., 1991; Jin et al., 1992; Greenberg and Kishiyama, 1993; Teng and Barer, 1995). Once the final contractile phase had stabilised, glybenclamide induced a further contraction. The contraction was not a non-specific effect of glybenclamide since it was not seen in the absence of hypoxia, whatever the contractile state of the tissue (fully relaxed, partially contracted, or contracted and then partially relaxed with vasodilator drugs that act by mechanisms not involving K_{ATP} channels). Thus the contraction to glybenclamide was a specific potentiation of the final contractile phase of the response to hypoxia, indicating that this phase is normally modulated by concurrent K_{ATP} channel-mediated vasorelaxation.

Despite the indications that hypoxia leads to K_{ATP} channel activation, these channels were not totally responsible for either of the relaxant components of the multiphasic response to hypoxia. The initial transient relaxation, seen when pre-contracted tissues were first exposed to hypoxia, was due to the release of NO from the endothelium, confirming previous findings (Greenberg and Kishiyama, 1993; Teng and Barer, 1995). The second relaxant phase, which was not due to NO release, was not abolished by glybenclamide; therefore, contrary to our expectation, the second relaxant phase could not be accounted for by K_{ATP} channel activation. In other studies the role of K_{ATP} channels in this phase of the response has either (a) not been examined (Teng and Barer, 1995), (b) been dismissed (Jin et al., 1992) or (c) been considered to partially account for the relaxation (Greenberg and Kishiyama, 1993). We did not attempt to quantify the relaxation in the absence and presence of glybenclamide since the size of this component of the response to hypoxia varied too much between tissues for us to take this approach. Hence we cannot say to what extent K_{ATP} channels may have contributed to this relaxation. Because we observed a relaxation that was resistant to both an NO synthase inhibitor and to glybenclamide, our data support the conclusion of Greenberg and Kishiyama (1993) that a third, as yet unidentified, vasodilator mechanism can occur in pulmonary arteries in response to hypoxia.

The mechanism whereby prolonged hypoxia might open K_{ATP} channels in rat pulmonary artery was not examined in this study and remains speculative, but it could conceivably reflect a reduction in intracellular ATP. In cultured vascular smooth muscle cells, Noack et al. (1992) showed that procedures which lower intracellular ATP caused an outward K^+ current involving the same population of K^+ channels as are opened by levcromakalim (i.e. K_{ATP} channels), and they proposed that, in vascular smooth muscle, prolonged hypoxia might reduce ATP enough for these K_{ATP} channels to open.

4.2. Consequences of hypoxia-induced vasorelaxation

Two consequences of the vasorelaxation induced by prolonged hypoxia were demonstrated in the present study. Firstly, hypoxia attenuated contractions to all of the vasoconstrictor spasmogens with the exception of endothelin. For those spasmogens that did not maximally contract the tissues under control conditions (i.e. angiotensin II and 5-HT), the contractions obtained under hypoxic conditions were minimal. The relative importance of the various

vasoconstrictors in controlling pulmonary vascular tone may therefore be altered in situations where there is severe prolonged hypoxia. In particular the importance of endothelin-1, relative to the other vasoconstrictors, may be increased.

Secondly in pre-contracted arteries, the sustained contractile component of the multiphasic response to hypoxia was moderated by concurrent K_{ATP} channel-mediated vasorelaxation. This observation in isolated pulmonary arteries complements the findings of Wiener et al. (1991) in isolated perfused lungs from ferrets, where moderate hypoxia produced a sustained vasoconstrictor response, but severe hypoxia (< 10 mmHg) produced vasoconstriction followed by vasodilatation (Wiener et al., 1991). The vasodilatation could be prevented by glybenclamide; hence it was concluded that when hypoxia is severe, hypoxic pulmonary vasoconstriction is suppressed by concomitant hyperpolarisation resulting from the opening of K_{ATP} channels (Wiener et al., 1991).

A third potential consequence of hypoxia-induced vasorelaxation involving K_{ATP} channels is that responses to K_{ATP} channel opening vasodilator drugs could be modified. Indeed in a previous study we found that the pulmonary vasorelaxant effects of the K_{ATP} channel openers, pinacidil and cromakalim, (but not all vasodilator drugs) were inhibited under hypoxic conditions (Wanstall, 1994). At the time we explained this observation with the tentative hypothesis that (i) hypoxia may have already opened the K_{ATP} channels and hyperpolarised the smooth muscle cells and (ii) as a result, any hyperpolarisation by K_{ATP} channel opening drugs may have been precluded (Wanstall, 1994). The second part of this hypothesis was supported by published data in cultured smooth muscle cells (Clapp and Gurney, 1992; Clapp et al., 1993); the first part of the hypothesis is now supported by the findings from the present study. The fact that the effects of K_{ATP} channel opening drugs are inhibited under hypoxic conditions (Wanstall, 1994) could explain why, in the present study, the effect of levcromakalim and hypoxia together was no greater than the effect of either treatment alone.

4.3. Summary and conclusions

Undoubtedly the most important response of pulmonary blood vessels to hypoxia is vasoconstriction. Nevertheless, as shown in this study, hypoxia can also relax pulmonary arteries, a response that is traditionally associated with systemic vessels. The data suggest that one of the mechanisms whereby hypoxia relaxes pulmonary arteries is through the opening of K_{ATP} channels.

The hypoxia-induced pulmonary vasorelaxation can inhibit not only the vasodilator effects of K_{ATP} channel opening drugs (Wanstall, 1994) but also the contractile effects of certain vasoconstrictor spasmogens (present study). These findings could conceivably have implications in the context of pulmonary hypertension, a condition that is often accompanied by hypoxaemia. For example, K_{ATP} channel opening drugs may have a role in the treatment of pulmonary hypertension, as an alternative to current vasodilator therapies (Wanstall et al., 1994; Wanstall, 1996); if so, their effectiveness may possibly be influenced by the level of hypoxaemia in individual patients. Also, because endothelin-1 (unlike the other vasoconstrictors examined in this study) was not attenuated by prolonged hypoxia, this peptide is likely to remain a highly effective pulmonary vasoconstrictor even under hypoxic conditions. This is of interest in light of the view that endothelin may be an important vasoconstrictor in the pathology of pulmonary hypertension (Stewart et al., 1991; Li et al., 1994) and that endothelin antagonists (whose effects are not altered by hypoxia; Wanstall, unpublished) may have a therapeutic role in the treatment of this disease (Oparil et al., 1995).

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