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Pulmonary versus systemic effects of vasodilator drugs: an in vitro study in isolated intrapulmonary and mesenteric arteries of neonatal piglets

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Abstract

The ability of several vasodilators to inhibit the responses to noradrenaline and U46619 (a thromboxane A_2 analog) in isolated pulmonary and mesenteric arteries of neonatal piglets was compared. In pulmonary arteries, acetylcholine produced endothelium-dependent relaxations (pIC_{50} = about 6.8) while, in mesenteric arteries, a relaxant ($\leq 10^{-7}$ M) or a contractile response ($\geq 10^{-6}$ M) was observed. Sodium nitroprusside produced relaxant effects in pulmonary and mesenteric arteries contracted by noradrenaline (pIC_{50} = 6.6 and 6.0, respectively) and U46619 (pIC_{50} = 5.4 and 6.7, respectively). ATP induced an endothelium-independent relaxation in pulmonary arteries (pIC_{50} = about 4) but in mesenteric arteries it produced weak relaxant effects. In resting mesenteric arteries, ATP induced a concentration-dependent contraction which was not observed in pulmonary arteries. Prostaglandin E_1 induced a concentration-dependent relaxation in pulmonary arteries (pIC_{50} = about 6). In mesenteric arteries, prostaglandin E_1 at $< 10^{-6}$ M produced a contractile effect whereas, at higher concentrations, a relaxant response was observed. The α-adrenoceptor antagonist tolazoline had no effect on arteries contracted by U46619 but relaxed arteries contracted by noradrenaline being slightly more potent in mesenteric than in pulmonary arteries (pIC_{50} = 5.1 and 4.8, respectively). Nifedipine ($> 10^{-7}$ M) relaxed both arteries, mesenteric being more sensitive than pulmonary arteries and noradrenaline more sensitive than U46619-induced contractions. In conclusion, differences in the relaxant effects for all vasodilators were found depending on the artery, the vasoconstrictor used or both. However, ATP was the only drug which, regardless of the concentration or vasoconstrictor used, produced greater relaxant effects in pulmonary than in mesenteric arteries.

Keywords: Pulmonary artery; Prostaglandin E_1; ATP; Acetylcholine; Tolazoline; Nifedipine; Sodium nitroprusside

1. Introduction

The normal adult pulmonary circulation is a low pressure, low resistance circuit (Barnes and Liu, 1995). In contrast, in the fetus, gas exchange occurs in the placenta and not in the lung and, therefore, right ventricular cardiac output is directed through the ductus arteriosus to the systemic circulation. Consequently, the fetal pulmonary vascular resistance is high and the pulmonary blood flow is low (Fineman et al., 1995). The transition from the fetal to the adult pulmonary circulation in the perinatal period is a delicate process which takes place at birth and in the following weeks (Fineman et al., 1995). Failure of the pulmonary circulation to undergo this transition results in persistent pulmonary hypertension of the newborn. Persistent pulmonary hypertension of the newborn is characterized by an increased pulmonary vascular resistance resulting in right to left shunting of blood across a patent foramen ovale and/or ductus arteriosus, severe hypoxemia and acidosis (Roberts and Shaul, 1993). Various intravenous vasodilators including the α-adrenoceptor blocker tolazoline (Stevenson et al., 1979; Ward, 1984; Gouyon and Francoise, 1992), prostaglandins I_2 and E_1 (Gouyon and Francoise, 1992), the Ca^{2+} channel blocker nifedipine (Simmoneau et al., 1981), $MgSO_4$ (Abu-Osba et al., 1992), acetylcholine (Tripp et al., 1980), the nitric oxide donor sodium nitroprusside (Benitz et al., 1985) and ATP (Fineman et al., 1990; Konduri and Woodard, 1991) have been used to reduce the increased pulmonary vascular resistance (Drummond and Lock, 1984; Kulik and Lock, 1984; Gouyon and Francoise, 1992; Roberts and Shaul, 1993). Since the magnitude of the shunt depends on the difference between pulmonary artery and aortic pressure, systemic vasodilation would increase the flow across the ductus

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reducing the blood supply to the lung and worsening the pulmonary gas exchange. Therefore, the ideal drug for the treatment of persistent pulmonary hypertension of the newborn should be a vasodilator with selectivity for pulmonary over systemic vessels (Roberts and Shaul, 1993; Drummond and Lock, 1984). Recently, the direct delivery of drugs to the lung by inhalation or endotracheal administration has been tested successfully in human newborns with nitric oxide (NO, Roberts et al., 1992; Kinsella et al., 1992) and tolazoline (Welch et al., 1995) and in animal experimental models with cGMP analogs (Lawson et al., 1995) and prostaglandin I_2 (Zobel et al., 1995) which reduce pulmonary artery pressure with minimum systemic effects. This strategy has the additional advantage that the best ventilated areas of the lung are those receiving higher concentrations of drugs and, therefore, those areas will receive higher flow allowing a higher gas exchange. However, this approach is not extensively available yet and may have other undesirable effects, particularly in chronic treatment.

The neonatal circulatory system responds quite differently to drugs than does the mature circulation but the effects of vasodilators in pulmonary neonatal vessels have been poorly investigated (Fineman et al., 1995). Therefore, the aim of the present work was to compare the ability of several vasodilators to inhibit the responses to noradrenaline and the thromboxane A_2 mimetic U46619 in the isolated pulmonary and mesenteric arteries of neonatal piglets.

2. Materials and methods

2.1. Tissue preparation

Male piglets (10–17 days of age, 4679 ± 267 g) obtained from the local abattoir were killed by exsanguination and the lungs and mesenteric beds were rapidly immersed in cold (4°C) Krebs solution (composition in mM: NaCl 118, KCl 4.75, $NaHCO_3$ 25, $MgSO_4$ 1.2, $CaCl_2$ 2.0, KH_2PO_4 1.2 and glucose 11) and transported immediately to the laboratory. Pulmonary and mesenteric arteries (i.d. 1–2 mm) were carefully dissected free of surrounding tissue and cut into rings of 2–3 mm of length (Pérez-Vizcaíno et al., 1994; Villamor et al., 1995). Two L-shaped stainless-steel wires were inserted into the arterial lumen and the rings were introduced in Allhin organ chambers filled with Krebs solution (gassed with 95% O_2 and 5% CO_2 at 37°C). One wire was attached to the chamber and the other to an isometric force-displacement transducer coupled to a signal amplifier (model PRE 206-4; Cibertec, Madrid, Spain) and connected to a Hewlett Packard computer via an A/D interface. Contractile tension was recorded by a REGXPC computer program (Cibertec). The rings were initially stretched to a resting tension of 0.5 *g* (pulmonary rings) or 2 *g* (mesenteric rings) and allowed to equilibrate for 60–90 min. During this period, tissues were re-stretched and washed every 30 min with warm Krebs solution. In some experiments, the endothelium was removed by gently rubbing the intimal surface of the rings with a metal rod. The presence of functional endothelium was tested by addition of acetylcholine (10^{-7} M) in arteries pre-contracted with 3×10^{-7} M noradrenaline. The ability of acetylcholine to induce relaxation of unrubbed rings was taken as an indicator of the presence of functional endothelium.

2.2. Experimental protocol

After equilibration, the rings were contracted with either 10^{-5} M noradrenaline or 10^{-6} M U46619. When the contractile response to each agonist reached a stable tension, cumulative concentration-response curves to acetylcholine, ATP, prostaglandin E_1, sodium nitroprusside, nifedipine and tolazoline were carried out by cumulative addition of drugs after a steady-state relaxant response was reached after each increment. In some arteries, the effects of vasodilators were evaluated in the presence of the nitric oxide synthase inhibitor N^G-nitro-L-arginine-methyl ester (L-NAME, 10^{-4} M) or in endothelium-denuded arteries. In another set of rings, the vasoconstrictor effects of ATP were tested in endothelium intact arteries under resting conditions.

2.3. Drugs

The following drugs were used: (−)-noradrenaline bitartrate, acetylcholine chloride, L-NAME, sodium nitroprusside, U46619, prostaglandin E_1, ATP-$MgCl_2$, tolazoline hydrochloride (Sigma, London, UK) and nifedipine (Bayer, Leverkusen, Germany). Drugs were dissolved in deionized distilled water (except nifedipine in absolute ethanol) and further dilutions were carried out in Krebs solution. Noradrenaline (10^{-2} M) was dissolved in 10^{-4} M ascorbic acid to prevent oxidation. The concentrations are expressed as final molar concentration in the tissue chamber.

2.4. Statistical analysis

Results are expressed as mean ± S.E.M. of measurements in *n* arteries. The vasoconstrictor and vasodilator responses were expressed in mg and as a percentage of the pre-contraction value, respectively. Individual cumulative concentration-response curves were fitted to a logistic equation. For the concentration-response of vasoconstrictors, the maximal effect (E_{max}) and the drug concentration exhibiting 50% of the E_{max} (pD_2, expressed as negative log molar) were calculated. For vasodilators, the negative log drug concentrations producing a 50% relaxation of the control contraction (pIC_{50} values) were calculated. Statistically significant differences were calculated by means of

an unpaired Student's t-test. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Effects of noradrenaline and U46619

The parameters of the concentration-response curves to noradrenaline (10^{-8}-10^{-4} M) and U46619 (10^{-10}-10^{-6} M) in pulmonary and mesenteric arteries are shown in Table 1. The maximal response (E_{max}) to both noradrenaline and U46619 was significantly greater in mesenteric than in pulmonary arteries ($P < 0.01$). In mesenteric arteries, noradrenaline induced greater maximal contractions than U46619 but the opposite occurred in pulmonary arteries. The pD_2 values for U46619-induced contractions were similar in both arteries ($P > 0.05$) whereas the pD_2 value to noradrenaline was greater in mesenteric than in pulmonary arteries ($P < 0.05$). The effects of vasodilators reported below were analyzed on the contractions induced by near-maximally effective concentrations of noradrenaline (10^{-5} M) and U46619 (10^{-6} M).

3.2. Vasodilator effects of acetylcholine

Addition of acetylcholine (10^{-8}-10^{-5} M) resulted in a concentration-dependent relaxation in pulmonary arteries pre-contracted with noradrenaline and U46619 (Fig. 1, Table 2). The relaxant effects of acetylcholine were more pronounced in arteries pre-contracted with noradrenaline as compared to U46619 ($P < 0.01$), so that acetylcholine fully relaxed arteries contracted by noradrenaline even when in arteries contracted by U46619 the maximal relaxation achieved was only 53 ± 5%. This relaxant effect was inhibited when the arteries were pre-treated with 10^{-4} M L-NAME or in endothelium-denuded arteries (not shown).

In mesenteric arteries, pre-contracted with either noradrenaline or U46619 the effects of acetylcholine were biphasic (Fig. 1). At 10^{-8} and 10^{-7} M, it produced a relaxant response (of similar magnitude than in pulmonary arteries pre-contracted with noradrenaline). However, when the concentration of acetylcholine was increased ($\geq 10^{-6}$ M), it produced a concentration-dependent contraction in mesenteric arteries that, at 10^{-5} M, averaged 23 ± 13 and 64 ± 32% over control tension in noradrenaline- and U46619-contracted vessels, respectively.

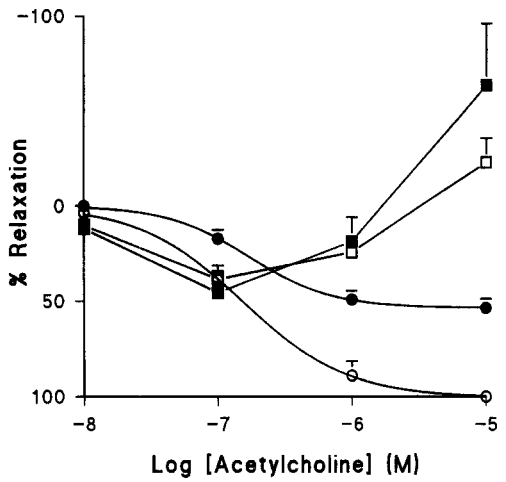

Fig. 1. Relaxant effects of cumulative addition of acetylcholine on pulmonary (circles) and mesenteric arteries (squares) pre-contracted with 10^{-5} M noradrenaline (open symbols) or 10^{-6} M U46619 (solid symbols) of neonatal piglets. Results are expressed as mean ± S.E.M. of 5–10 experiments. Abscissa, % relaxation; ordinate, log acetylcholine concentration (M).

3.3. Vasodilator effects of sodium nitroprusside

The relaxant effect of sodium nitroprusside (10^{-8}-10^{-4} M) was tested in endothelium-denuded arteries. Sodium nitroprusside induced a concentration-dependent relaxation in pulmonary and mesenteric arteries (Fig. 2). However, there were differences in the relaxant potency depending on the artery and the vasoconstrictor employed. Pulmonary arteries contracted by noradrenaline and mesenteric arteries contracted by U46619 were the most sensitive to sodium nitroprusside whereas pulmonary arteries contracted by U46619 were the least (Table 2). Furthermore, sodium nitroprusside was unable to fully relax U46619-induced contractions in pulmonary arteries, so that the highest concentration tested (10^{-4} M) produced a maximal relaxation of 64 ± 5%.

3.4. Vasodilator effects of ATP and effects on resting tension

ATP (10^{-6}-10^{-3} M) induced a concentration-dependent relaxation in pulmonary arteries contracted by either noradrenaline or U46619 ($P > 0.05$ noradrenaline vs. U46619; Fig. 3). However, in mesenteric arteries contracted by noradrenaline, ATP produced only a weak relax-

Table 1
Parameters (pD_2 and E_{max}) of the concentration-response curves to noradrenaline and U46619 in pulmonary and mesenteric arteries

	Pulmonary arteries			Mesenteric arteries		
	pD_2	E_{max} (mg)	n	pD_2	E_{max} (mg)	n
Noradrenaline	6.52 ± 0.07	923 ± 80	17	6.07 ± 0.11 [a]	5273 ± 718 [b]	8
U46619	7.31 ± 0.15 [d]	1279 ± 145 [c]	11	7.11 ± 0.13 [d]	2083 ± 285 [a,d]	7

[a] and [b] $P < 0.05$ and $P < 0.01$ mesenteric vs. pulmonary arteries. [c] and [d] $P < 0.05$ and $P < 0.01$ noradrenaline vs. U46619, respectively.

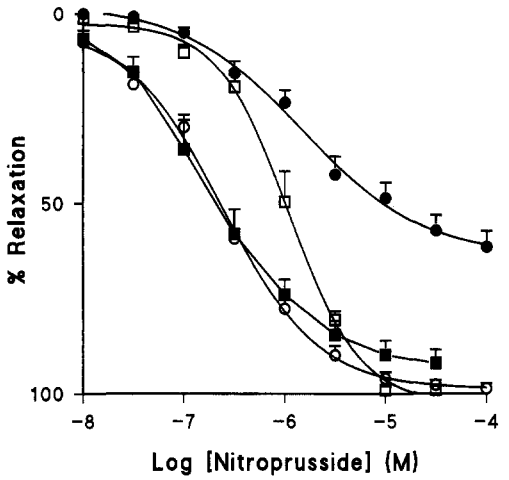

Fig. 2. Relaxant effects of cumulative addition of sodium nitroprusside on pulmonary (circles) and mesenteric arteries (squares) pre-contracted with 10^{-5} M noradrenaline (open symbols) or 10^{-6} M U46619 (solid symbols) of neonatal piglets. Results are expressed as mean ± S.E.M. of 7–9 experiments. Abscissa, % relaxation; ordinate, log nitroprusside concentration (M).

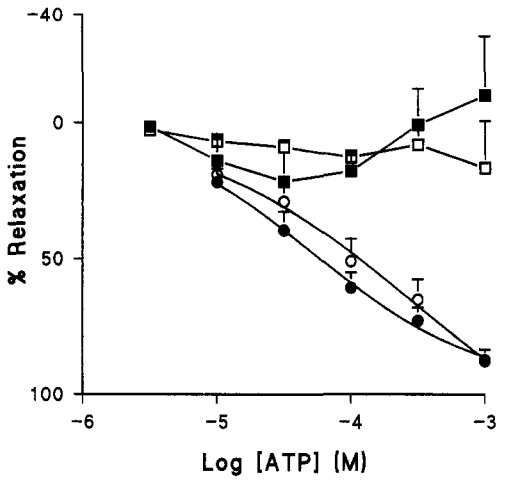

Fig. 3. Relaxant effects of cumulative addition of ATP on pulmonary (circles) and mesenteric arteries (squares) pre-contracted with 10^{-5} M noradrenaline (open symbols) or 10^{-6} M U46619 (solid symbols) of neonatal piglets. Results are expressed as mean ± S.E.M. of 5–7 experiments. Abscissa, % relaxation; ordinate, log ATP concentration (M).

ant effect whereas, in those contracted with U46619, a biphasic effect was observed. At concentrations of $\leq 10^{-4}$ M, ATP produced a relaxant effect while, at higher concentrations, a progressive increase in contractile force was observed, so that at the highest concentration tested reached tension values similar to control values. The effects of ATP were also tested in endothelium-denuded pulmonary arteries and in rings pre-treated for 20 min with L-NAME and then contracted by U46619. In both cases, ATP produced a relaxant effect similar to that observed in control endothelium intact pulmonary arteries (not shown).

Increasing concentrations of ATP (10^{-8}-10^{-3} M) produced minimal or no effect in resting pulmonary arteries ($n = 6$; Fig. 4). In contrast, in mesenteric arteries, ATP ($\geq 10^{-6}$ M) produced a concentration-dependent contraction ($n = 5$; Fig. 4). These contractions were fast but transient, reaching a peak in about 1–2 min and were followed by a slower decay to a lower tone.

3.5. Vasodilator effects of prostaglandin E_1

Addition of prostaglandin E_1 (10^{-8}-10^{-5} M) to pulmonary arteries resulted in a concentration-dependent relaxation (Fig. 5). The maximal relaxant effect was greater when the pulmonary arteries were contracted by noradrenaline than by U46619 ($P < 0.05$; Table 2). In mesenteric arteries, the response to prostaglandin E_1 was biphasic. At low concentrations, prostaglandin E_1 produced a contractile effect which was maximum at 10^{-7}-3×10^{-7} M while, at higher concentrations, prostaglandin E_1 produced a relaxant effect, so that, at 10^{-5} M, it produced almost full relaxation when the arteries were contracted by noradrenaline but only 41 ± 11% when contracted by U46619.

Table 2
Parameters of the concentration-response curves to acetylcholine, sodium nitroprusside, ATP, prostaglandin E_1 and tolazoline calculated from Figs. 1–3 and 5–7

	Pulmonary arteries						Mesenteric arteries					
	Noradrenaline			U46619			Noradrenaline			U46619		
	pIC_{50}	Max (%)	n	pIC_{50}	Max (%)	n	pIC_{50}	Max (%)	n	pIC_{50}	Max (%)	n
Acetylcholine	6.8 ± 0.2	101 ± 2	5	5.9 ± 0.4 [d]	53 ± 5 [d]	5	B.E.	B.E.	7	B.E.	B.E.	8
Nitroprusside	6.6 ± 0.1	100 ± 1	9	5.4 ± 0.3 [d]	61 ± 4 [d]	9	6.0 ± 0.1 [b]	106 ± 4	7	6.7 ± 0.2 [b,d]	92 ± 4	7
ATP	3.9 ± 0.2	87 ± 4	6	4.1 ± 0.1	87 ± 4	7	N.E.	N.E.	7	N.E.	N.E.	7
Prostaglandin E_1	6.1 ± 0.1	96 ± 3	9	5.7 ± 0.2	67 ± 6 [d]	10	5.8 ± 0.1	87 ± 7	8	> 5	41 ± 11 [b,d]	7
Tolazoline	4.8 ± 0.1	91 ± 3	7	N.E.	N.E.	7	5.1 ± 0.1 [a]	88 ± 3 [a]	7	N.E.	N.E.	5
Nifedipine	5.0 ± 0.1	51 ± 8	5	> 5	15 ± 3 [d]	7	5.7 ± 0.1 [b]	75 ± 5 [a]	7	> 5	28 ± 8 [d]	6

pIC_{50} is the negative logarithm of concentration which relaxed 50% and Max is the maximal relaxant effect achieved with the highest concentration of vasodilator tested.

B.E., biphasic effect; relaxation and contraction depending on drug concentration (see text and figures for details). N.E., minimum or no effect.

[a] and [b] $P < 0.05$ and $P < 0.01$ mesenteric vs. pulmonary arteries. [d] $P < 0.01$ noradrenaline vs. U46619, respectively.

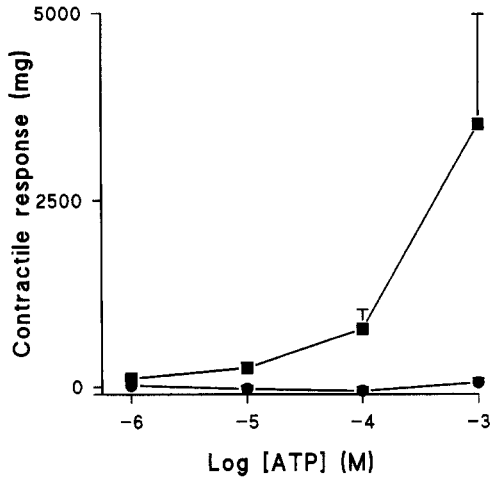

Fig. 4. Contractile effects of cumulative addition of ATP on pulmonary (circles) and mesenteric arteries (squares) on resting tension. Mesenteric arteries responded to ATP with a fast contraction reaching a peak in about 1–2 min and were followed by a decay in tone to a lower sustained level (peak contractile values are shown). Results are expressed as mean ± S.E.M. of 6 experiments. Abscissa, % relaxation; ordinate, log ATP concentration (M).

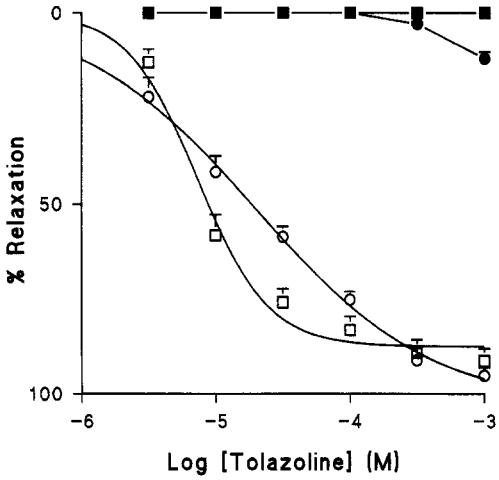

Fig. 6. Relaxant effects of cumulative addition of tolazoline on pulmonary (circles) and mesenteric arteries (squares) pre-contracted with 10^{-5} M noradrenaline (open symbols) or 10^{-6} M U46619 (solid symbols) of neonatal piglets. Results are expressed as mean ± S.E.M. of 6–7 experiments. Abscissa, % relaxation; ordinate, log tolazoline concentration (M).

3.6. Vasodilator effects of tolazoline

The α-adrenoceptor antagonist tolazoline (10^{-6}-5×10^{-4} M) fully relaxed pulmonary or mesenteric arteries contracted by noradrenaline (Fig. 6), this effect being slightly more potent in mesenteric arteries ($P < 0.05$; Table 2). However, tolazoline had no effect on arteries contracted by U46619.

3.7. Vasodilator effects of nifedipine

Fig. 7 shows that, at concentrations of $\leq 10^{-7}$ M, nifedipine produced no relaxant effect on pulmonary or mesenteric arteries. At higher concentrations, it produced a concentration-dependent relaxation in both arteries, even when the maximal relaxant effect could not be reached at the maximal concentration tested (10^{-5} M). Higher concentrations could not be used because the vehicle (ethanol) had significant effects at these concentrations. The relaxant responses to nifedipine were more pronounced when the arteries were contracted by noradrenaline than by U46619 ($P < 0.05$) and in mesenteric than in pulmonary arteries ($P < 0.05$).

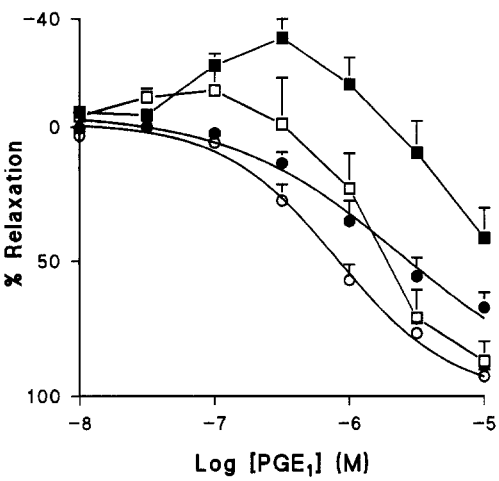

Fig. 5. Relaxant effects of cumulative addition of PGE_1 on pulmonary (circles) and mesenteric arteries (squares) pre-contracted with 10^{-5} M noradrenaline (open symbols) or 10^{-6} M U46619 (solid symbols) of neonatal piglets. Results are expressed as means ± S.E.M. of 7–10 experiments. Abscissa, % relaxation; ordinate, log PGE_1 concentration (M).

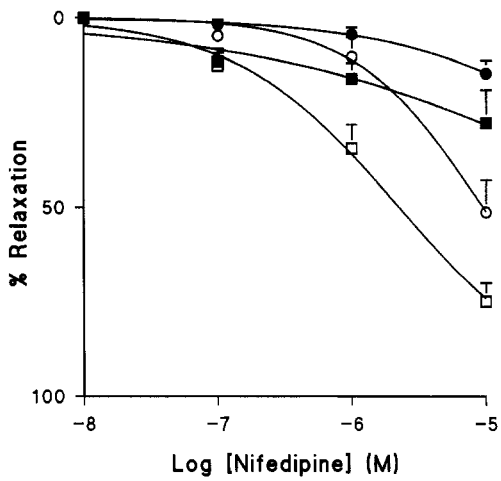

Fig. 7. Relaxant effects of cumulative addition of nifedipine on pulmonary (circles) and mesenteric arteries (squares) pre-contracted with 10^{-5} M noradrenaline (open symbols) or 10^{-6} M U46619 (solid symbols) of neonatal piglets. Results are expressed as mean ± S.E.M. of 5–7 experiments. Abscissa, % relaxation; ordinate, log nifedipine concentration (M).

4. Discussion

In the present study we have compared the effects of six vasodilators (acetylcholine, sodium nitroprusside, ATP, prostaglandin E_1, tolazoline and nifedipine) in isolated pulmonary and mesenteric arteries of neonatal piglets. For any of the vasodilators studied, we have found differences in their relaxant effects depending on the artery, the agonist used to contract the artery or both. ATP was the only drug which, at any concentration and regardless of the contractile agonist used, produced relaxant effects more marked in pulmonary than in mesenteric arteries.

The responses of isolated arteries to vasodilator drugs depend on the species, vascular bed, age, sex, pre-existing tone, endothelial preservation, vasoconstrictor used to increase tone and arterial diameter among other factors. Therefore, we must rise the following methodological considerations. We have studied the effects of vasodilators under conditions of a high vascular tone, i.e. after inducing maximal or near-maximal contractions, which presumably reflect what happens in persistent pulmonary hypertension of the newborn. Noradrenaline and the thromboxane A_2 mimetic U46619 were chosen as contractile agonists since noradrenaline is considered to be one of the most important factors regulating systemic and pulmonary vascular tone (Bülbring and Tomita, 1987; Barnes and Liu, 1995) and thromboxane A_2 has been associated with several forms of persistent pulmonary hypertension of the newborn (Dobyns et al., 1994). The same concentrations of vasoconstrictors were used in pulmonary and mesenteric arteries and, therefore, the vasodilator effects were not evaluated under equieffective concentrations of noradrenaline and U46619 for a given artery. Mesenteric arteries of a similar diameter than pulmonary arteries were chosen as representatives of systemic arteries. However, the effects of vasodilators on the whole systemic vascular resistance is the sum of the effects in all vascular beds and, thus, extrapolation of mesenteric arteries to an universal systemic artery has to be done with caution.

Acetylcholine has been reported to produce endothelium-dependent vasodilatation and both endothelium-dependent and -independent contraction (Furchgott and Zawadki, 1980; Altiere et al., 1986). In the present study, acetylcholine induced a concentration-dependent relaxation in pulmonary arteries and this effect was inhibited by the nitric oxide synthesis inhibitor L-NAME indicating an acetylcholine-induced nitric oxide release from the endothelium (Furchgott and Zawadki, 1980). However, when the arteries were pre-contracted by U46619, acetylcholine was unable to induce full relaxation. In contrast, in mesenteric arteries, acetylcholine produced relaxation at concentrations of $\leq 10^{-7}$ M, but contraction at higher concentrations. Therefore, at high concentrations, acetylcholine showed greater relaxant effects on pulmonary than on mesenteric arteries, although its relaxant effect was significantly less marked when the arteries were pre-contracted by U46619.

The vasodilator effects of sodium nitroprusside have been attributed to the release of nitric oxide which, in turn, stimulates soluble guanylate cyclase and increases the intracellular levels of cGMP (Ignarro and Kadowitz, 1985; Feelisch, 1991). The effects of this drug were analyzed in endothelium denuded arteries to avoid interferences with endothelial release of nitric oxide. Addition of sodium nitroprusside produced full vasorelaxant effects in mesenteric arteries contracted by either U46619 or noradrenaline and in pulmonary arteries contracted by noradrenaline but, as occurred with acetylcholine, it induced only partial relaxation in pulmonary arteries contracted by U46619. Thus, as recently reported (Pérez-Vizcaíno et al., 1996), activation of thromboxane A_2 receptors by U46619 in pulmonary arteries seems to reduce the sensitivity to nitric oxide.

ATP had no effect on resting tension but relaxed pulmonary arteries pre-contracted by noradrenaline or U46619. In contrast, addition of ATP ($> 10^{-6}$ M) to mesenteric arteries at resting tone induced a concentration-dependent contraction. The vasoactive effects of ATP have been attributed to the activation of membrane P_2-purinoceptors. Activation of P_{2x}-purinoceptors located on smooth muscle mediate contraction in both rat and human pulmonary arteries whereas P_{2y}-purinoceptors mediating relaxation are located on the endothelium in rat and on the smooth muscle in human pulmonary arteries (Liu et al., 1989a,b). The present results show that ATP-induced relaxation in neonatal piglet pulmonary arteries are endothelium- and nitric oxide-independent. The purinoceptor subtype mediating this effect is unknown but based on the similarities of the response with human pulmonary arteries it might be tempting to speculate that it is mediated by P_{2y}-purinoceptors located on smooth muscle. In contrast to human pulmonary arteries (Liu et al., 1989a), ATP produced minimal contractile effect in piglet pulmonary arteries at resting tone. At present, we do not know if differences are species- or age-dependent. In pre-contracted mesenteric arteries, ATP produced both relaxant and contractile effects. A weak relaxant response was observed at low concentrations ($\leq 10^{-4}$ M) whereas, at higher concentrations, ATP produced a weak contractile effect, so that the average tension level was not significantly different to the initial tension value. Therefore, the vasodilator effect of ATP was selective for pulmonary over mesenteric arteries regardless of the agonist used to contract the arteries.

Prostaglandin E_1 is a non-selective agonist of prostanoid EP_1, EP_2 and EP_3 receptors (Coleman et al., 1994). In general, EP_1 and EP_3 receptor subtypes mediate contraction of smooth muscle, and EP_2 receptors mediate smooth muscle relaxation. Therefore, both prostaglandin E_1-induced vasodilation and vasoconstriction have been reported (Kadowitz et al., 1976; Bergström et al., 1968; Qian

et al., 1994). In the present study, prostaglandin E_1 relaxed pulmonary arteries but this effect was significantly more pronounced when the arteries were pre-contracted by noradrenaline. In contrast, in mesenteric arteries, low concentrations of prostaglandin E_1 induced a small contractile response whereas, at higher concentrations, a relaxation was observed. Therefore, prostaglandin E_1 showed a weak selectivity for pulmonary over mesenteric arteries.

Tolazoline is considered a non-selective α-adrenoceptor blocker (Ruffolo et al., 1991) that exhibits other non-adrenoceptor-mediated vasodilators effects (Drummond and Lock, 1984). In the present study, tolazoline almost fully relaxed pulmonary and mesenteric arteries pre-contracted with noradrenaline, which consistent with its α-adrenoceptor blocking properties and this effect was slightly but significantly more potent in mesenteric than in pulmonary arteries. Tolazoline, however, had no effect in pulmonary or mesenteric arteries contracted by U46619, which suggested that a direct pulmonary vasodilator effect unrelated to α-adrenoceptor blockade was absent.

The potency of the L-type Ca^{2+} channel blocker nifedipine to inhibit the contractions induced by noradrenaline or other agonists is highly variable, depending on the role of Ca^{2+} entry through L-type Ca^{2+} channels in the contractile response (Cauvin et al., 1983; Godfraind et al., 1986). In the present study, only at very high concentrations of nifedipine ($\geq 10^{-6}$ M) induced relaxant responses in noradrenaline- or U46619-precontracted arteries. However, at these concentrations, it is very unlikely that the vasodilator effects of nifedipine can be related to Ca^{2+} entry blockade. The relaxant response to nifedipine was more marked in both pulmonary or mesenteric arteries pre-contracted by noradrenaline as compared to U46619 contracted vessels. Nevertheless, mesenteric were more sensitive than pulmonary arteries to nifedipine.

Since lowering pulmonary artery pressure, while maintaining systemic vascular resistance and good cardiac output, is crucial for newborns with persistent pulmonary hypertension of the newborn, a search for selective pulmonary vasodilators has been constant in the last two decades (Roberts and Shaul, 1993). Most clinical studies in neonates suffering persistent pulmonary hypertension of the newborn, however, have been carried out in small number of patients, were not randomized, and no direct measurements of pulmonary artery pressure were made, so that arterial pO_2 or clinical improvement was used to indirectly evaluate pulmonary vasodilation. Therefore, conclusions regarding drug pulmonary selectivity cannot be drawn and most data come from animal models of persistent pulmonary hypertension of the newborn. Tolazoline, the most widely used drug in the treatment of persistent pulmonary hypertension of the newborn, produced systemic hypotension in $> 50\%$ of patients (Stevenson et al., 1979; Ward, 1984; Starling et al., 1981; Gouyon and Francoise, 1992) and, therefore, it can be considered as a poor pulmonary selective drug. Similar systemic deleterious effects have been reported in animal models of pulmonary hypertension after infusion of acetylcholine (Tripp et al., 1980), nifedipine (Dickstein et al., 1984) or prostaglandin E_1 (Tripp et al., 1980; Starling et al., 1981). The limited use of sodium nitroprusside in persistent pulmonary hypertension of the newborn has rendered variable results (Benitz et al., 1985). The present in vitro results with tolazoline and nifedipine demonstrated that systemic arteries dilate at least as much as pulmonary arteries, supporting the poor selectivity observed in clinical studies. The results obtained with acetylcholine, prostaglandin E_1 or sodium nitroprusside are difficult to interpret in terms of pulmonary vs. systemic selectivity due to the biphasic (contractile and relaxant) responses or to agonist-dependent differences. ATP has demonstrated selective pulmonary vasodilating effects in animal models of pulmonary hypertension (Konduri and Woodard, 1991; Fineman et al., 1990) and our in vitro results also provide evidence of its selective pulmonary vasodilator effect. Indeed, ATP was able to induce contractile responses in mesenteric but not in pulmonary arteries under resting conditions. Very recently, the administration of low doses of ATP in pulmonary hypertensive newborns and infants produced a decrease in pulmonary vascular resistance without effects on systemic blood pressure, suggesting that ATP may be a selective pulmonary vasodilator, although at higher doses it produced mild systemic effects (Brook et al., 1995).

In conclusion, the vasodilators studied exhibited differences in the relaxant effects depending upon the artery and/or the agonist used to contract the vessel. However, ATP was the only drug which, at all concentrations and regardless of the contracting agent used, relaxed the pulmonary artery but not the mesenteric artery.

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