Effects of Alpha Adrenergic Blockade and Tissue Catecholamine Depletion on Pulmonary Vascular Response to Hypoxia

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ABSTRACT The highly reactive pulmonary vascular bed of the neonatal calf was utilized to determine whether the hypoxic pulmonary pressor response is modified by α-adrenergic blockade with phenoxybenzamine (Group A) or by tissue catecholamine depletion with reserpine (Group B). In addition, in Group A, the effects of hypoxia on the pulmonary circulation were compared and contrasted with those of *l*-norepinephrine (α-receptor stimulator) and isoproterenol (β-receptor stimulator).

In Group A, changes in pulmonary vascular resistance were calculated from measurements of appropriate pressures and of pulmonary blood flow (electromagnetic flowmeter). The increase in pulmonary vascular resistance produced by hypoxia was not diminished by α-adrenergic blockade. However, blockade abolished the pulmonary vasoconstrictor effect of norepinephrine. During hypoxic pulmonary vasoconstriction, the administration of either norepinephrine or isoproterenol lowered the pulmonary vascular resistance both before and after α-blockade. While this may be a true vasodepressor effect of these drugs it may also reflect passive changes in the pulmonary vessels secondary to an increased pulmonary blood flow.

The pulmonary vascular response to hypoxia in the reserpinized calves (Group B) was tested under three circumstances: (*1*) in the awake animal, (*2*) in the anesthetized animal prepared in the same way as those in Group A, and (*3*) during constant flow perfusion of the left lower lobe pulmonary artery. From these studies it was concluded that tissue catecholamine depletion did not diminish the pulmonary vascular response to hypoxia.

Thus, neither α-adrenergic blockade nor tissue catecholamine depletion prevents the hypoxic pulmonary pressor response. Furthermore, α-blockade prevents the pulmonary vasoconstrictor response to norepinephrine but not to hypoxia. Therefore it is concluded that hypoxic pulmonary vasoconstriction is not mediated through adrenergic receptor stimulation or release of endogenous catecholamines.

INTRODUCTION

The mechanism by which hypoxia increases pulmonary vascular resistance remains elusive. 15 yr ago it was suggested that the catecholamines were involved in this response (1, 2). Subsequent investigations have been inconclusive in either supporting or refuting this hypothesis. Several investigators have concluded that blockade of the sympathetic nervous system reduces or abolishes the pulmonary pressor response to hypoxia (3–9). There is equally strong evidence to dispute this

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(10–13). To explore the problem further, we selected the newborn calf whose highly reactive pulmonary vascular bed yields pressure changes of larger magnitude than seen in any other experimental animal in which studies of this nature have been performed (14–17).

This study was designed primarily to determine whether the hypoxic pulmonary pressor response is modified by either α-adrenergic blockade with phenoxybenzamine or by depletion of tissue stores of norepinephrine with reserpine. An additional objective was to compare the pulmonary vascular effects of hypoxia with those of sympathomimetic drugs.

METHODS

The experiments were performed on two groups of calves which will be described separately as Group A and Group B.

Group A

Five calves aged 24–72 hr, and weighing 23–48 kg were used. After anesthesia with intravenous pentobarbital sodium in a dose of 15–20 mg/kg, we performed endotracheal intubation and administered positive pressure respiration with a Harvard respiratory pump using a tidal volume of 350–500 ml and a frequency of 15–20 breaths per min. The expiratory tube from the pump was placed under 2–5 cm of water to maintain expansion of the lungs when the chest was opened. Ventilation was adjusted to maintain arterial blood P_{CO_2} levels between 40 and 50 mm Hg. As a result the pH was usually in the range of 7.350–7.450 and did not vary by more than ± 0.050 during any experiment. Via the left external jugular vein we passed a 7F Lehman catheter into the pulmonary artery using the pressure pulse contour as a guide to its position. A 7F NIH catheter was passed retrograde into the aorta via the left carotid artery to measure systemic blood pressure.

With the animal lying on its right side a left thoracotomy was performed. The ductus arteriosus was ligated and a Statham electromagnetic flowmeter probe (Statham Instruments, Los Angeles, Calif.) was placed around the main pulmonary artery. This provided a direct measure of pulmonary blood flow since it avoided considerations of shunting through the foramen ovale or ductus arteriosus in these newborn animals (18). A 6F Lehman catheter was introduced into the pulmonary artery by direct puncture and guided into the "wedge" position. A satisfactory wedge position was assumed if the resultant pressure pulse had a characteristic atrial contour. Furthermore, at the commencement of each experiment, this pressure did not differ more than a few mm Hg from the direct left atrial pressure. A polyethylene catheter, introduced into the pulmonary artery by direct puncture, was used as a route for drug administration. A 7F NIH catheter was passed into the left atrium via its appendage. To confirm that this catheter had not passed into the right atrium through the foramen ovale or into a pulmonary vein, the catheter was advanced through the mitral valve into the left ventricle, then slowly withdrawn until an atrial pressure pulse appeared; a blood sample with an oxygen tension corresponding to that of the aorta further confirmed the left atrial position.

Pressures were measured in the pulmonary artery and "wedge" position, left atrium, and aorta with Statham p23Db pressure transducers with zero reference at heart level. A Medicon (Statham Instruments, Los Angeles, Calif.) 0–5000 pulsed field electromagnetic blood flowmeter measured blood flow in the main pulmonary artery, and the electrically integrated mean flow was recorded. The flow probes and flowmeter were calibrated in vitro (19) and the response was shown to be linear with flow. Recording of pressures and flow were made with an Electronics for Medicine DR 8 oscillographic recorder (Electronics for Medicine, Inc., White Plains, N. Y.). Systemic arterial blood was analyzed immediately after collection for pH, P_{O_2} and P_{CO_2} using appropriate Radiometer (Rinn Corp., Chicago, Ill.) electrodes at 38°C. Pulmonary vascular resistance was expressed in resistance units of mm Hg per liter per minute per 25 kg body weight. The equation used for this calculation was $PVR = [(\overline{PA} - \overline{LA})/\dot{Q}] \times \text{weight}/25$ where PVR, pulmonary vascular resistance units; $\overline{PA}$, pulmonary arterial mean pressure, mm Hg; $\overline{LA}$, left atrial mean pressure, mm Hg; $\dot{Q}$, pulmonary blood flow in liters per minute; and weight is expressed in kilograms.

The pulmonary vascular responses to hypoxia and to norepinephrine (α-adrenergic receptor stimulator) and isoproterenol (β-adrenergic receptor stimulator) were compared before and after α-adrenergic blockade with phenoxybenzamine. Hypoxia was induced by ventilating both lungs with a gas mixture of 10% oxygen in nitrogen which lowered the P_{IO_2} from 120 to 55 mm Hg (PB in Denver = 625 mm Hg) with no change in minute ventilation. *l*-Norepinephrine 24 μg, and isoproterenol 4 μg, each in solution in 3 ml of 5% dextrose in water, were injected rapidly into the pulmonary artery. These maneuvers were applied in a variable sequence, each preceded by a stable control period on room air. Each drug was also injected after 2 min of a subsequent period of hypoxia.

Phenoxybenzamine, in a dose of 1.5–2.0 mg/kg, diluted in 200 ml of normal saline was then infused over a 10 min period into the pulmonary artery. After 1 hr, the conversion of the biphasic systemic pressure response to epinephrine to a purely vasodepressor response was presumed to be evidence of α-adrenergic blockade (20). The earlier maneuvers were then repeated.

Group B

Three calves, 2–4 days old, and weighing 34–40 kg, were each given intramuscular reserpine, 0.1 mg/kg per day for 2 days and studied 48 hr after the first dose. This dose has been demonstrated to be adequate for de-

pletion of myocardial catecholamine stores in the dog (21). To confirm the adequacy of this dose in the present study, two methods were used: (*a*) With the animal awake, the heart rate and systemic blood pressure responses to graded intravenous doses of tyramine were determined (21). After thoracotomy the left atrial appendage was excised and frozen rapidly. Its norepinephrine content was determined by the trihydroxyindole method as described by Chidsey and associates (22).

The pulmonary pressor response to hypoxia was studied in each animal under three different circumstances: (*1*) with the animal awake, (*2*) in the anesthetized animal with intact pulmonary circulation in which changes in pulmonary vascular resistance could be calculated as in Group A, and (*3*) during constant flow perfusion of a portion of the pulmonary vasculature.

(*1*) With the animal lying on its right side, the left external jugular vein and left common carotid artery were exposed under local anesthesia with 1% of lidocaine hydrochloride. A 7F NIH cardiac catheter was introduced into the carotid artery and passed retrograde into the aorta, and a 7F Lehman catheter passed via the external jugular vein into the pulmonary artery using the pressure pulse contour as a guide to its position. The heart rate and systemic pressure responses to intravenous tyramine were then determined at three dosage levels: 1, 10, and 100 μg/kg (22). Then after a period of control observations with the animal breathing room air, a polyethylene mask was applied over the snout and the animal was given 10% oxygen in nitrogen to breathe for a period of 1–2 min. The pulmonary arterial and systemic pressure responses were recorded.

(*2*) The animal was then prepared in the same way as those in Group A with minor modifications. The dose of pentobarbital required for anesthesia was only 50–75% of that needed in the nonreserpinized animals. The methods of measuring pulmonary blood flow and pressures were the same as those used in Group A. However, the left atrial appendage was excised for norepinephrine assay soon after thoracotomy, and left atrial pressures were accordingly measured indirectly from the left ventricular end-diastolic pressures. Catheterization of the left ventricle was performed by advancing the aortic catheter retrograde through the aortic valve. As before, systemic arterial blood gases were analyzed and changes in pulmonary vascular resistance were determined in response to ventilation with 10% O_2 and 100% O_2.

(*3*) After these observations on the intact cardiopulmonary circulation, the pulmonary circulation to the left lower lobe was then isolated and perfused. A large cannula was introduced into the right atrium via the right external jugular vein and connected to a Watson-Marlow roller pump. This, in turn, led to a cannula which was inserted into the left pulmonary artery distal to the upper lobe branch. Thereby a partial bypass of the right ventricle was achieved. A polyethylene catheter was introduced via the lingular branch of the left pulmonary artery into the lower lobe branch to record the perfusion pressure. A vascular clamp was applied to the left pulmonary artery distal to the upper lobe branch to assure isolation of the circulation to the lower and lingular lobes. The procedure of tying a cannula directly into the perfused vessel could conceivably interrupt nerve pathways in the vessel wall. However, we have observed in other animals that the pulmonary pressor response to hypoxia is no different when this method of cannulation is employed when compared with indirect cannulation of the left lower lobe via a lingular artery. Perfusion was established with a minimal interruption of blood flow to the left lower lobe, at a constant flow rate of 525 ml/min. The airway to the lobe was not isolated and both lungs were ventilated as previously.

Pressures were measured in the perfused vessel (LLPA), the main pulmonary artery, and the left ventricle. Left ventricular blood was analyzed for pH, P_{O_2}, and P_{CO_2}. After a steady control pressure had been recorded in the perfused vessel, observations were made during ventilation of both lungs with 10% O_2 and 100% O_2.

The significance of changes in the pulmonary vascular resistance in Groups A and B, and of pulmonary arterial pressure changes in the awake and the perfused preparations in Group B was assessed with the Fisher *t* test, and the corresponding *P* value is indicated.

RESULTS

The systemic arterial blood P_{O_2} ranged from 44.5 to 55.0 mm Hg during ventilation with room air. These values are within the range we have observed in normal awake calves of the same age in Denver. Ventilation with 10% oxygen in nitrogen reduced the systemic arterial blood P_{O_2} to 18–24 mm Hg. In a few animals in which pulmonary venous blood was also obtained simultaneously, its P_{O_2} was higher than that of arterial blood, which indicated that varying degrees of right-to-left shunting through the foramen ovale were present. Therefore the arterial blood P_{O_2} was not a reliable index of either pulmonary arterial, alveolar, or pulmonary venous P_{O_2} levels. For this reason we have not attempted to correlate systemic P_{O_2} levels with changes in pulmonary vascular resistance.

Group A

In Tables I and II data are presented for isolated measurements taken from rapidly changing dynamic states. In most instances these measurements correspond to the maximum change in resistance produced by each maneuver as explained below.

I. Before α-Adrenergic Blockade

(*a*) *Hypoxia* (*Fig. 1*). The initial phase of the response to hypoxia was characterized by an

TABLE I

Hemodynamic Effects of Hypoxia, Norepinephrine, and Isoproterenol, at Normal pH, before, and after Alpha Adrenergic Blockade*

	Before phenoxybenzamine											After phenoxybenzamine								
								Hypoxia									Hypoxia			
	Norepinephrine		Isoproterenol		Hypoxia			Isoproterenol		Norepinephrine		Norepinephrine		Hypoxia			Isoproterenol		Norepinephrine	
Calf—Wt Parameter	Pre	Post	Pre	Post	Pre	1 min	3 min	Pre	Post	Pre	Post	Pre	Post	Pre	1 min	2 min	Pre	Post	Pre	Post
1. 48																				
$P_{\overline{PA}}$	22	35	31	31	35	52	65	75	44	61	61	41	35	43	70	56	65	52	56	44
$P_{\overline{W}}$	13	14	15	12	13	16	16	20	13	15	15	16	14	14	14	11	11	5	11	10
$P_{\overline{LA}}$	5	5	8	8	8	8	9	8	6	8	8	4	3	3	3	3	2	1	3	2
$P_{\overline{AO}}$	129	174	118	99	119	110	86	83	78	75	103	77	101	70	40	27	28	32	27	62
$\dot{Q}_{PA}$	3.36	3.36	3.60	4.32	4.08	4.32	4.56	4.32	5.04	4.56	5.04	3.84	3.84	3.84	2.88	1.44	1.68	2.88	1.44	3.12
R_{PA}	9.8	17.1	12.3	10.2	12.7	19.2	23.6	29.8	14.4	22.1	20.2	18.4	15.9	20.0	44.7	70.7	72.0	34.0	70.7	25.9
2. 34																				
$P_{\overline{PA}}$	38	47	35	28	30	51	65	62	46	65	66	30	25	23	40	50	40	32	50	43
$P_{\overline{W}}$	7	7	7	7	6	6	7	7	7	7	7	6	7	6	6	6	5	5	6	7
$P_{\overline{LA}}$	4	4	3	3	3	3	3	4	3	3	3	2	2	2	2	2	2	2	2	2
$P_{\overline{AO}}$	142	172	128	120	134	128	102	80	80	102	126	70	97	65	57	44	36	39	44	72
$\dot{Q}_{PA}$	5.76	5.76	6.00	6.48	5.76	5.76	5.52	5.04	6.00	5.52	6.00	2.64	3.60	3.12	3.12	2.16	0.72	2.16	2.16	3.84
R_{PA}	8.0	10.2	7.2	5.3	6.4	11.3	15.2	15.6	9.8	15.3	14.3	14.4	8.7	9.1	16.6	30.2	71.8	18.9	30.2	14.6
3. 34																				
$P_{\overline{PA}}$	52	85	52	48	52	67	90	81	57	90	104	55	71	44	69	61	71	53	61	65
$P_{\overline{W}}$	13	21	15	13	14	14	18	20	14	18	23	15	18	12	15	12	13	11	12	14
$P_{\overline{LA}}$	5	2	5	4	4	4	5	5	5	5	0	4	1	4	2	5	5	5	5	4
$P_{\overline{AO}}$	87	140	101	87	98	96	64	81	74	64	122	77	98	66	59	41	55	60	41	64
$\dot{Q}_{PA}$	3.84	3.60	4.08	4.80	4.32	4.08	2.88	3.36	5.28	2.88	4.08	4.08	4.32	4.32	3.12	1.20	2.40	4.32	1.20	2.88
R_{PA}	16.6	31.3	15.6	12.5	15.1	21.0	40.1	30.7	13.6	40.1	34.7	17.0	22.3	12.6	29.2	63.5	36.9	15.1	63.5	28.8
4. 32																				
$P_{\overline{PA}}$	42	47	42	35	37	56	92	91	63	92	87	44	42	40	79	67	72	57	57	56
$P_{\overline{W}}$	17	18	18	17	18	18	20	20	19	20	20	22	21	19	24	26	38	36	26	26
$P_{\overline{LA}}$	7	7	7	7	7	7	7	7	7	7	6	6	6	5	5	6	6	5	6	5
$P_{\overline{AO}}$	137	170	112	97	132	134	98	105	76	98	124	77	92	77	62	44	57	47	44	62
$\dot{Q}_{PA}$	2.40	2.52	2.52	2.76	2.64	2.64	2.40	2.52	3.24	2.40	2.76	2.88	3.24	2.76	2.64	1.20	2.64	3.24	1.20	2.76
R_{PA}	18.7	20.4	17.8	13.1	14.6	23.8	45.3	42.6	22.1	45.3	37.6	17.0	14.2	16.3	35.8	65.0	32.0	20.5	65.0	23.7
5. 23																				
$P_{\overline{PA}}$	62	80	61	54	63	86	72	75	54	72	94	59	68	58	83	73				
$P_{\overline{W}}$	20	20	22	20	20	20	22	23	23	22	23	20	20	20	20	22				
$P_{\overline{LA}}$	13	13	13	13	13	13	13	12	11	13	13	13	13	12	12	12				
$P_{\overline{AO}}$	97	129	90	91	102	97	56	56	52	56	75	66	83	73	65	41				
$\dot{Q}_{PA}$	2.16	2.04	3.00	3.12	3.12	2.52	1.08	1.20	1.92	1.08	2.04	3.60	3.96	3.36	3.00	1.20				
R_{PA}	20.9	30.2	14.7	12.1	14.7	26.7	50.3	48.3	20.6	50.3	36.5	11.8	12.8	12.6	21.8	46.7				

Abbreviations: $P_{\overline{PA}}$, mean pulmonary arterial pressure; $P_{\overline{W}}$, mean "wedge" pressure; $P_{\overline{LA}}$, mean left atrial pressure; $P_{\overline{AO}}$, mean aortic pressure. All pressures in mm Hg. $\dot{Q}_{PA}$, pulmonary arterial blood flow, liters/min. R_{PA}, units of pulmonary vascular resistance in mm Hg/liter per min per 25 kg.

* The sequence of administration of hypoxia, norepinephrine, and isoproterenol was not the same in each animal and is unrelated to the order of presentation in the table.

TABLE II

Changes in Pulmonary Vascular Resistance Caused by Hypoxia, Norepinephrine, and Isoproterenol before and after α-Adrenergic Blockade*

	Before phenoxybenzamine								After phenoxybenzamine					
			10% O_2		NE during		IP during	1 hr after		10% O_2		NE during		IP during
Calf	NE‡	IP‡	1 min	3 min	10% O_2	10% O_2	10% O_2	blockade	NE	1 min	3 min	10% O_2	10% O_2	10% O_2
1	+7.3	−2.1	+6.5	+10.9	−2.1	+17.3	−15.4	+6.0	−2.5	+24.7	+50.7	−44.8	+53.6	−38.0
2	+2.2	−1.9	+4.9	+8.8	−0.9	+9.2	−5.8	+3.0	−5.7	+7.5	+21.1	−15.6	+60.5	−52.9
3	+14.7	−3.1	+5.9	+25.0	−5.4	+16.3	−17.1	−2.0	+5.3	+16.6	+50.9	−34.7	+24.1	−21.8
4	+1.7	−4.7	+9.2	+30.7	−7.8	+23.9	−20.5	0	−2.8	+19.5	+48.7	−41.3	+18.0	−11.5
5	+9.3	−2.6	+12.0	+35.6	−13.8	+35.7	−27.7	−2.5	+1.0	+9.2	+34.1			
Mean	+7.3	−2.9	+7.7	+22.2	−6.0	+20.5	−17.3	+1.0	−0.5	+15.5	+41.1	−34.1	+39.0	−31.1
SEM	±2.4	±0.5	±1.3	±5.3	±2.3	±4.5	±3.6	±3.5	±2.0	±3.2	±6.1	±6.5	±10.6	±9.1
P	<0.05	<0.01	<0.01	<0.02	<0.1	<0.01	<0.01	NS	NS	<0.01	<0.01	<0.02	<0.05	<0.05

Abbreviations: NE, norepinephrine; IP, isoproterenol; NS, not significant.

* Pulmonary vascular resistance expressed in units of mm Hg/liter per min per 25 Kg. Changes in resistance calculated from Table I.

increase in mean pulmonary arterial pressure, averaging 19 mm Hg, with little change in pulmonary blood flow during the 1st min. During the 2nd and 3rd min the mean pulmonary arterial pressure rose to a level averaging 33 mm Hg above control values. At this time, the flow had decreased markedly in animals 3 and 5, and in the latter animal there was an associated fall in pressure. The calculated pulmonary vascular resistance increased progressively in all animals and this increase was statistically significant after 1 min as well as after 2–3 min. After 1 min of hypoxia, when the flow had not decreased significantly in any of the animals, the resistance had increased by 62% of control values. After a further 1–2 min of hypoxia the increment in resistance average 175% of control values while the flow was still relatively unchanged in three of the five animals.

(*b*) *Norepinephrine* (*Figs. 1, 2, and 3*). An injection of 24 μg of *l*-norepinephrine into the pulmonary artery consistently caused an increase in the pulmonary arterial pressure within 8 sec of the injection, which reached a maximal effect within 12–15 sec. The pressure rise averaged 16 mm Hg. Pulmonary blood flow measured at the time of the maximum rise in pressure remained unchanged in

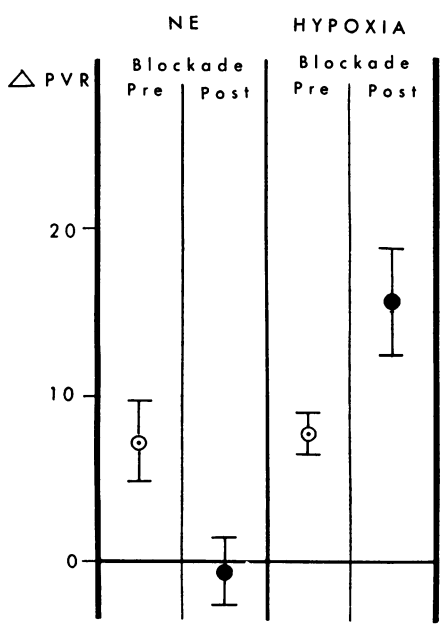

FIGURE 1 Comparison of effects of norepinephrine (*NE*) and hypoxia before (⊙) and after (●) α-adrenergic blockade with phenoxybenzamine. Mean changes in pulmonary vascular resistance (△ *PVR*), expressed in resistance units of mm Hg/liter per minute per 25 kg are shown; vertical lines represent SEM. The data are taken from tables I and II, and the values illustrated for △ PVR during hypoxia are those obtained in the early phase of the pulmonary vascular response during which flow was relatively unchanged. Blockade abolished the response to NE but not to hypoxia.

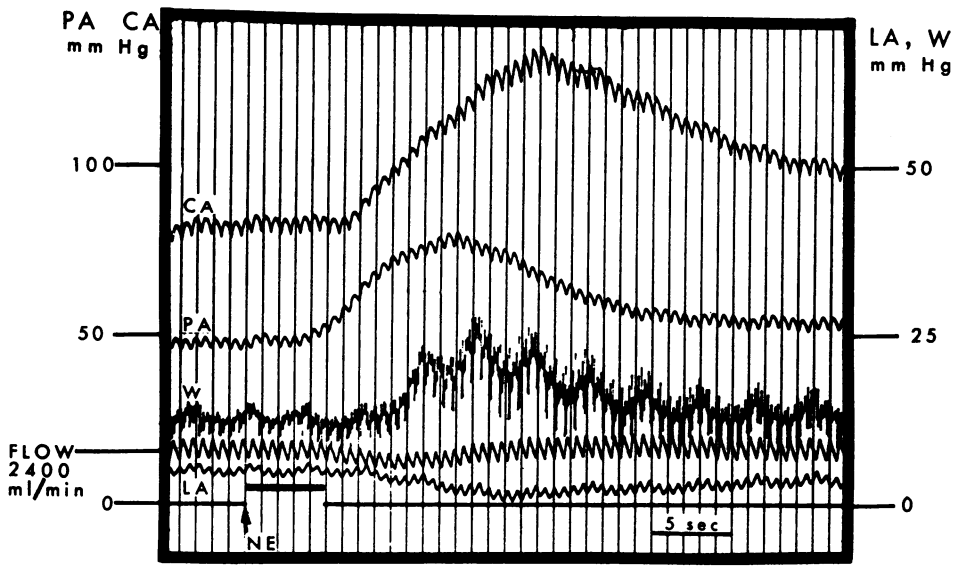

FIGURE 2 Pulmonary pressor response to injection (arrow) of norepinephrine (*NE*) into pulmonary artery (*PA*). PA mean pressure begins to rise before aortic (*CA*) and "wedge" (*W*) pressures. The decrease in left atrial pressure (*LA*) indicates that a systemic "back-pressure" mechanism is not operative.

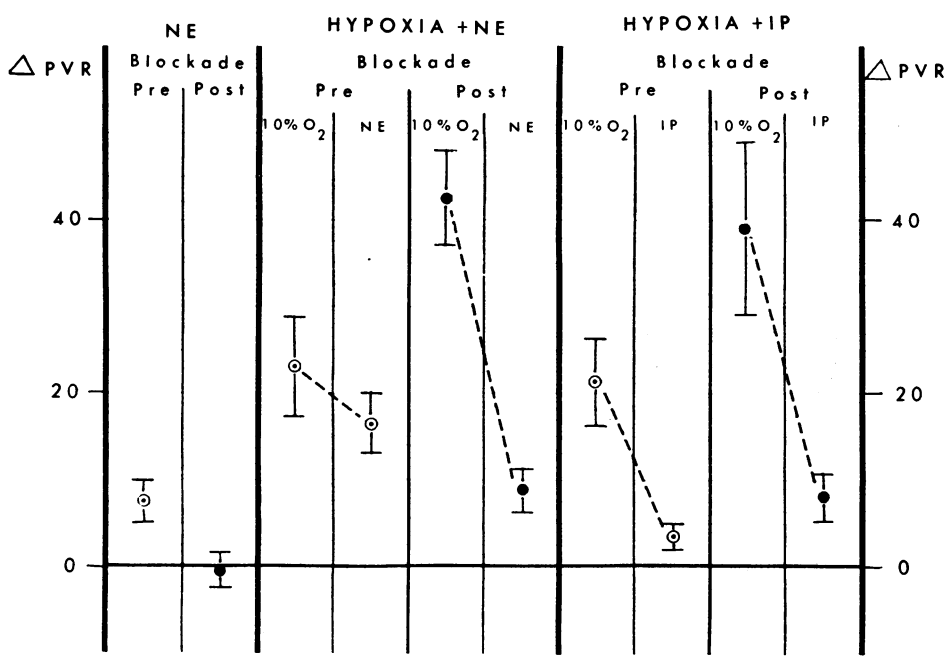

FIGURE 3 Effects of norepinephrine during normoxia (*NE*) compared with its effects during hypoxia (*HYPOXIA + NE*) and with those of isoproterenol during hypoxia (*HYPOXIA + IP*). Mean changes in pulmonary vascular resistance (△ *PVR*) expressed in units of mm Hg/liter per minute per 25 kg are indicated; vertical bars represent SEM. Effects compared in each instance before (⊙) and after (●) α-adrenergic blockade with phenoxybenzamine. Blockade abolished the pulmonary vasoconstrictor response to NE but not to hypoxia. Both NE and IP decreased PVR when given during hypoxic pulmonary vasoconstriction, an effect which was greater after blockade.

one animal, increased slightly in three, and decreased in one. The left atrial pressure did not change in four animals, and decreased by 3 mm Hg in one despite a consistent increase in systemic blood pressure in all animals to levels averaging 39 mm Hg above control values. The pulmonary arterial wedge pressure did not change in two animals and increased in three. When the wedge pressure did increase, this occurred after the pulmonary arterial pressure had increased (Fig. 2). The calculated pulmonary vascular resistance was increased significantly in all animals ($P < 0.05$), the average increment being 50% of control values.

The effect of an injection *l*-norepinephrine was observed during ventilation with 10% oxygen, at a time when the pulmonary vascular resistance was increased. This resulted in no change in pulmonary arterial pressure in one animal, an increase in two, and a decrease in two, while the pulmonary blood flow increased in all. The calculated pulmonary vascular resistance decreased in all animals but the decrease was not significant ($P < 0.1$).

(*c*) *Isoproterenol.* An injection of isoproterenol into the pulmonary artery caused a decrease in pulmonary arterial pressure in four animals but no change in one, while the flow was increased in all. The calculated pulmonary vascular resistance was significantly decreased ($P < 0.01$) to a level averaging 25% lower than the control value. When the drug was injected during ventilation with 10% oxygen, at a time when the pulmonary vascular resistance was increased, a profound consistent decrease in pulmonary vascular resistance resulted, with the level returning almost to control values (Fig. 3). This decrease was highly significant ($P < 0.01$).

II. After α-Adrenergic Blockade with Phenoxybenzamine

(*a*) *Hypoxia* (*Fig. 1*). In all animals the hypoxic pulmonary pressor response persisted. During the initial phase of the response to hypoxia the increase in pulmonary arterial mean pressure averaged 27 mm Hg and was associated with little or no change in pulmonary blood flow in animals 2, 4, and 5. However, after 1.5–2 min, pulmonary blood flow had decreased markedly in all animals accompanied by a decrease in pressure in animals 1, 3, 4, and 5. Calculated pulmonary vascular resistance increased progresively in all animals and this increase was statistically significant ($P < 0.01$). The final increment in resistance was 290% above control values. This calculation is made in the presence of extremely low flow rates. In the 1st min of hypoxia, before flow was significantly decreased, the increment in resistance averaged 106% above control values. These results indicate that the increment in pulmonary vascular resistance produced by hypoxia before a decrease in pulmonary blood flow was not diminished by α-adrenergic blockade.

(*b*) *Norepinephrine* (*Figs. 1 and 3*). Injection of *l*-norepinephrine into the pulmonary artery produced no significant change in pulmonary vascular resistance in contrast to its pulmonary vasoconstrictor effect before α-adrenergic blockade. When the drug was injected during ventilation with 10% oxygen, at a time when the pulmonary vascular resistance was increased, a large, significant decrease in resistance was noted ($P < 0.02$) (Fig. 3). This effect was greater than that which had occurred before α-adrenergic blockade.

(*c*) *Isoproterenol* (*Fig. 3*). An injection of isoproterenol into the pulmonary artery during ventilation with 10% oxygen, at a time when the pulmonary vascular resistance was increased, resulted in a profound, significant decrease in resistance ($P < 0.05$) which was of greater magnitude than that observed before α-adrenergic blockade.

Group B, reserpinized calves

Assessment of Norepinephrine Depletion

In none of the animals did tyramine provoke an increase in heart rate or in systemic pressure. The absence of these responses indicates depletion of tissue stores of norepinephrine since the primary hemodynamic effects of tyramine result from liberation of endogenous catecholamines (21). The average norepinephrine content of the left atrial appendages was 0.01 μg/g whereas in normal calves of the same age in this laboratory this value is 1.56 μg/g (SD = 0.66, $P < 0.05$). This provides further evidence that reserpine had adequately depleted the tissue stores of norepinephrine.

(*a*) *Awake animals* (*Table III*). The pulmonary arterial pressure rose consistently and markedly during hypoxia. The average elevation in mean pressure was 32 mm Hg ($P < 0.01$) which represents a 91% increment in pressure. In a pre-

TABLE III

*Hypoxic Pulmonary Pressor Response in Awake Reserpinized Calves**

Calf	Wt	Inspired gas % O_2	$P_{\overline{PA}}$ mm Hg	P_{AO} mm Hg	$\Delta P_{\overline{PA}}$ mm Hg
	kg				
1.	40	21	21	$\overline{105}$	
		10	53	$\overline{88}$	+32
2.	40	21	50	140/95	
		10	80	100/35	+30
3.	34	21	33	93/57	
		10	67		+34
Mean					+32
P					< 0.01

* Abbreviations as in Table I.

vious investigation from this laboratory (17) it was shown that in eight normal newborn calves studied awake, the same hypoxic stimulus induced an increment in pressure of 21 mm Hg (SD = 9.6) which represented a 51% increment. Therefore the pulmonary pressor response to hypoxia was not diminished in the awake reserpinized animal.

(*b*) *Anesthetized animals* (*Table IV*). During anesthesia the pulmonary blood flows were generally lower in the reserpinized animals (Table IV) than in normal anesthetized animals (Table I). The flows in the reserpinized animals decreased further during hypoxia. Nevertheless, hypoxia induced an increment in pressure averaging 21 mm Hg (compared with 33 mm Hg in control animals, Table I) which represents an increment of 35% (compared with 77% in the control animals). The calculated pulmonary vascular resistance increased by an average of 27.6 U ($P < 0.05$) (compared with 22.2 U in the control group (Table II). However, this represented an average increment in resistance of only 106% compared with a 175% increment in the controls. In two reserpinized animals in which the pulmonary vascular resistance produced by hypoxia was compared with that during ventilation with 100% oxygen, hypoxia was noted to induce an increment in resistance of 195%. Therefore, the pulmonary

TABLE IV

*Hemodynamic Effects of Hypoxia in Anesthetized Reserpinized Calves. Observations during Each of Two Periods of Hypoxia in Each Animal**

Calf	Wt	Inspired gas % O_2	Pa_{O_2} mm Hg	$P_{\overline{PA}}$	$P_{\overline{W}}$	P_{LV}	$\dot{Q}_{PA}$	R_{PA}	ΔR_{PA}‡
	kg								
1.	40	21	55.8	45	11	105/13	2.94	17.4	
		10	20.8	74	19	72/8	2.52	41.9	+24.5
		21	43.8	44	12	86/7	3.08	19.2	
		10	20.8	66	17	57/–4	2.24	50.0	+30.8
2.	40	21	47.0	79	27	102/13	2.38	44.4	
		10	19.5	89	28	87/15	1.82	65.1	+19.5
		21	47.0	62	23	70/12	2.10	38.1	
		10	19.5	80	20	78/15	1.82	57.1	+19.0
		100	282.0	58	19	115/16	4.06	16.6	
3.	34	21	47.9	62	25	108/18	1.96	30.5	
		10	20.0	75	26	78/14	1.26	65.8	+35.3
		100	280.0	52	23	122/13	2.10	25.3	
		21	42.3	56	23	96/11	2.10	29.1	
		10	20.2	72	25	86/11	1.26	65.8	+36.7
		100	280.0	48	22	103/12	2.10	23.3	
Mean									+27.6
P									< 0.05

* Abbreviations as in Table I; P_{LV}, left ventricular pressure, mm Hg; Pa_{O_2}, systemic arterial oxygen tension.

‡ ΔR_{PA} represents the increase in pulmonary vascular resistance in resistance units (mm Hg/liter per min per 25 kg) produced by ventilation with 10% O_2 after ventilation with 21% O_2.

TABLE V

*Pressures in Pulmonary Artery, Perfused at Constant Flow, before and during Hypoxia in Reserpinized Calves**

Calf	Wt	Inspired gas % O_2	Pa_{O_2} mm Hg	$P_{\overline{LLPA}}$	$P_{\overline{RPA}}$	P_{LV}	$\dot{Q}_{RPA}$	$\Delta P_{\overline{LLPA}}$‡ 10% O_2–100% O_2	$\Delta P_{\overline{LLPA}}$‡ 10% O_2–21% O_2
	kg								
1.	40	21	60.0	41	77	121/8	2.38		
		10	25.5	64	101	79/6	1.12		+23
		100	410.0	56	89	114/7	2.10		
		10	27.2	83	103	84/6	1.12	+27	
2.	40	21	46.0	32	50	66/14	2.52		
		10	22.0	45	67	61/12	1.12		+13
		100		32	52	70/12	3.22		
		10		60	67	58/13	1.82	+28	
3.	34	21	40.0	35	37	54/10	0.84		
		10	18.9	47	61	64/10	1.12		+12
		100	160.0	33	28	68/11	1.12		
		10	20.0	45	45	57/10	1.26	+12	
Mean								+22	+16
P	*P*							< 0.05	< 0.05

* Abbreviations: $P_{\overline{LLPA}}$, mean pressure in perfused pulmonary artery; $P_{\overline{RPA}}$, $\dot{Q}_{RPA}$, pressure and flow, respectively, in nonperfused portion of pulmonary artery. Pressures in mm Hg, flow in liter/min.

‡ $\Delta P_{\overline{LLPA}}$ 10% O_2—100% O_2 represents the difference in mean pressure in the perfused pulmonary artery produced by ventilation with 10% O_2 after ventilation with 100% O_2. Similarly, 10% O_2—21% O_2 represents the pressure difference on changing from room air to 10% O_2.

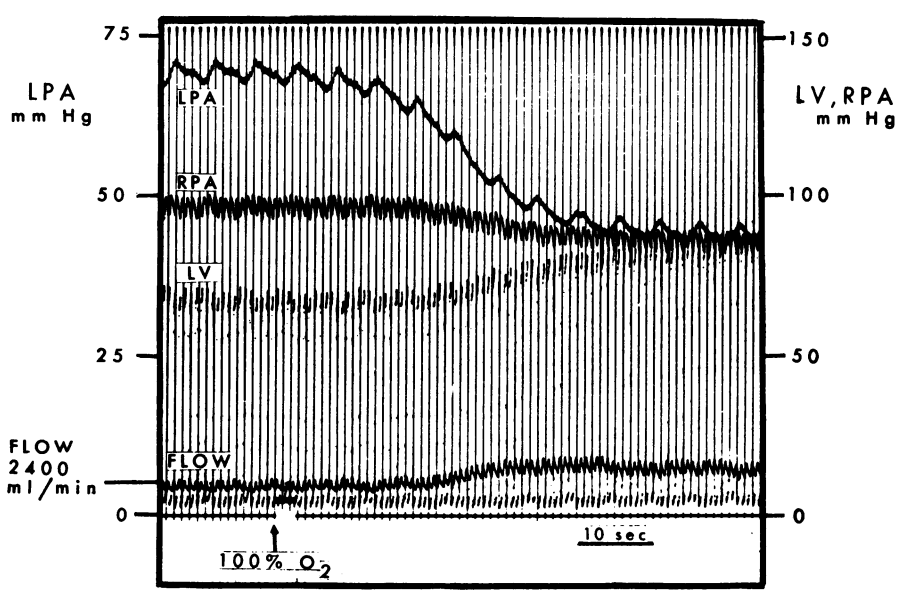

FIGURE 4 Pulmonary pressor response to hypoxia persisted in reserpinized calf. Mean pressure in pulmonary artery perfused at constant flow (*LPA*), when elevated by hypoxia, decreased markedly after commencement (arrow) of ventilation with 100% O_2. In the nonperfused portion of the pulmonary artery (*RPA*) the pressure decrease was not as great, but the accompanying increase in RPA flow (*FLOW*) resulted in a decrease in the calculated pulmonary vascular resistance in the intact lung as well. LV, left ventricle.

pressor response to hypoxia was preserved in the anesthetized reserpinized animals, but the calculated percentile increases in pressure and resistance were decreased. This was a consequence of the initially elevated pressures and resistances associated with flows which were lower than those recorded in control animals.

(*c*) *Perfused LLPA* (*Table V*). The LLPA was perfused at the same constant flow throughout each experiment. Therefore changes in pressure in that artery represent changes in pulmonary vascular resistance. By using this constant flow preparation we avoided the problems resulting from the low cardiac output in the anesthetized reserpinized animals in the aforementioned experiment and the further decreases in pulmonary blood flow during hypoxia. Hypoxia consistently elevated the LLPA pressure by an average of 16 mm Hg above values during ventilation with room air ($P < 0.05$), and an average of 22 mm Hg above values during ventilation with 100% O_2 ($P < 0.05$) (Fig. 4). This response is the same as that observed in normal animals during LLPA perfusion at comparable flow rates (16 mm Hg, SEM ± 2.9) (23). Similarly, the percentile increment of 44% is the same as the increment of 43% in normal animals.

DISCUSSION

We have investigated the potential role of the catecholamines and α-adrenergic receptors in the pulmonary pressor response to hypoxia. We have approached the problem by using the intact pulmonary circulation of the newborn calf to compare the effects of hypoxia and norepinephrine, and to determine the influence of either α-adrenergic blockade or of catecholamine depletion on the hypoxic pulmonary pressor response. Since the intact pulmonary circulation was subject to changes in blood flow, additional information was obtained in one group of animals by perfusing a portion of the pulmonary vascular bed at a normal constant rate of flow.

In the highly reactive pulmonary vascular bed of the newborn calf, hypoxia produced large increases in pulmonary arterial pressure. During the early phase of the response to hypoxia (1st min) pulmonary blood flow remained relatively constant, but later the blood flow decreased. Consequently, calculated pulmonary vascular resistance increased primarily as a result of the rise in pressure during the early phase of the response, with a further increase resulting from the subsequent decrease in blood flow. This secondary increase in resistance could represent simply a passive narrowing of pulmonary blood vessels after the decrease in blood flow (24, 25). On the other hand, the earlier increase in resistance in the absence of a change in pulmonary blood flow is more likely to indicate active vasoconstriction. Therefore, we will confine our argument to those changes in resistance which were associated with only minor changes in flow.

During the 1st min of hypoxia the pulmonary vascular resistance increased by 62% of control values before α-adrenergic blockade, with a further increment in resistance and no diminution in flow in three of the five animals. Since the increment in resistance in response to hypoxia after phenoxybenzamine was 106%, it is apparent that this vasoconstrictor response was not changed by blockade.

Similarly, the pulmonary pressor response to hypoxia in awake animals after catecholamine depletion (91% increment in pressure) was clearly not reduced when compared with normal awake animals (51% increment) (17). When the reserpinized animals were anesthetized, the low cardiac output resulted in an increased calculated pulmonary vascular resistance. Consequently, the percentile increment in resistance during hypoxia was less than that observed in normal anesthetized animals although in absolute units, the increase in calculated resistance was at least as great. When the problem of diminished flow was resolved by perfusing a portion of the pulmonary vascular bed at a constant normal flow rate, both the absolute and the percentile increments in resistance during hypoxia were identical with comparable observations in normal animals (23). A consideration of these three sets of observations in the reserpinized animals indicates that catecholamine depletion did not diminish the pulmonary vascular response to hypoxia.

Many investigators, using dogs or cats, have reported that the hypoxic pulmonary pressor response was reduced or abolished by pharmacological or surgical sympathectomy (3–9). However, other work may be cited in which the pulmonary vascular response to hypoxia persisted in

dogs, cats (10–12), calves (13), and humans (26) after similar maneuvers. An analysis of these diverse conclusions indicates that reported changes in pulmonary arterial pressures and resistances in most species were usually of small magnitude and often difficult to interpret. The large changes in pulmonary arterial pressures and resistances which we have obtained in the newborn calf have helped to resolve this uncertainty by providing strong evidence that neither α-adrenergic blockade nor tissue catecholamine depletion prevents or diminishes the pulmonary vascular response to hypoxia.

Norepinephrine produced a significant increase in pulmonary vascular resistance with little change in pulmonary blood flow in all animals before α-adrenergic blockade, which implied pulmonary vasoconstriction. This effect was qualitatively similar to the early response to hypoxia. However, after the administration of phenoxybenzamine, norepinephrine failed to increase the pulmonary vascular resistance whereas the pulmonary vascular response to hypoxia persisted. This selective blocking of the effects of norepinephrine but not of hypoxia suggests that they acted through different mechanisms.

When norepinephrine was injected during hypoxic pulmonary vasoconstriction, both before and after α-adrenergic blockade, it caused a decrease in the calculated pulmonary vascular resistance. This was due almost entirely to an increase in pulmonary blood flow which may have opened passively some segments of the vascular bed that had been closed. However, this effect was more pronounced after blockade than before, an observation which suggests that α-adrenergic blockade may have unmasked the β-receptor–stimulating properties of norepinephrine (27). Norepinephrine is known to have a vasodepressor effect on the systemic circulation when administered during vasoconstriction, probably by a peripheral action (28), and it is conceivable that a similar mechanism operates in the pulmonary circulation. A further argument favoring a vasodilator effect of norepinephrine is our observation (23) that in the presence of constant flow perfusion of a portion of the pulmonary vasculature, the drug lowered the pulmonary vascular resistance in the presence of pulmonary vasoconstriction induced by a combination of hypoxia and acidosis. Similarly, isoproterenol is known to produce pulmonary vasodilation (23, 29), and it is of interest that during hypoxic pulmonary vasoconstriction its effects resembled those of norepinephrine. Again, however, it is possible that in our study the decrease in resistance caused by isoproterenol was in part due to its consistent effect in increasing blood flow.

From the results of the present study, we may conclude that neither α-adrenergic blockade nor depletion of tissue catecholamine stores prevents the hypoxic pulmonary pressor response. Moreover, this study has indicated that the pulmonary vasoconstrictor effects of hypoxia and norepinephrine are mediated differently. Thus, this pharmacological approach in a suitable experimental model implies that hypoxic pulmonary vasoconstriction is not mediated through adrenergic receptor stimulation or release of endogenous catecholamines.

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