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Attenuation of Hypoxic Pulmonary Vasoconstriction by Verapamil in Intact Dogs[1] (39271)

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Alveolar hypoxia may cause pulmonary vasoconstriction either by direct depolarization of the vascular smooth muscle cell membrane (electro-mechanical excitation-contraction coupling), or by releasing a vasoactive chemical mediator (pharmaco-mechanical coupling). Despite attempts to isolate a chemical mediator, no substance has been shown to fulfill all the requirements for mediating hypoxic vasoconstriction. If the pulmonary vasoconstriction induced by hypoxia is due to a direct action on smooth muscle, then it should be susceptible to blockade of transmembrane Ca^{2+} influx, and the contraction elicited by membrane depolarization. Since verapamil has been demonstrated to block Ca^{2+} influx in both cardiac and vascular smooth muscle in response to actions of depolarizing agonists (1, 2), it might abolish or at least attenuate hypoxic pulmonary vasoconstriction. Recent studies have shown that verapamil does reduce the pulmonary pressor response to hypoxia in isolated, blood-perfused rat lungs (3, 4). The present study was designed to test the hypothesis of a direct action of hypoxia causing pulmonary vasoconstriction, in intact dogs, by blocking Ca^{2+} influx into vascular smooth muscle cells with verapamil.

Materials and methods. Mongrel dogs, obtained in Denver, Colorado (1,600 m, P_B = 625 mm Hg), were anesthetized with sodium pentobarbital (30 mg/kg, iv) and intubated with a cuffed endotracheal tube. The animals breathed spontaneously through a low resistance respiratory valve. Polyethylene catheters were positioned in the main pulmonary artery and in the abdominal aorta for pressure determinations, and an infusion catheter was placed in the superior vena cava. Pulmonary arterial wedge pressure was determined with a Swan-Ganz catheter,[2] positioned in a peripheral pulmonary artery. Mean blood pressures, cardiac output (dye dilution), pulmonary vascular resistance, and total systemic resistance were computed as previously described (5). Endtidal CO_2 was monitored with an infrared CO_2 analyzer (Beckman, model LB-1) and, during hypoxia, was maintained at the control level by manual addition of 100% CO_2 to the inspired gas.

Cardiovascular responses to isocapnic hypoxia were determined in eight dogs during 20-min exposures to 10% O_2. Control measurements were first obtained while the animals breathed 30% O_2. With the onset of hypoxia, heart rate and mean blood pressures were recorded each minute, while cardiac output, pulmonary vascular resistance, and total systemic resistance were measured at 3, 5, 10, 15, and 20 min of hypoxia. Arterial blood gas tensions and pH were determined with appropriate electrodes (Radiometer blood gas analyzer, model 27) during both 30% O_2 and 10% O_2 breathing.

After the completion of the control 20-min hypoxic exposures, verapamil hydrochloride[3] (0.5 mg/kg; 10^{-6} M/kg) was administered iv to each of the eight dogs. The hypoxic exposures were then repeated (between 10 and 20 min after verapamil), and the pulmonary vascular responses were compared to the control hypoxic responses. In another group of four dogs, 10-min hypoxic exposures were given repetitively after verapamil administration to determine the time course of verapamil's effect on hypoxic pulmonary vasoconstriction.

The pulmonary perfusion pressure (pulmonary arterial–pulmonary arterial wedge pressure) response to another vasoconstrictor agent, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), which acts primarily by pharmaco-mechanical excitation-contraction coupling, was also

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[2] Edwards Laboratories, Santa Ana, California.

[3] Knoll Pharmaceutical Co., Whippany, N.J.

determined before and after verapamil. Intravenous injections of $PGF_{2\alpha}$ (2 μg/kg) were administered to four dogs while pressure was monitored continuously.

Results. Baseline systemic and pulmonary hemodynamics were altered following the administration of verapamil (Table I). Cardiac output and stroke volume were both increased while mean systemic arterial pressure and total systemic resistance were both reduced. Heart rate was unchanged by verapamil. Although pulmonary vascular resistance was unchanged, pulmonary perfusion pressure was increased in the treated animals.

Verapamil attenuated the pulmonary vasoconstrictor responses to hypoxia (Fig. 1). The maximum rise in pulmonary vascular resistance was 3.3 units (+87%) at 5 min of the control hypoxic exposure, but was only 1.4 units (+38%) after verapamil (Table I). The peak increase in pulmonary perfusion pressure was also attenuated, with an increase of 13 mm Hg (+124%) during the control hypoxic exposure, and an increase of only 6 mm Hg (+47%) during the postverapamil hypoxic exposure (Table I). The most pronounced attenuation of hypoxic pulmonary vasoconstriction occurred during the first hypoxic exposure postverapamil. Thereafter, the increase in pulmonary vascular resistance during the subsequent hypoxic exposures (in four dogs) gradually returned toward the preverapamil response. The half-life of this attenuated response was found to be approximately 1 hr. The reduced hypoxic responses were not attributable to decreased hypoxic stimuli since arterial oxygen tensions were similar during the control (Pa_{O_2} = 37 ± 0.7 mm Hg) and postverapamil (Pa_{O_2} = 39 ± 0.8 mm Hg) hypoxic exposures. Cardiac output, heart rate, and stroke volume during hypoxia were unchanged by verapamil, whereas systemic arterial pressure and total systemic resistance during hypoxia were significantly lower after verapamil (Table I).

Pulmonary pressor responses to $PGF_{2\alpha}$ were unaltered by the dose of verapamil

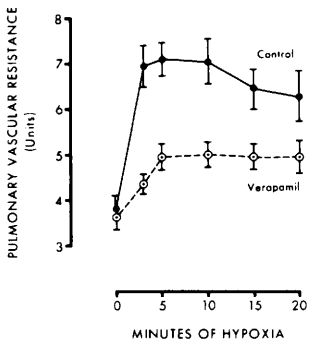

FIG. 1. Attenuated pulmonary vascular resistance response to hypoxia following pretreatment with verapamil (0.5 mg/kg). Values are mean ± SEM.

TABLE I. SYSTEMIC AND PULMONARY HEMODYNAMICS DURING NORMOXIA AND HYPOXIA BEFORE AND AFTER VERAPAMIL.[a]

	Before verapamil			After verapamil		
		Hypoxia			Hypoxia	
	Control	5 Min	20 Min	Control	5 Min	20 Min
Cardiac output (liter/min)	2.9 ± 0.2	3.3 ± 0.2	3.6 ± 0.3	4.0 ± 0.4*	3.6 ± 0.3	4.1 ± 0.4
Heart rate (beats/min)	136 ± 8	142 ± 8	143 ± 7	132 ± 14	128 ± 12	132 ± 10
Stroke volume (ml)	21.6 ± 1.7	23.4 ± 2.2	25.8 ± 2.5	30.3 ± 2.0*	27.7 ± 1.2	31.4 ± 2.0
Systemic arterial pressure (mm Hg)	134 ± 7	152 ± 9	149 ± 7	112 ± 10*	125 ± 10*	129 ± 9*
Total systemic resistance (units)	47.2 ± 2.8	47.7 ± 3.4	42.6 ± 3.7	30.0 ± 3.5*	36.3 ± 2.6*	32.1 ± 1.8*
Pulmonary perfusion pressure (mm Hg)	11 ± 1	24 ± 2	22 ± 1	14 ± 2*	18 ± 2*	20 ± 2
Pulmonary vascular resistance (units)	3.8 ± 0.3	7.1 ± 0.4	6.3 ± 0.6	3.6 ± 0.2	5.0 ± 0.3*	5.0 ± 0.3*

[a] All values are mean ± SE.

* Values "after verapamil" are significantly different from comparable "before verapamil" values at $P < 0.05$.

used in these experiments. Pulmonary perfusion pressure increased from 11 ± 1 to 33 ± 3 mm Hg during control normoxic conditions, and from 14 ± 1 to 32 ± 3 mm Hg after verapamil administration.

Discussion. Systemic vasodilatation was observed after verapamil administration, a finding that has been demonstrated in both intact animals and perfused organ systems (6–8). Verapamil has also been shown to be a negative chronotropic and inotropic agent (6, 7), however, in this study cardiac output was increased while heart rate was unchanged. Pulmonary circulatory variables were relatively unchanged, with only slight increases in pulmonary perfusion pressure and unchanged pulmonary vascular resistances. These changes in baseline hemodynamics indicate that verapamil had a greater effect on the systemic circulation than on the pulmonary circulation or heart of the anesthetized dog. The more profound actions of verapamil on the systemic circulation can be attributed to the greater amount of vascular smooth muscle in systemic vasculature.

Attenuation of hypoxic responses by verapamil suggests that hypoxic pulmonary vasoconstriction involves transmembrane Ca^{2+} influx (electro-mechanical coupling). A reduction in the hypoxic stimulus could have resulted in the attenuated response; however, arterial oxygen tensions were similar, indicating that the hypoxic stimulus was unchanged by verapamil. It is possible that verapamil produced a nonspecific action upon the vasculature, making it unresponsive to all vasoconstrictors. A reduction in intrinsic pulmonary reactivity would have reduced both the hypoxic response and the $PGF_{2\alpha}$ response equally. However, the pulmonary pressor responses to $PGF_{2\alpha}$ were unchanged by verapamil, indicating that the action of verapamil was specific for hypoxia. Furthermore, $PGF_{2\alpha}$-induced pulmonary vasoconstriction has been shown to result from pharmaco-mechanical coupling and to utilize primarily intracellular Ca^{2+} stores (4), lending further support to verapamil's blocking actions on Ca^{2+} influx (1, 2).

Since verapamil acts on the membranes of smooth muscle cells to antagonize the influx of extracellular Ca^{2+}, it may also act on membranes of other cells. Mast cells contain large amounts of vasoactive substances that can be released following influx of extracellular Ca^{2+} (9, 10). Since the mast cell has been implicated in mediating the hypoxic response by releasing vasoactive substances (11), the attenuation of the hypoxic response by verapamil may be due to its action on mast cells, preventing the release of a chemical mediator. However, until a chemical mediator of hypoxic pulmonary vasoconstriction is described, the smooth muscle cell membrane must still be considered the primary site of action of verapamil.

Summary. The hypothesis that hypoxic pulmonary vasoconstriction is mediated directly by depolarization of the vascular smooth muscle was tested in anesthetized dogs. Pulmonary vascular responses to hypoxia were first determined in eight dogs during 20-min exposures to 10% O_2. Each animal was then treated with verapamil (0.5 mg/kg, iv), to block transmembrane Ca^{2+} influx in an attempt to abolish the vasoconstrictor responses to hypoxia. The hypoxic exposures were then repeated, and the pulmonary vascular responses were compared to the control responses. Verapamil administration attenuated hypoxic pulmonary vasoconstriction, but did not abolish the responses to hypoxia. Pulmonary vascular resistance increased 87% during the control hypoxic exposure, but increased only 38% during hypoxia after verapamil. The response to another vasoconstrictor, prostaglandin $F_{2\alpha}$, was not reduced by verapamil, indicating a different mechanism of mediation. These results suggest that the pulmonary vasoconstrictor response to alveolar hypoxia, in the intact dog, involves transmembrane Ca^{2+} influx, and are consistent with the idea that hypoxia acts primarily by directly depolarizing vascular smooth muscle, rather than acting indirectly through a chemical mediator.

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