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0031-3998/84/1812-1262$02.00/0
PEDIATRIC RESEARCH

Vol. 18, No. 12
Printed in U.S.A.

The Effect of Calcium Antagonists on Hypoxic Pulmonary Hypertension in the Piglet

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ABSTRACT. Cardiovascular responses to the calcium antagonists verapamil and nifedipine were evaluated in a piglet model of hypoxic pulmonary hypertension. All animals were mechanically ventilated and paralyzed. Cardiac output (CO), pulmonary artery (Ppa) and aortic blood pressure (AoP), pulmonary wedge pressure, right atrial pressure (Pra), and arterial blood gases were measured prior to and after pulmonary hypertension was induced by hypoxia and after administration of calcium-blocking agents. Results were compared to a control group of piglets subjected to a similar period of hypoxia. Verapamil infusion (0.15 mg/kg) resulted in a rapid decrease in Ppa, AoP and pulmonary vascular resistance ($p < 0.05$) which returned to baseline values by 15 min. Nifedipine (100 μg/kg) resulted in a decrease in Ppa at 1 min ($p < 0.05$) which remained significantly lower than controls throughout the study period. AoP declined precipitously during the same time period ($p < 0.01$). No significant change in Ppa was noted when nifedipine was administered at a dose of 10 μg/kg. For the most part, these drugs have a transient vasodilatory action on pulmonary as well as systemic circulation in this animal model; however, they might in higher doses be associated with significant systemic hypotension. For this reason, the use of these drugs in the treatment of hypoxic pulmonary hypertension in the neonate should be approached with caution. (*Pediatr Res* 18:1262–1265, 1984)

Abbreviations

AoP, aortic blood pressure
CO, cardiac output
Ppa, pulmonary artery pressure
Ppaw, pulmonary wedge pressure
Pra, right atrial pressure
PVR, pulmonary vascular resistance
SVR, systemic vascular resistance

Received August 4, 1983; accepted May 7, 1984.
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This work was supported in part by National Institutes of Health Grants 1RO1 HD14940-03 and 5RO1 HL25023-03, March of Dimes Research Grant 6-303, and University of Miami Project: New Born.

Pulmonary hypertension with secondary right to left shunting through the foramen ovale and/or ductus arteriosus is a common problem in the neonatal period. It has been associated with hyaline membrane disease (9), transient tachypnea of the newborn (4), meconium aspiration (8), fetal hypoxia (20, 22), and

neonatal pneumonia (10). These conditions are similar in that hypoxia, which is a significant component of each disorder, may initiate or contribute to the development of pulmonary hypertension. Therapies employed to treat the resulting pulmonary hypertension have included tolazoline and mechanical hyperventilation with induction of alkalosis. The latter treatment has been shown to reduce pulmonary artery pressure in neonates (18). Despite these approaches, in many cases the pulmonary hypertension and hypoxemia persist, resulting in a high mortality.

It has been postulated that hypoxia results in pulmonary vasoconstriction secondary to smooth muscle membrane depolarization and transmembrane influx of extracellular calcium (2, 3, 6, 14). It has also been speculated that alkalosis secondary to hyperventilation may reduce hypoxia-induced pulmonary vasoconstriction by decreasing extracellular ionized calcium (1, 19). In view of these hypotheses, the present study was undertaken to evaluate the effect of the calcium antagonists, verapamil and nifedipine, on hypoxic pulmonary hypertension in a piglet model.

MATERIALS AND METHODS

Piglets ages 6 to 30 days were anesthetized with 30 mg/kg of pentobarbital given intraperitoneally. A femoral artery and vein were cannulated and used for AoP measurement, blood sampling, and drug infusions. The left external jugular vein was cannulated and the catheter was advanced into the right atrium for measurement of pressure and injection of iced saline which was used for measurement of cardiac output. A 5F Swan-Ganz thermodilution catheter was introduced into the right external jugular vein and advanced under fluoroscopy into the left pulmonary artery. CO was measured by thermodilution using a cardiac output computer (9510-A, Edwards Laboratory, Santa Ana, CA) and corrected for weight. This catheter was also used for measurement of Ppa and Ppaw. Heparinized normal saline (10 units/cc) was infused continuously through the pulmonary artery catheter. Vascular pressures were measured with pressure transducers (model P23; Gould-Statham Instruments, Hato Rey, PR) and recorded on a multichannel recorder (model 5 polygraph, S2-925T25; Grass Instrument, Quincy, MA).

Tracheostomy was performed and the piglets were ventilated with a Sechrist ventilator (model IV-100 B infant ventilator; Sechrist Industries, Anaheim, CA). The ventilator was set at a peak inflation pressure of 12 cm H_2O, end expiratory pressure of 2 cm H_2O, inspiratory time of 0.5 s, a respiratory rate of 35/min, and an Fio_2 of 0.21. Rate and peak inspiratory pressure were adjusted to maintain the $Paco_2$ between 35 and 40 mm Hg.

Arterial blood gases were measured prior to and after hypoxia was induced and 30 min after calcium antagonists were given (pH/Blood Gas Analyzer 113, Instrument Laboratory Inc., Lexington, MA). Rectal temperature was recorded continuously (Yellow Springs Instrument Co., Yellow Springs, OH), and maintained at 38.0° C by means of a radiant warmer.

Arterial blood and pulmonary artery pressures were measured continuously. Cardiac ouput and pulmonary wedge and right atrial pressures were measured prior to and after hypoxia, and at 5, 15, and 30 min after verapamil or nifedipine was given. PVR was calculated using the formula: (Ppa − Ppaw)/CO/kg SVR calculated using the formula: (AoP − Pra)/CO/kg. The animals were paralyzed with pancuronium bromide using an initial intravenous dose of 0.1 mg/kg followed by an infusion of 0.3 mg/kg/h. After 30 min, baseline cardiovascular measurements and arterial blood gases were obtained. The Fio_2 was decreased to 0.10–0.14 to attain a $Pao_2 < 50$ mm Hg.

Measurements were repeated after 30 min. The animals were then allowed to stabilize for an additional 30 min before baseline hypoxia measurements were made.

After these measurements, nine piglets ($\bar{x} \pm$ SD; weight, 3302 ± 1562 g; age, 18 ± 6 days) received verapamil HCl (Knoll Pharmaceutical Co., Whippany, NJ) at 0.15 mg/kg infused into the right atrium over 2 min while AoP and Ppa were measured continuously. Six piglets (weight, 4378 ± 699 g; age, 19 ± 4 days) received nifedipine, three receiving a dose of 10 μg/kg and three a dose of 100 μg/kg. Four piglets (weight, 3596 ± 1284 g; age, 17 ± 10 days) did not receive any drug and comprised the control group.

Nifedipine (Pfizer Pharmaceuticals, New York) was prepared 2 h prior to the experiment by dissolving the agent in 95% ethanol and water, and combining the solutions with polyethylene glycol. The solution was protected from light until infused.

Comparisons between the mean value before and after hypoxia, and drug infusion were performed using the paired t test. Per cent change from hypoxic baseline was compared at 1, 5, 15, and 30 min post-drug infusion between treatment and control animals by two sample t test. Results are expressed as mean ± SD.

RESULTS

Hypoxia induced significant increases in mean Ppa (16.0 ± 6.3 to 28.0 ± 6.2 mm Hg; $p < 0.001$), PVR (59.1 ± 34.0 to 112.2 ± 39.8 mm Hg/liter/min/kg; $p < 0.001$), and mean AoP (101.3 ± 15.9 to 113.0 ± 13.4 mm Hg; $p < 0.002$). The following comparisons relate per cent change from hypoxic baseline to time post-calcium antagonist administration with comparable time periods in the control animals.

Verapamil administration was associated with a decrease in Ppa and AoP at 1 ($p < 0.01$) and 5 min ($p < 0.05$) postinfusion (Fig. 1). By 15 min, these differences had disappeared and Ppa

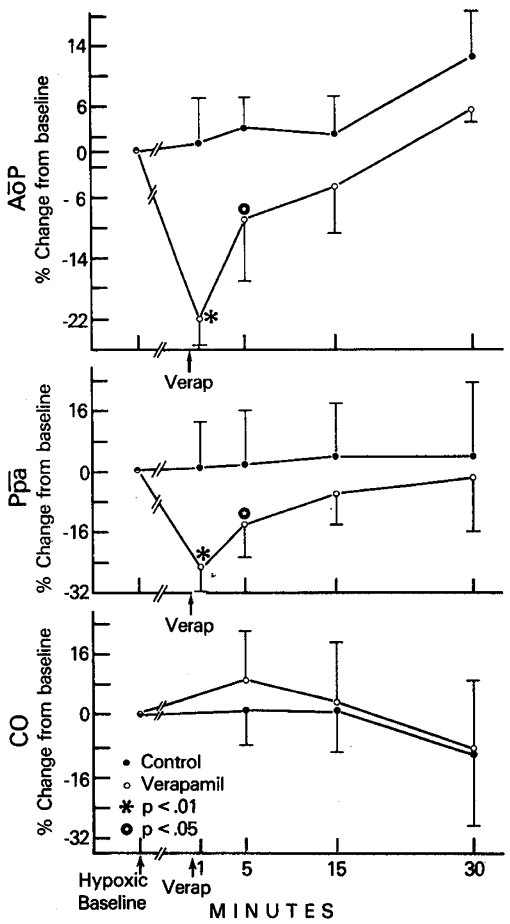

Fig. 1. Mean arterial and pulmonary artery pressure measurements, and cardiac output during hypoxia and after verapamil infusion. The time scale represents time after verapamil administration. Values are expressed as $\bar{x}$ (±SD) per cent change from hypoxic baseline ($n = 9$). Significance refers to differences from control animals.

approximated hypoxic baseline. PVR and SVR were also significantly decreased by 5 min ($p < 0.05$), but were similar to baseline by 15 min. Cardiac output increased at 5 min but was not different from control animals.

Nifedipine (10 μg/kg) did not result in a significant drop in Ppa (Fig. 2). However, piglets receiving 100 μg/kg of nifedipine displayed a significant decrease in Ppa ($p < 0.05$) which was different from hypoxic baseline throughout the monitored period. PVR and SVR were significantly decreased at 5 min ($p < 0.05$). Nifedipine-treated animals displayed progressive deterioration over the study period reflected by a significant decline in AoP ($p < 0.01$) in all animals. Changes in acid-base status were not different from control animals irrespective of drug or dosage.

The ratio of PVR to SVR (Fig. 3) shows no statistically significant change with verapamil or nifedipine (10 μg/kg). This finding indicates that the action of these drugs was nonspecific in that they cause a decrease in systemic as well as pulmonary artery pressure. There was a significant increase in this ratio when a dose of 100 μg/kg of nifedipine was infused ($p < 0.01$). This was related to a more pronounced decrease in SVR compared to PVR.

Four piglets received multiple doses of verapamil (0.15 mg/kg per dose at 10-min intervals). The mean total dose used was 0.41 mg/kg (range, 0.30–0.60 mg). The same patterns of response noted in Figure 1 were observed in all animals for each dose. Two additional animals received verapamil as an infusion. One of these received a dose of 0.01 mg/kg/min for 30 min. Mean blood pressure decreased from 41 to 37 mm Hg and Ppa fell from 36 to 31 mm Hg. The remaining piglet received incremental doses of verapamil beginning at 0.001 mg/kg/min increasing to 0.015 mg/kg/min at which point the AoP declined from 92 to 69 mm Hg and the Ppa remained essentially unchanged.

One animal received a total of 40 μg/kg of nifedipine (10, 10, and 20 μg/kg infusions at 10-min intervals). This resulted in a fall in AoP of 48 mm Hg and decrease in Ppa of 3 mm Hg.

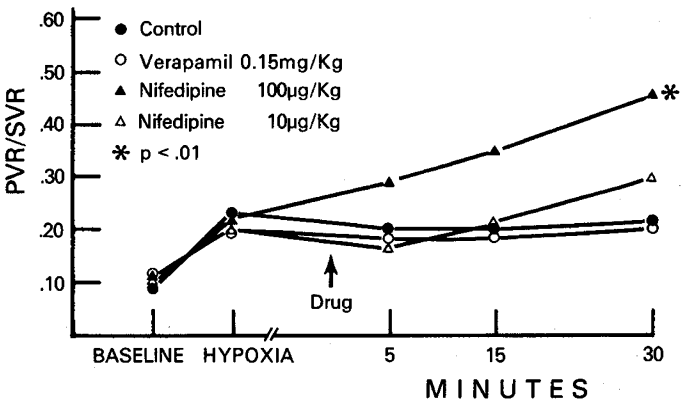

Fig. 3. Ratio of PVR to SVR during hypoxia and after drug administration. The ratio at 30 min in the nifedipine 100-μg group was significantly greater ($p < 0.01$) than the control group.

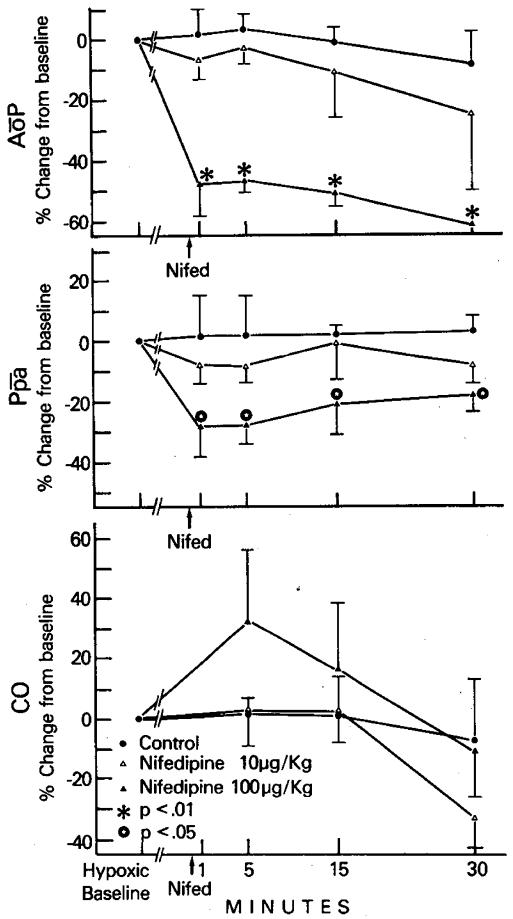

Fig. 2. Mean arterial and pulmonary artery pressure measurements, and cardiac output during hypoxia and after nifedipine infusion. The time scale represents time after nifedipine administration. Values are expressed as $\bar{x}$ (±SD) per cent change from hypoxic baseline. Significance refers to differences from control animals. Each study group is comprised of three animals.

DISCUSSION

The exact mechanism by which hypoxia induces pulmonary hypertension is unknown. It has been suggested that pulmonary vasoconstriction in the presence of hypoxia might result from membrane depolarization and transmembrane influx of extracellular calcium, or a transmitter-induced release of calcium from an intracellular pool (3, 16). These postulations have led to the suggestion that the pressor response to hypoxia may be inhibited by agents that reduce or block calcium entry across the cell membrane (16). In support of this theory, investigators have demonstrated that pulmonary vasoconstriction secondary to alveolar hypoxia is inhibited by verapamil, a calcium antagonist (16, 17). Calcium antagonists probably act in this situation by not only reducing membrane permeability to calcium, but also by depressing the rate at which intracellular stores of calcium are replenished from extracellular sources (2, 3, 6, 7, 14, 23).

Calcium antagonists have been used with varying success to treat adults who have primary pulmonary hypertension (5, 13, 24). In addition, patients with chronic airflow obstruction and acute respiratory failure with severe pulmonary hypertension secondary to hypoxic vasoconstriction have responded favorably to calcium antagonists (12, 13, 21).

Pulmonary hypertension accompanies a number of neonatal disorders. Frequently, the precipitating mechanism for pulmonary hypertension in these disorders is hypoxia before, at, or soon after birth. The present study attempted to look at treatment of hypoxia-induced pulmonary hypertension in a young animal model to ascertain if therapy with calcium antagonists could ameliorate pulmonary hypertension. However, caution must be exercised in extrapolating the results of these animal data to the human neonate in that pulmonary hypertension secondary to hypoxia was produced in normal lungs, and that right-to-left shunting through the foramen ovale and ductus arteriosus have not been demonstrated in similar age piglets. Although shunts were not ruled out in the subjects of this study, we have recorded cardiac output simultaneously using a Swan-Ganz catheter placed in the pulmonary artery and a thermodilution catheter placed in the descending aorta in 26 piglets ages 6 to 28 days with hypoxic pulmonary hypertension without demonstrating a shunt. While these data emphasize the differences in physiology between this species and the human neonate, in whom shunts

are common, they suggest that the cardiac output data are reliable.

The present data reveal that while there is an early drop in Ppa in animals treated with verapamil and nifedipine (100 μg/kg), the response is short-lived. In addition, the response is nonspecific as reflected by the absence of a significant change in the PVR/SVR ratio for verapamil and nifedipine (10 μg/kg). The higher dose of nifedipine resulted in an increase in PVR/SVR, indicating a more pronounced decrease in SVR in relation to PVR.

The initial dose of nifedipine (100 μg/kg) used in this experiment was based on work done in experimental animals (15). This dose, however, resulted in marked clinical deterioration manifested by significant decline in arterial blood pressure. Consequently, a lower dose (10 μg/kg) was used. Furthermore, the action of both verapamil and nifedipine seems extremely short-lived. The explanation of this is unclear in view of the noted half-lives of verapamil (3 to 7 h) and nifedipine (5 h) in humans (11). Plasma levels of these drugs were not obtained in the present study and, therefore, it is not clear whether the short duration of action could have been due to a short half-life in these animals. Although the number of animals treated with multiple doses of these drugs is limited, these preliminary findings do not support the benefit of multiple doses. However, the use of continuous infusions of these drugs combined with the use of a peripheral pressor agent may be beneficial.

In conclusion, these drugs have for the most part a transient, vasodilatory action on pulmonary as well as systemic circulation. In addition, they may result in significant clinical deterioration. For these reasons, it is unlikely that they could play a significant role in reducing hypoxic pulmonary hypertension in the neonate.

Acknowledgments. The authors wish to express their thanks to Ralph N. Cicalese for his excellent technical assistance, to Dr. George B. Bikhazi and Ignatius Leung for their assistance, to Leticia Hernandez and Patricia Ayon de Lujan for their secretarial support, and to Dr. Mary Jane Jesse for her critical review of this manuscript.

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