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EFFECT OF VERAPAMIL ON THE PULMONARY VASOCONSTRICTOR ACTION OF PROSTAGLANDIN $F_{2\alpha}$ AND A SYNTHETIC PGH_2 ANALOGUE

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1 The vasopressor response to prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and to ((15S)-hydroxy-11α, 9α-(epoxymethano)-prosta-5Z, 13E-dienoic acid) (U-46619) in the canine isolated lung lobe was significantly attenuated following the administration of verapamil.

2 The pressor response to arachidonic acid (AA) was not affected by the presence of verapamil.

3 The pulmonary pressor effect of $PGF_{2\alpha}$ and U-46619 is dependent, at least in part, on Ca^{2+} influx into vascular smooth muscle cells.

4 The pulmonary pressor response to AA cannot be attributed to $PGF_{2\alpha}$ or to endoperoxide intermediates but to some other product dependent on intracellular calcium stores.

Introduction

In the dog, the bisenoic prostaglandin precursor, arachidonic acid (AA), has a profound pressor effect on the circulation of the perfused pulmonary lobe (Wicks, Rose, Johnson, Ramwell & Kot, 1976). One of the end-products of AA metabolism, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), and the endoperoxide analogue, U-46619, are vasoconstrictors in the canine pulmonary circulation (Rose, Kot, Ramwell, Doykos & O'Neil, 1976). The pressor effects of AA, $PGF_{2\alpha}$ and U-46619 on the pulmonary circulation are the result of their action, direct or indirect, on vascular smooth muscle. $PGF_{2\alpha}$ has been shown to produce contraction of helical strips of canine pulmonary artery and veins (Kadowitz & Hyman, 1974), and also induces a rise in pulmonary arterial pressure in the isolated lung lobe of the dog perfused at constant flow. The contraction of vascular smooth muscle is intimately related to the mobilization of extracellular and/or intracellular calcium ions (Ca^{2+}) (Devine, Somlye & Somloy, 1972; Deth & van Breeman, 1974; Hurwitz, Joiner & von Hagen, 1976). The relative importance of the two major pools of calcium in the pulmonary vascular contraction induced by certain prostaglandins remains unclear.

Verapamil is a Ca^{2+} antagonist, which blocks entry of extracellular Ca^{2+} into the smooth muscle cell (Kohlhazdt, Bauer, Krause & Fleckenstein, 1972). The purpose of this study was to determine the effect of verapamil on the magnitude of the pressor response induced by AA, $PGF_{2\alpha}$ and U-46619 in the canine pulmonary circulation.

Methods

Mongrel dogs of either sex were anaesthetized with sodium pentobarbitone (30 mg/kg) and maintained on positive-pressure ventilation by endotracheal intubation. Ventilation rate was 15/min and the tidal volume was adjusted for each dog, according to size, within a range of 225 to 250 ml. The right femoral artery was catheterized for systemic arterial pressure recording and as a source of blood for priming the extracorporeal pump.

A left thoracotomy was performed and the circulation to the left lower lung was isolated by cannulating the lobar artery and vein. The isolated lobe was perfused with autologous citrated (10% vol/vol of 3.8% citrate) blood with a peristaltic pump. Pulmonary venous blood drained passively into a reservoir maintained at 37°C and recirculated through the isolated lobe.

Mean lobar arterial and venous pressures were monitored at the inflow cannulae, respectively. Mean lobar arterial pressure was maintained at 9 to 21 mmHg by varying flow rate through the lobe. Mean venous pressure was maintained at 0 to 5 mmHg by manual adjustment of the height of the outflow cannula.

$PGF_{2\alpha}$ was obtained from the Upjohn Company. Stock solutions (1 mg/ml in ethanol) were evaporated to dryness under nitrogen and dissolved in 0.9% w/v NaCl solution (saline) to produce 100 μg/ml of $PGF_{2\alpha}$. Arachidonic acid (5,8,11,14-

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eicosatetraenoic acid, 99% pure and obtained from porcine liver) was obtained from Sigma. The sodium salt of arachidonic acid was prepared daily by dissolving with sodium carbonate (100 mM) during constant stirring under nitrogen in the absence of light. The resulting solution (10 μg/ml) was used only when water clear. U-46619 ((15S)-hydroxy-11α,9α-(epoxymethano)-prosta-5Z, 13E-dienoic acid) was generously provided by Dr G.L. Bundy of the Upjohn Company. It was dissolved in ethanol to provide a 1 mg/ml solution. It was diluted in saline to 10 μg/ml. Verapamil was obtained from Knoll Pharmaceuticals and prepared daily by dissolving in 0.85% saline. The resulting verapamil concentration was 10 μg/ml.

The test substances were administered by bolus injection directly into the inflow cannula. Doses of these substances were as follows: $PGF_{2\alpha}$ 1.25 μg/kg, AA 100 μg/kg, U-46619 0.1 μg/kg and verapamil, 0.5 μg/kg. The $PGF_{2\alpha}$, AA and U-46619 doses have been previously shown to produce consistent, submaximal responses in the isolated lung lobe preparation. The dose of verapamil used did not alter pulmonary arterial pressure. Verapamil was administered at 1 min intervals prior to $PGF_{2\alpha}$, arachidonic acid and U-46619. The system was allowed to return to control conditions after a test injection (approximately 10 min) before another agent was administered. Student's *t* test was used for the statistical analysis of data and significance was set at the $P<0.01$ level.

Results

The pulmonary pressor action of $PGF_{2\alpha}$ was determined in 8 dogs before the administration of verapamil. The mean increase in lobar arterial pressure was 60.3 ± 9.2% (Figure 1). Lobar venous pressure did not change. Following verapamil administration in the same animals, $PGF_{2\alpha}$ increased lobar arterial pressure 35.6 ± 1.6%, which represented a significant attenuation of the pulmonary pressor response.

The pulmonary pressor action of U-46619 was evaluated in another 8 dogs. The mean increase in lobar arterial pressure was 45.7 ± 6.1% before and 28.3 ± 5.6% after verapamil administration, which also represented a significant attenuation of the pulmonary pressor response. There was no evidence of tachyphylaxis in the absence of verapamil with either $PGF_{2\alpha}$ or U-46619.

In an additional 5 dogs, the pulmonary pressor action of AA was evaluated before and after verapamil. Before verapamil administration, AA (100 μg/kg) induced a 77.0 ± 20.8% increase in lobar arterial pressure (Figure 1). After verapamil the pulmonary pressor response to AA was slightly increased (85.9±20.6%), but the change was not significantly different from the responses observed before verapamil.

Additional experiments were performed to determine whether the attenuation of the $PGF_{2\alpha}$ pulmonary pressor response could be attributed to the saline vehicle in which the verapamil was dissolved. In this group of animals, $PGF_{2\alpha}$ increased lobar arterial pressure 38.6 ± 10.1% before and 31.8 ± 8.5% after the administration of the saline vehicle. The difference was not significant.

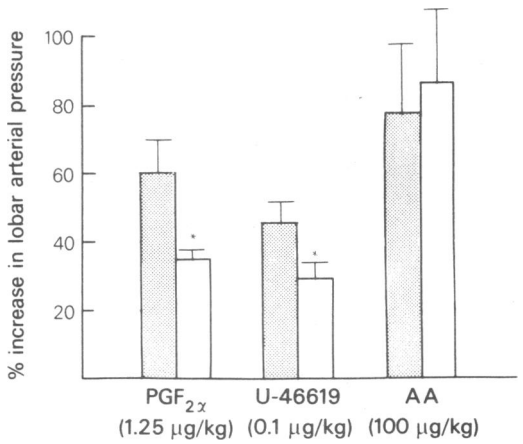

Figure 1 Mean percentage changes in lobar arterial pressure produced by bolus injections of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), U-46619 and arachidonic acid (AA) before (stippled columns) and after (open columns) administration of verapamil (0.5 μg/kg). Doses of each substance are indicated below each set of columns. Values expressed are means; vertical lines show s.e.mean. *Significance less than $P=0.005$.

Discussion

The isolated perfused lung lobe preparation of the dog was selected for this study, because changes in pressure within the system directly reflect alterations in vascular smooth muscle tone. Although $PGF_{2\alpha}$ and U-46619 are known pulmonary vasoconstrictors in many animal species, the precise biochemical mechanisms responsible for their vasoconstrictor action remain undefined. Presumably the vascular smooth muscle response involves the mobilization of extracellular and/or intracellular calcium ions.

In this study we demonstrated that the vasopressor effect of $PGF_{2\alpha}$ and U-46619 in the canine isolated lung lobe was significantly attenuated following the administration of verapamil. Since verapamil blocks transport of calcium from the extracellular to the intracellular environment (Kohlhazdt *et al.*, 1972), it appears that the pulmonary pressor response of $PGF_{2\alpha}$ and U-46619 is dependent, at least in part, on

Ca^{2+} influx into vascular smooth muscle cells. $PGF_{2\alpha}$ and U-46619 may attach to cell receptors and elicit Ca^{2+} influx into the cell. The sudden availability of Ca^{2+} intracellularly is responsible for the vasoactive characteristics attributed to $PGF_{2\alpha}$ and U-46619. The inability of verapamil to block completely the $PGF_{2\alpha}$ and U-46619 pulmonary pressor response suggests that they also have the ability to release intracellular calcium stores which are inaccessible to verapamil.

On the other hand, the pressor response to AA was not affected by the presence of verapamil. The pressor action of AA has been shown to be blocked by aspirin and indomethacin (Wicks *et al.*, 1976), indicating that biosynthetic conversion of AA to other prostanoic products is necessary for its vascular action. Two $PGF_{2\alpha}$ analogues, N-dimethylamine $PGF_{2\alpha}$ and N-dimethylamide $PGF_{2\alpha}$, antagonize the vasoconstrictor response to $PGF_{2\alpha}$ in the isolated lung lobe of the dog (Fitzpatrick, Alter, Corey, Ramwell, Rose & Kot, 1978). However, these two analogues did not antagonize the AA response. The failure of verapamil to attenuate the pressor response to AA indicates that the pulmonary vasoconstrictor action cannot be attributed to $PGF_{2\alpha}$, or to endoperoxide intermediates, but rather to the generation of some other product whose primary action on pulmonary vascular smooth muscle is mediated by release of intracellular calcium stores.

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