VASCULAR EFFECTS OF ARACHIDONATE IN LUNGS/Wicks et al.

SUMMARY We compared the effects of arachidonic acid (AA), the biseonic prostaglandin precursor, with those of prostaglandin F\textsubscript{3\alpha} (PGF\textsubscript{3\alpha}) and norepinephrine (NE) on pulmonary vascular resistance in the isolated (in situ), perfused canine lung lobe. AA (100 \mu g/kg) produced a significant increase in the pressure gradient (93.3 ± 8.4%), as did NE (1 \mu g/kg, 41.6 ± 3.2%). Aspirin (25 mg/kg) completely blocked the pulmonary vascular effect of AA, but did not affect the response to PGF\textsubscript{3\alpha}. Linoleic acid, a control fatty acid, did not produce pulmonary vasoconstriction. The pressor effect of AA was not blocked by pretreatment with phenolamine, propranolol, cyproheptadine, or atropine. The use of an artificial perfusate free of cellular elements did not prevent the vasoconstrictor action of AA. The times to onset of action of the three agents were similar, and short. These results suggest that AA is converted into vasoactive intermediates or a prostaglandin, and the vasoactive intermediates or the prostaglandin act directly on precapillary pulmonary vascular smooth muscle rather than through platelet, plasma, adrenergic, or cholinergic mechanisms.

THE CARDIOVASCULAR actions of the biseonic prostaglandins, prostaglandin E\textsubscript{2} (PGE\textsubscript{2}) and F\textsubscript{3\alpha} (PGF\textsubscript{3\alpha}), have been investigated intensively in several species of animals.

PGE\textsubscript{2} produces systemic vasodilation and a depressor response in the dog, whereas PGF\textsubscript{3\alpha} is a moderately active peripheral vasoconstricting agent. Both compounds have a direct positive inotropic effect on the heart. The precursor of the biseonic prostaglandins, arachidonic acid (AA), is released from cell phospholipids and other lipid sources and is converted into endoperoxide intermediates and eventually into PGE\textsubscript{2} and PGF\textsubscript{3\alpha}. Recent studies have shown that AA (300 \mu g/kg) produces a systemic depressor response in dogs which is equivalent to that caused by a dose of 5 \mu g/kg of PGE\textsubscript{2}.

Information about the selective action of AA on different
segments of the circulatory system is lacking. The specific aims of this investigation were: (1) to determine the effects of AA on the canine pulmonary circulation; (2) to determine whether the response represented a direct action of AA; and (3) to assess the role of the platelet in the cardiovascular actions of AA in light of studies which describe the platelet-aggregating potential of AA

Methods

Twenty-eight mongrel dogs, unselected as to sex or weight, were anesthetized with intravenous sodium pentobarbital (30 mg/kg) and maintained on positive-pressure ventilation (room air) by endotracheal intubation. Ventilation rate was 15/min and the tidal volume was adjusted for each dog within a range of 225–300 ml. Expiration was passive.

A thoracotomy was performed in the 4th or 5th left intercostal space. We isolated the circulation to the left lower lung lobe from the remainder of the pulmonary circulation by cannulating the lobar artery and vein with polyethylene catheters. The bronchus was left intact to maintain ventilation of the lobe. The isolated lobe was perfused with autologous citrated (10% vol/vol of 3.8% sodium citrate) blood by means of a peristaltic pump. Pulmonary venous blood was drained into an open, siliconized reservoir maintained at 37°C and was recirculated through the isolated lobe. The volume of the system was approximately 200 ml.

Mean lobar arterial and venous pressures were monitored at the inflow and outflow cannulas, respectively. Flow rate through the lobe was adjusted manually to maintain mean lobar artery pressure between 12 and 16 mm Hg. This was accomplished with a flow rate between 6 and 7 ml/kg per min. The height of the outflow cannula was adjusted to produce a mean lobar venous pressure of 0–5 mm Hg.

We recorded airway pressure, which was used as an index of airway resistance, by inserting a needle into the endotracheal tube. Systemic arterial pressure was monitored through an indwelling femoral arterial catheter. All pressures were recorded continuously with a multichannel direct-writing recorder. Additional anesthetic agent, when needed, was administered through a femoral venous catheter.

Measurements of \( P_{O_2} \), \( P_{CO_2} \), and \( pH \) were made at intervals to ensure that ventilation and perfusion were adequate. The ranges of these were: \( P_{O_2} \), 118–123 mm Hg; \( P_{CO_2} \), 22–29 mm Hg; and \( pH \), 7.40–7.47.

Arachidonic acid (5,8,11,14-eicosatetraenoic acid, >99% pure and obtained from porcine liver) and linoleic acid (>99% pure) were obtained from Sigma. The sodium salts of these acids were prepared by dissolving them in 100 mM sodium carbonate with constant stirring under nitrogen, in the absence of light. The solutions were prepared daily and were used only if they were clear.

Prostaglandins ( tromethamine salts ) were supplied by Upjohn. Saline solutions of the prostaglandins were made daily from stock ethanol solutions. The sodium salt of acetylsalicylic acid (Merck) was prepared in modified Tyrode’s solution and the \( pH \) was adjusted to 7.35 with sodium hydroxide. Norepinephrine (norepinephrine bitartrate, Levophed) was diluted in normal saline (75 \( \mu g/ml \)).

\( \alpha \)-Adrenergic blockade was achieved with phentolamine (Regitine) at doses of 100–200 \( \mu g/kg \) (7.5–15 \( \mu g/ml \) of blood in the system). \( \beta \)-Adrenergic antagonism was achieved with propranolol hydrochloride (Inderal), 100 \( \mu g/kg \) (7.5 \( \mu g/ml \) of blood in the system). Cyproheptadine (Periactin), an antihistamine and antiserotonin agent, was used at a dose of 55 \( \mu g/kg \) (4 \( \mu g/ml \) of blood in the system). Atropine sulfate (200 \( \mu g/kg \) or 15 \( \mu g/ml \) of blood) was used as an anticholinergic agent. Blockades were tested with the appropriate challenge drugs in doses of 1–5 \( \mu g/kg \).

Challenge drugs and test substances were administered as bolus injections directly into the infow cannula just proximal to the point of its insertion in the lobar artery. Injection volumes were 0.1–0.4 ml. Blocking agents were administered directly into the reservoir. Sufficient time was permitted for blockade to become effective. The system was allowed to return to control conditions after a test injection (approximately 5 minutes) before another agent was administered.

In the first series of experiments (17 animals), the pulmonary vascular effects of PGF\(_{2\alpha}\) (1 \( \mu g/kg \)), norepinephrine (NE) (1 \( \mu g/kg \)) and AA (100 \( \mu g/kg \)) were studied in the blood-perfused, isolated lobe. In the second group (nine animals), the effects of these agents on pulmonary vascular reactivity were studied in blood-perfused lobes pretreated with aspirin (25 mg/kg). In the third group (five animals) pulmonary vascular responses to these compounds were first studied in the blood-perfused lobe and then immediately after replacement of the blood with a dextran-based artificial perfusate (Perfufex, Pharmacia) containing 2.5 mm CaCl\(_2\) and 25 mm NaH\(_2\)CO\(_3\).

For statistical analysis of the data obtained, we used Student's \( t \)-test. Significance was set at the 0.05 level.

Results

RESPONSES OF BLOOD PERFUSED LOBES TO AA

Dose-response relationships for AA were established in the blood-perfused lung. The pressor response to AA showed a nearly linear relationship up to a dose of 150 \( \mu g/kg \). The threshold dose for this system was between 1 and 10 \( \mu g/kg \). On the basis of these results, a dose of 100 \( \mu g/kg \) of AA was used in all subsequent studies on isolated lobes. This dose resulted in a reproducible pressor response, from which recovery occurred within 5 minutes.

Through trial, we selected a dose of PGF\(_{2\alpha}\) of 1 \( \mu g/kg \) as one which elicited a reproducible pulmonary pressor response. Since a dose of NE of 1 \( \mu g/kg \) produced an equipressor response, this dose was continued throughout this study. Recovery from the pressor responses to both agents was complete in 5 minutes.

Figure 1A demonstrates the pulmonary vascular response to a dose of AA of 100 \( \mu g/kg \). AA produced a mean increase of 93.3 ± 8.4% (SE) in lobar artery pressure at the peak of the response. Lobar vein and airway pressures did not change in any experiments. Systemic arterial pressure also
remained stable, indicating that there was no significant transfer of AA from the isolated lobe circulation into the systemic circulation. PGF$_{2 \alpha}$ and NE also produced pressor responses in the isolated lobe: these were 41.2 ± 6.5% and 41.6 ± 3.2%, respectively, at a dose of 1 µg/kg. Lobar vein, airway, and systemic arterial pressures were unchanged in both cases. The percentage changes in pulmonary artery pressure caused by AA, PGF$_{2 \alpha}$ and NE are summarized in Figure 2.

**ASPIRIN BLOCKADE**

The tracing shown in Figure 1B demonstrates the effects of pretreatment with aspirin on the pulmonary vascular response to AA. Aspirin did produce a small reduction (1 mm Hg) in baseline perfusion pressure in many preparations. The pulmonary pressor response to AA was completely eliminated in every preparation pretreated with aspirin. In most cases a slight reduction (3-4 mm Hg) in pulmonary artery pressure occurred within 6 seconds after administration of AA. Comparable changes in pulmonary artery pressure were produced by the control fatty acid, linoleic acid; this finding suggests that this was a nonspecific effect of fatty acid in the presence of this anti-inflammatory agent.

Responses to PGF$_{2 \alpha}$ and NE were not blocked by aspirin (Fig. 2). The response to PGF$_{2 \alpha}$ was essentially unchanged (44.8 ± 7.4%) but the response to NE was diminished significantly (18.7 ± 1.0%).

**OTHER BLOCKING AGENTS**

Phentolamine, propranolol, atropine, and cyproheptadine were tested and none blocked or attenuated the pulmonary vascular response to AA.

**ARTIFICIAL PERFUSION**

During perfusion of the isolated lobe with an artificial perfusate free of cellular elements, the pulmonary pressor response to AA was as great (98.4 ± 31.6%) as in the blood-perfused lobe. The pulmonary vascular response to NE, 1 µg/kg, was also unaltered by substitution of the artificial perfusate but the pressor effect of PGF$_{2 \alpha}$ was diminished significantly to 10.0 ± 0.95%.

**TIME OF ACTION RELATIONSHIPS**

The times to onset of action for the substances were quite similar and rapid (mean, 5.5-7 seconds). This time was significantly greater (mean, 12 seconds) only for PGF$_{2 \alpha}$ in the artificially perfused lobe. The time to peak effect of each substance was similar between groups (mean, 21-31 seconds) except for AA (mean, 59 seconds) and PGF$_{2 \alpha}$ (mean, 45 seconds) in the lobe pretreated with aspirin. Duration of response (not shown here) was variable. In all cases, this did not exceed 5 minutes.

**Discussion**

In contrast to its systemic hypotensive effect, AA produced a rapid and marked increase in pulmonary artery...
pressure in the isolated blood-perfused dog lung lobe. This dose-related increase in pressure gradient occurred in the presence of constant flow and indicates that the rise in pressure was the direct result of an increase in pulmonary vascular resistance. The characteristics of this response were similar to those obtained with much smaller doses of PGF_{2\alpha} and NE. The times to onset and peak action of each of the agents were very similar; this finding strongly suggests a direct action of both AA and PGF_{2\alpha}.

However, the pressor action of AA was blocked by aspirin; this indicates that biosynthetic conversion of AA to endoperoxides, prostaglandins, or other products is necessary for its vascular action. Hamberg and Samuelsson demonstrated that conversion of AA into polar derivatives in a sheep seminal vesicle preparation was complete within 15 seconds, but formation of PGE_{2} (measured indirectly) was complete only after 2 minutes. Moreover, Hamberg and Samuelsson recently reported that administration of AA to the isolated artificially perfused guinea pig lung yielded only approximately 3% PGE_{2} and 5% PGF_{2\alpha} and more than 90% of other non-prostanoid compounds. In a later report, Hamberg and co-workers showed that biosynthesized endoperoxide prostaglandin intermediates had potent smooth muscle-stimulating abilities, both in vitro and in vivo.

These data, in conjunction with the rapid onset of action of AA in the lung lobe, suggest that prostaglandin intermediates are the agents responsible for the pulmonary pressor response. Conversion of AA into PGF_{2\alpha} may contribute to this response, although the time for synthesis of PGF_{2\alpha} seems to be greater than the 6-second time to onset of AA action.

Our results with PGF_{2\alpha} compare favorably with the results of studies by others on dogs and other species. Lonigro and Dawson reported that PGF_{2\alpha} exerts most of its effect on pulmonary vessels upstream to the capillaries and that degradation of PGF_{2\alpha} seems to be downstream from the site of action. In the present study, the times to onset of action of both AA and PGF_{2\alpha} were identical, and strongly suggest that the sites of action are the small pulmonary arteries.

The decreased pressor response to NE in the presence of aspirin cannot be adequately explained on the basis of our data. However, Kadowitz and others have shown that F series prostaglandins in subvasoactive doses increase vascular reactivity to NE. In addition, NE has been shown to cause the release of prostaglandins from several tissues. Either of these mechanisms might account for this decreased pressor response to NE, since generation of endogenous prostaglandins would be blocked by aspirin pretreatment with aspirin.

Experiments with the other blocking agents demonstrate that \(\alpha\) - and \(\beta\)-adrenergic, cholinergic, and serotonin receptors do not mediate the response to the active compounds synthesized from AA. Rather, other receptor sites are involved in the response to these vasoactive compounds.
Silver and colleagues demonstrated that a large dose of AA administered to rabbits intravenously produced death as a result of extensive blockage of the pulmonary circulation by platelet aggregates. No measurements of pulmonary artery pressure were made in these experiments. To rule out mechanical obstruction of small pulmonary vessels as the mechanism responsible for the pulmonary pressor response in our experiments, the isolated lobe was perfused with an artificial perfusate free of cellular elements. The pulmonary vascular responses to AA and NE were not significantly different when a perfusate free of cellular elements was used. Mechanical obstruction did not account for the rise in pulmonary artery pressure. Moreover, conversion of AA into active intermediates does not require the presence of platelets or other blood cellular elements. Other tissues, such as vascular endothelium, must be a source of prostaglandins.

An adequate explanation for the diminished response to PGF by platelet aggregates. No measurements of pulmonary artery pressure were made in these experiments. To rule out mechanical obstruction of small pulmonary vessels as the mechanism responsible for the pulmonary pressor response in our experiments, the isolated lobe was perfused with an artificial perfusate free of cellular elements. The pulmonary vascular responses to AA and NE were not significantly different when a perfusate free of cellular elements was used. Mechanical obstruction did not account for the rise in pulmonary artery pressure. Moreover, conversion of AA into active intermediates does not require the presence of platelets or other blood cellular elements. Other tissues, such as vascular endothelium, must be a source of prostaglandins.

An adequate explanation for the diminished response to PGF during use of an artificial perfusate is lacking. Binding of PGF to the dextran base, or absence of a necessary blood component for its action, could explain the reduced responsiveness in this artificial system. In addition, PGF may be metabolized by erythrocytes to the potent, smooth muscle-stimulating 15-keto PGF. In the absence of erythrocytes this prostaglandin metabolite may not be formed.

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