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Inhibition of Prostaglandin-Induced Pulmonary Vasoconstriction by Organic Acid Transport Inhibitors (40120)[1]

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Prostaglandins (PGs) are largely inactivated upon a single passage through the pulmonary circulation (1). Movement of PGs into the cell is required for metabolism of these compounds (2, 3). Several investigators (4–7) have presented evidence supporting the concept of carrier mediated transport of PGs into various tissues including lung. For example, Bito and Baroody (5) demonstrated that $PGF_{2\alpha}$ uptake and metabolism by the perfused rat lung could be largely blocked with the organic acid transport inhibitors probenecid and bromcresol green (GBr). Bakhle *et al.* (8) made similar observations with PGE_2 in the presence of BGr or thymol blue (0.01 m*M*). However, no studies have been made of the consequence of such inhibition by organic acid transport inhibitors on the hemodynamic actions of these PGs. Using the isolated perfused canine lung lobe, we have explored the possibility that probenecid and bromcresol green could modify the previously demonstrated pulmonary pressor activity of the bisenoic PG precursor arachidonic acid (AA) (9), a synthetic PG endoperoxide analogue (10, 11), and $PGF_{2\alpha}$ (12). Norepinephrine was used to test the reactivity of the preparation and also to ascertain whether probenecid or BGr inhibited the pulmonary pressor response to this catecholamine.

Materials and methods. Mongrel dogs (11–18 kg) of either sex and unknown age were anesthetized with sodium pentobarbital (30 mg/kg iv). The left lower lobe of the lung was perfused as described previously (9). Autologous citrated blood was pumped at constant flow rate into the cannulated lobar artery and was collected from the lobar vein into a reservoir to be recycled. The lung was ventilated with ambient air.

The sodium salt of arachidonic acid (5,8,-11,14-eicosatetraenoic, 99% pure; NuChek Prep. Inc., Elysian, MN) was prepared daily by dissolving in sodium carbonate (100 m*M*) with constant stirring under nitrogen in the absence of light. The resulting solution (10 mg/ml) was used only if clear. The endoperoxide analogue used in this study was obtained from The Upjohn Co., (Kalamazoo, MI): (15s)-hydroxy-11α, 9α epoxymethano prosta 5Z, 13E-dienoic acid, (abbreviated 11–9 EM) was prepared daily by diluting stock solution with saline to a final concentration of 20 μg/ml. The tromethamine salt of $PGF_{2\alpha}$ (The Upjohn Co.) was prepared in saline (100 μg/ml) from stock ethanol solutions. Norepinephrine (NE; Levophed-Winthrop Laboratories, New York, N.Y.) was diluted in saline (100 μg/ml) just prior to use. Probenecid (Merck, Sharp and Dohme Laboratories, Rahway, NJ) was prepared by dissolving in NaOH (2 *N*). The pH was adjusted to 7.4 with monopotassium phosphate buffer to a final concentration of 10 mg/ml. Bromcresol green (Fisher Scientific, Springfield, NJ) was prepared similarly except that it was dissolved in NaOH (1 *N*) and the final concentration was 1 mg/ml. Indomethacin (Merck, Sharp and Dohme) was dissolved in an aqueous sodium carbonate solution to a final concentration of 100 mg/ml.

Pressor agents were administered as bolus injections directly into the inflow cannula just proximal to the point of its insertion in the lobar artery. Responses to these substances were recorded for the following doses (μg/kg): AA, 100; 11–9 EM analogue, 0.5; $PGF_{2\alpha}$, 1; and NE, 1. The inhibitors were added directly to the reservoir and were circulated through the isolated lobe to allow equilibration before being challenged with the pressor agents. The final concentrations (μg/ml) of the inhibitors used in these experiments were as follows: indomethacin, 100;

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probenecid, 425 and 850, and BGr, 6, 12 and 24. The pressor agents, with the exception of 11-9 EM, were administered in the presence of each inhibitor at the concentrations indicated. The order of administration of these agents was randomized for each concentration of inhibitor. The 11-9 EM was given only in the presence of the highest concentration of each inhibitor. Vascular responses to the pressor substances were reported as mean percentage changes from control perfusion pressure ± SEM. Results were analyzed with Students' *t* test, and differences were considered significant if $P < 0.05$.

Results. Administration of each inhibitor resulted in a transient 0–2 mm Hg drop in baseline perfusion pressure which returned to control before pressor agents were injected. The results of experiments conducted with probenecid are summarized in Fig. 1A. The mean control pulmonary pressor response to AA of 66 ± 9% was reduced significantly to 33 ± 8% by probenecid (425 μg/ml). The response to AA was not attenuated further by higher concentrations of probenecid. Like AA, the pulmonary pressor response to $PGF_{2\alpha}$ was diminished significantly from 45 ± 9% to 20 ± 10% by probenecid (425 μg/ml). The $PGF_{2\alpha}$ pressor response was almost completely inhibited by higher concentrations of probenecid (850 μg/ml). The mean control pulmonary pressor response to 11-9 EM of 156 ± 24% was not inhibited significantly (125 ± 41%) by probenecid up to 850 μg/ml. In six animals, the mean control pulmonary pressor response to NE was 36 ± 4%. This response was reduced significantly by probenecid at only the higher dose (850 μg/ml).

The effects of BGr as an inhibitor of the pulmonary pressor activity of these four compounds are summarized in Fig. 1B. In contrast to probenecid, the response to AA was not reduced significantly (66 ± 9% vs. 57 ± 11%) by BGr (24 μg/ml). In five experiments the mean response to 11-9 EM (164 ± 26%) was not inhibited significantly by BGr (24 μg/ml).

The response to $PGF_{2\alpha}$ was diminished significantly from a mean of 57 ± 7% to a mean of 31 ± 9% by BGr (6 μg/ml). This inhibitory action was not augmented appreciably by higher concentrations of BGr. At a 12 μg/ml of BGr, the pressor response to $PGF_{2\alpha}$ was 29 ± 7% and was virtually unchanged at 24 μg/ml of BGr.

The pulmonary pressor response to NE was not reduced significantly by BGr at the highest concentration used. As anticipated, indomethacin blocked the pulmonary pressor response to AA in every instance ($n = 5$). It

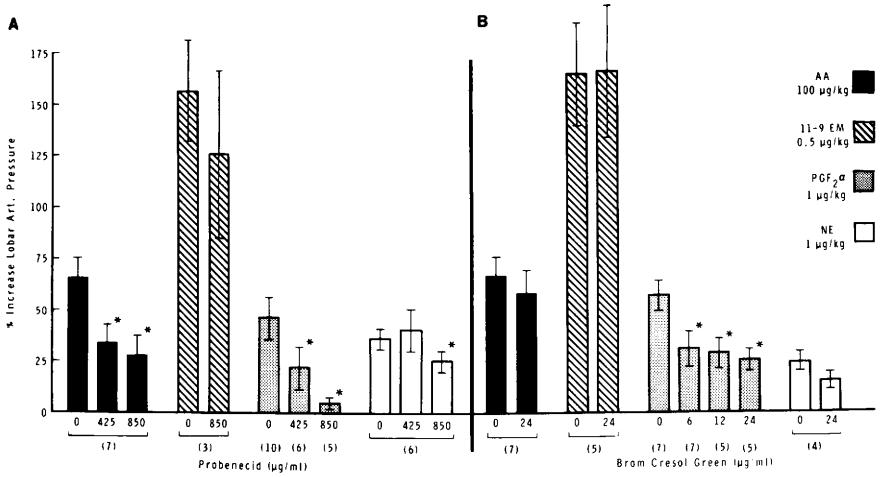

FIG. 1. The pulmonary vasopressor responses to arachidonic acid (AA), the PG endoperoxide analogue (11–9 EM), $PGF_{2\alpha}$, and norepinephrine (NE) in the absence and presence of probenecid (Fig. 1A) and bromcresol green (Fig. 1B). Each inhibitor alone did not change the baseline perfusion pressure (14–15 mm Hg). The dose of each pressor agent is shown to the right of the second panel and the concentration of the inhibitor is shown below each bar. The SEM is illustrated on each bar and the number of observations (n) is indicated below each group of bars. The asterisks indicate a significant difference ($P < 0.05$) from each control pressor response.

caused a slight (20%), though not significant enhancement of the 11-9 EM pressor action ($n = 3$). The responses to $PGF_{2\alpha}$ and NE ($n = 3$ each) were not altered after indomethacin.

Discussion. The pulmonary pressor effects of AA and $PGF_{2\alpha}$ were inhibited by probenecid, a clinically useful organic acid transport inhibitor. At the highest concentration, probenecid inhibited the action of NE, but did not reduce the pulmonary pressor response to the 11-9 EM.

The pulmonary pressor effect of AA is due to vasoactive products formed from AA since it is blocked by PG synthetase inhibitors such as aspirin (9). The vasoactive products of AA include endoperoxides, PGs (13), and thromboxanes (14). Probenecid has no PG synthetase inhibitory properties (15), therefore the antagonism of AA action by probenecid is due to a mechanism other than PG synthetase inhibition. One possible mechanism is the inhibition of uptake of AA into the cell, preventing the conversion of AA into active products. Probenecid is a competitive organic acid transport inhibitor in the kidney, and has been described as a PG transport inhibitor in the rat lung (5), chicken and rat kidney (7, 16) and other tissues (4). A second possible mechanism is that probenecid may interfere with the release of vasoactive products of AA from the cells. Hamberg and others (13) have shown that a large fraction of the products formed in the lung following injection of AA are released into the circulation, whereupon they may act at downstream sites. A third possible mechanism, that probenecid may act directly as a competitive inhibitor of the pulmonary pressor products formed from AA, was explored by examining the effects of probenecid on responses to the endoperoxide analogue and $PGF_{2\alpha}$.

The naturally occurring endoperoxides PGG_2 and PGH_2 are unstable and are not generally available in sufficient quantities for work in dogs. The stable endoperoxide analogue 11-9 EM (17) has been found to share some pulmonary vasoconstrictor and airway responses with the natural endoperoxides. PGG_2 and PGH_2 constrict feline pulmonary artery and increase airway resistance in the guinea pig (18); the PGH_2 analogue increases pulmonary artery pressure (10) and airway resistance (19) in the dog. Probenecid did not inhibit the action of the 11-9 EM, indicating that the analogue has access to its site of action.

The pulmonary pressor response to $PGF_{2\alpha}$ was blocked in a dose related manner by probenecid. Since most evidence from receptor binding studies point to the cell membrane as the site of action of $PGF_{2\alpha}$, uptake into the cell may not be necessary for $PGF_{2\alpha}$ action. Therefore, probenecid may inhibit $PGF_{2\alpha}$ action at the cell membrane, but the present data are not sufficient to determine whether this inhibition is competitive.

Since Bito and Baroody (5) reported that probenecid inhibits the PG catabolizing enzyme 15-hydroxy PG dehydrogenase in the rat lung, an enhancement of $PGF_{2\alpha}$ action in the dog lung was expected. Instead, an inhibition of its pressor action was observed. It is very unlikely that the dehydrogenase activity is enhanced by probenecid in the dog lung, therefore the inhibition of $PGF_{2\alpha}$ action by probenecid is independent of the PG catabolizing enzymes.

Thus, the mechanism by which probenecid inhibits the effect of AA in the lung remains unclear. Although $PGF_{2\alpha}$ action is blocked, this explains only part of the inhibition since Hamberg *et al.* (13) reported that $PGF_{2\alpha}$ compromises only a small fraction (<6%) of the products of exogenous AA released from the perfused guinea pig lung. Other enzymes or vasoactive products of the AA metabolic pathway may be affected by probenecid.

The pulmonary pressor response to NE was inhibited significantly only at the highest concentration of probenecid. This observation demonstrates that the pulmonary vasculature was still reactive to a catecholamine at probenecid levels that inhibited AA and $PGF_{2\alpha}$.

BGr inhibited only the pulmonary pressor response to $PGF_{2\alpha}$. This indicator dye achieved approximately 50% inhibition at only 6 μg/ml. At comparable concentration of BGr, Bito and Baroody (5) found a 95.7% inhibition of $PGF_{2\alpha}$ uptake in the perfused rat lung. Perhaps BGr at this concentration of 6 μg/ml is interfering maximally with the response to $PGF_{2\alpha}$, although not causing complete inhibition. Since nearly complete inhibition of $PGF_{2\alpha}$ can be achieved with

probenecid and not with BGr, and AA is inhibited by probenecid and not by BGr, these substances may act in different manners that are unrelated.

Summary. Probenecid and bromcresol green (BGr), reported to be PG transport inhibitors, were tested for their effectiveness in modifying the vasopressor responses of arachidonic acid (AA), an endoperoxide analogue, $PGF_{2\alpha}$ and norepinephrine in the isolated perfused canine lung lobe. Only the pulmonary pressor action of AA was blocked by pretreatment with indomethacin. Probenecid attenuated AA responses and blocked those of $PGF_{2\alpha}$ in a dose-related manner. BGr had no antagonistic activity against AA and inhibited $PGF_{2\alpha}$ action by 50%. Probenecid and BGr appear to inhibit $PGF_{2\alpha}$ differently. Neither blocked the pressor action of the endoperoxide analogue, and neither attenuated the norepinephrine pressor action at PG inhibiting concentrations. The use of organic acid transport inhibitors represents a potentially useful approach in studying the mechanisms of action of AA and its metabolic products.

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