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PRELIMINARY COMMUNICATIONS

METABOLISM OF PROSTAGLANDIN A_1 BY THE PERFUSED RABBIT LUNG

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Prostaglandins E_1, E_2 and $F_{2\alpha}$ have been shown to be extensively metabolized upon a single passage through the pulmonary vascular bed both in vivo (1,2,3,4) and in vitro (5,6). Prostaglandin A_2 has also been reported to be significantly metabolized in rat (3) and guinea pig (5) lung while Horton and Jones (7) concluded that prostaglandin A_2 passes unchanged through the lungs of cats and dogs. Similar experiments with prostaglandin A_1 (PGA_1) have indicated that there is no loss of this substance upon passage through the pulmonary vasculature of cat (7) or dog (1) lung.

With the exception of a recent study by Hook and Gillis (6) all of the above experiments utilized bioassay for detection and quantification of the prostaglandin removal. The bioassay cannot differentiate between endogenously produced and administered prostaglandins; also it is possible that the response of the bioassay organ may be modified by materials other than prostaglandins that may be released from the lung. Therefore, we have examined the fate of tritium labelled prostaglandin A_1 during its passage through the rabbit lung vascular bed.

Materials and Methods

Right and left lungs from mature male rabbits (2-3 kg) were perfused independently with Kreb's solution at 37°C through the pulmonary arteries at a flow rate of 10 ml/min in the manner described by Gillis and Iwasawa (8). Throughout the entire period of perfusion the lungs were kept in a static inflated state. In some experiments one lung was perfused at 4°C while the other lung from the same animal was perfused simultaneously at 37°C. Thus, one lung of the pair served as a control while the contralateral lung was the experimental organ for determining the effect of temperature on the fate of PGA_1 in the perfused lung. After a 15 minute period of perfusion of Kreb's solution, the perfusion medium was changed to Kreb's solution containing 0.28 M ^{3}H-PGA_1 (New England Nuclear) and perfusion was continued for an

additional 8-15 minutes.

The prostaglandin in 40 to 60 ml each of lung effluent and perfusion fluid was extracted by the procedure of Gilmore, Vane and Wyllie (9), as modified by Hook and Gillis (6). This procedure involves acidification of each of the solutions to pH 2.5 with HCl and subsequent extraction with ethyl acetate. The organic phase is then evaporated to dryness and the residue redissolved in anhydrous methanol.

Aliquots of the methanol solutions were spotted on E. Merck Silica Gel 60 thin layer chromatography plates and developed in the organic phase of the following solvent system in the presence of a saturated atmosphere: ethyl acetate/acetic acid/2,2,4-trimethylpentane/water (100:10:30:100). Unlabelled PGA_1 was spotted on top of the radioactive prostaglandins and after the chromatogram was run, visualization of PGA_1 was achieved by spraying the plates with a 3.5% solution of phosphomolybdic acid in isopropyl alcohol and heating at 115°C for ten minutes. Measured sections (0.5-1.0 cm) of the TLC plate between the origin and the solvent front were then scraped into scintillation vials containing 10 ml of Bray's scintillation phosphor (10) and the radioactivity measured in a Packard Tri Carb liquid scintillation counter (Model 3320). Data are reported as means $\pm$ standard error.

Results and Discussion

Figure 1 illustrates the recovery of radioactivity in scrapings of a typical chromatogram in which extracts from lung effluent and perfusion fluid were run. Total recovery of radioactivity from the TLC plates varied between 70 and 80 percent. The radioactivity at Rf 0.44 corresponded to the location of marker PGA_1 as visualized with phosphomolybdic acid. A second peak of radioactivity consistently was found on the chromatograms of lung effluent corresponding to an Rf of 0.54. This was absent from the chromatograms of perfusion fluid. An average of 52 $\pm$ 2.5 percent (n=8) of the combined radioactivity of both peaks was located at the Rf corresponding to the unidentified substance.

Because the radioactivity at the higher Rf may represent a metabolite of PGA_1, experiments were conducted to determine the effects of hypothermia on the formation of the substance. These experiments (Table 1) demonstrate that hypothermia significantly ($p < .01$) decreases radioactivity at the Rf corresponding to the possible metabolite. The unidentified substance accounted for 62 $\pm$ 4 percent of total radioactivity at both locations in chromatograms from effluent of lungs perfused at 37°C. In contralateral lungs simultaneously perfused at 4°C, this value dropped to 10 $\pm$ 2 percent while concomitantly, the

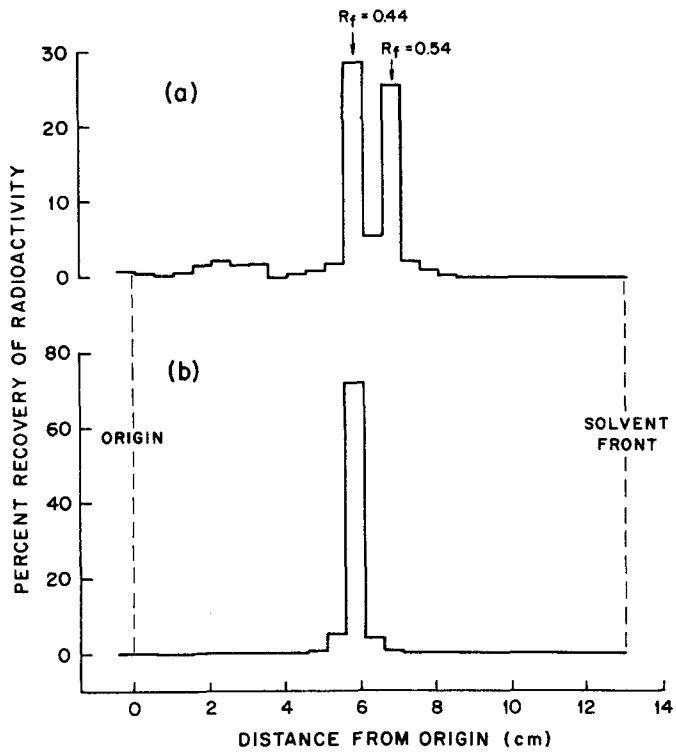

Figure 1: Recovery of radioactivity from thin layer chromatograms of (a) lung effluent and (b) perfusion solution.

amount of radioactivity at the Rf location corresponding to unchanged PGA_1 increased.

We also consistently found that 35-40 percent of the total radioactivity in effluent of lungs perfused with PGA_1 was not extractable into ethyl acetate. In further study of this phenomena, the aqueous effluent was evaporated to dryness after ethyl acetate extraction. The residue was taken up in a small volume of methanol and spotted on Merck Silica Gel 60 TLC plates which were

Table 1

Percent Radioactivity Recovered from Chromatograms at Rf Values Corresponding to those of PGA_1 and Unidentified Substance

Perfusion Temp.	PGA_1	Unidentified Substance (U.S.)	U.S. / PGA_1 + U.S.
37°C (n = 4)	21.4 ± 2.8	35.6 ± 4.3	.62 ± .04
4°C (n = 4)	59.6 ± 1.8	6.0 ± 1.0	.10 ± .02

All values are means + S.E.

Lungs were perfused for 8-15 minutes and the effluent collected. Extraction and chromatographic separation of PGA_1 and unidentified substance are explained in the methods.

then developed in the solvent system described above. The radioactivity did not move from the origin, indicating that it was associated with a relatively polar compound. This observation was consistent with the low solubility of the compound(s) in ethyl acetate. Formation of this polar product was prevented by perfusion of PGA_1 at 4°C, in which case all radioactivity in lung effluent was extracted into ethyl acetate.

The absence of any evidence of metabolism in previous reports (1,7) might be explained if the substance formed has biological activity similar to that of PGA_1. If this is true then clearly the bioassays used previously, which measure only the disappearance of PGA_1 "activity" during passage of this substance through lung vasculature, would not have revealed disappearance of PGA_1 and formation of the new substance. We are presently conducting experiments to isolate and identify these substances and to compare their biological properties with those of PGA_1.

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