METABOLITES FORMED AFTER INTRAVENOUS ADMINISTRATION OF FREE OR ALBUMIN-BOUND PROSTAGLANDIN E_2 IN THE RAT*

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1. Introduction

Recent reports [1, 2] indicated the presence of seven metabolites of prostaglandin E_2 (PGE_2) and five metabolites of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) in the urine of rats after intravenous injection with radioactive prostaglandins. These results suggested that PGE_2 and $PGF_{2\alpha}$ are converted into three groups of metabolites via three separate pathways which involve oxidation of the hydroxyl group at C-15 [3], reduction of the trans double bond [3], β [4] and ω oxidations. Previous studies from this laboratory have established the binding *in vitro* of PGE_2, $PGF_{2\alpha}$ and prostaglandin A_2 (PGA_2) to plasma albumin [5, 6]. Studies with rats that received intravenous injections of prostaglandin $PGF_{2\alpha}$ and PGA_2 [7] showed this binding to significantly modify the *in vivo* metabolism of these prostaglandins as judged from the disappearance of the injected prostaglandins from the blood circulation and the appearance of their metabolites. This report describes differences in the *in vivo* metabolism of intravenously injected free and albumin-bound PGE_2.

2. Materials and methods

Prostaglandins E_2, $F_{2\alpha}$, 15-keto E_1, A_2 and 15-keto A_1 were kindly provided by Dr. John E. Pike (The Upjohn Co., Kalamazoo, Mich., USA). ^{3}H-labeled arachidonic acid, specific activity 1.22 Ci/mmole was obtained from New England Nuclear (Boston, Mass., USA) and used for the biosynthesis of ^{3}H-labeled PGE_2 using a purified enzyme preparation from sheep senimal vesicles prepared as described elsewhere [8].

Rats weighing approx. 100 g were divided into two groups of 4 rats each, anesthetised with ether and injected into the femoral vein with 0.2 ml of either PGE_2-saline solution (0.2 mg/ml) or PGE_2-rat plasma solution (0.2 mg/ml). Each injected aliquot contained 2×10^5 cpm of ^{3}H-labeled PGE_2 [5]. Blood (1.5–2.0 ml) was withdrawn by heart puncture into a heparinized syringe 30–45 sec after injection. Plasma was immediately isolated, 50 μl aliquots taken for determination of total radioactivity and the remaining portion extracted for prostaglandins. A mixture of prostaglandins containing $F_{2\alpha}$, E_2, 15-keto E_1, A_2 and 15-keto A_1 (20 μg each) was added to the extract and thin-layer chromatography performed using chloroform–tetrahydrofurane–acetic acid (10:2:1) [9] as developing solvent. The above mentioned prostaglandins were run as reference compounds in each chromatographic separation. Plates were divided into zones according to the mobilities of these standards, each zone scraped into a scintillation vial and counted in Bray solution [10]. The counts were corrected for quenching by adding tritiated toluene as an external standard. Further details on the preparation of the injected PGE_2-containing solutions and on the method of prostaglandins extraction from plasma has been described previously [7].

* Part IV of a series "Interaction of Prostaglandins with Blood Plasma Proteins" from this laboratory.

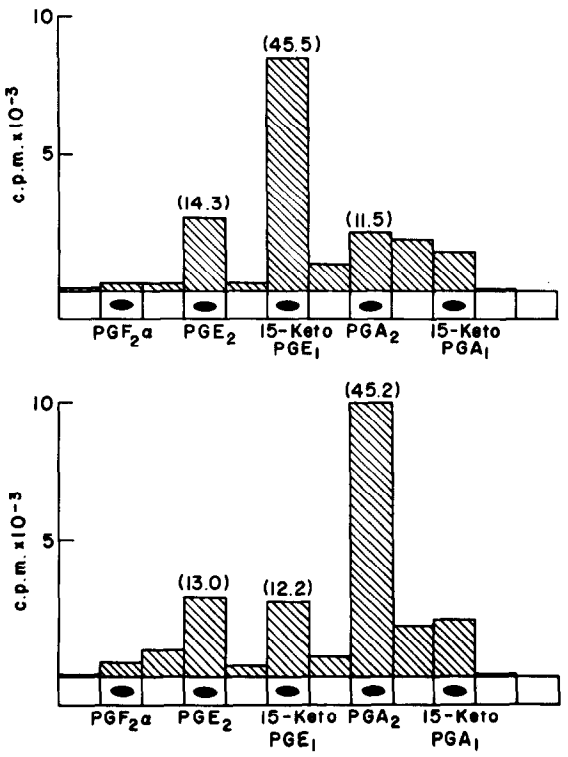

Fig. 1. Radioactivity distribution among prostaglandin metabolites following thin-layer chromatography of lipid extracts obtained from rat plasma after intravenous injection of either PGE_2-saline solution (top figure) or PGE_2-rat plasma solution (bottom figure). Values in parenthesis above bars represent % of total radioactivity in the indicated zones.

3. Results and discussion

The total radioactivity recovered in the blood (assuming a total blood volume of 10 ml for the 100 g rat used) was found to be 16–20% in rats which received PGE_2-saline solution, and 24–28% in rats injected with PGE_2-rat plasma solution. The distribution of the radioactivity among the various prostaglandin metabolites is shown in fig. 1. The data in each part of the figure was obtained from a plasma extract taken from a single rat. Similar results were obtained for the remaining three rats in each group, respectively. Significant differences were observed in the relative amounts of 15-keto PGE_2 and PGA_2 in the plasma of rats in the two groups. In the plasma of rats injected with PGE_2-saline solution, the major metabolite formed was 15-keto PGE_2 (45.6%), while PGA_2 was only a minor component (11.4%). In contrast, with rats injected with PGE_2-rat plasma solution (i.e. PGE_2 mostly bound to rat albumin) the major metabolite was PGA_2 (45.2%) while 15-keto PGE_2 was a minor component (12.2%). The possible chemical conversion of PGE_2 to PGA_2 during the extraction procedure was checked for by adding radioactive PGE_2 to rat plasma followed by extraction and thin-layer chromatography. Approx. 0.5–1% of the recovered radioactivity was associated with the PGA_2 zone, indicating negligible conversion of PGE_2 to PGA_2 during the extraction.

The conversion of PGE type prostaglandins in plasma to their 15-keto derivatives during passage through the lungs is considered to be a part of the normal metabolic fate of these protaglandins. An enzyme carrying out this reaction (a prostaglandin 15-OH dehydrogenase) has been partially purified from guinea pig lungs [11]. The results described here indicate that the metabolic fate of intravenously injected PGE_2 depends on whether PGE_2 is injected in the free form, or bound to albumin. The binding of PGE_2 to rat albumin prior to its injection into the blood appears to reduce its availability for the 15-OH dehydrogenase enzyme, thereby decreasing the rate of formation of 15-keto PGE_2. A reduced availability of PGE_2 as a result of albumin binding was also observed in previous studies from this laboratory [5] which showed the binding of PGE_2 to human serum albumin to render PGE_2 inactive in producing contractions on isolated gerbil colon *in vitro*. Further studies are in progress in order to determine what, if any, are the metabolic relationships between the decreased formation of 15-keto PGE_2 and the apparent concurrent increase in the amount of PGA_2 formed when PGE_2 is administered bound to plasma albumin.

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