METABOLISM OF PROSTAGLANDIN E\textsubscript{1} IN STOMACH, JEJUNUM CHYLE AND PLASMA OF THE DOG AND THE RAT*

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Accepted November 22, 1972

Abstract—The metabolic degradation of PGE\textsubscript{1} was studied by the incubation of \textsuperscript{3}H\textsuperscript{-}PGE\textsubscript{1} with rat and dog plasma and thoracic lymph and the homogenates of various rat and dog tissues. It was observed that rat and dog plasma metabolize very little PGE\textsubscript{1} even when incubated for one hr. Very little metabolic degradation of PGE\textsubscript{1} was found by dog thoracic lymph. In contrast, rat and dog lung and kidney metabolize PGE\textsubscript{1} very rapidly, and approx. 95\% of PGE\textsubscript{1} was converted to a less polar metabolite within 20 min of incubation. The metabolic degradation of PGE\textsubscript{1} was also rapid in the homogenates of rat stomach and jejunum, but the velocity of this reaction in these homogenates was slower than that in the rat or dog lung and kidney homogenates.

It has been well-established that i.v. administration of prostaglandins not only stimulates gastrointestinal motility in different species of animals in vivo and in vitro (1, 2), but also reduces gastric acid secretion in conscious dogs (3, 4) and humans (5), and in the isolated rat and frog stomach mucosa (6, 7). Prostaglandins are found to be biosynthesized in the stomach and intestinal walls by either neuronal or chemical stimulations (1, 2, 8-10), and may play an important role on a physiological feedback mechanism in the gastrointestinal tract (2, 10). Little information is available however, on the metabolic degradation of prostaglandins in the gastro-intestinal tract and thoracic lymph. Parkinson et al. (11) found that orally administered prostaglandin E\textsubscript{1} (PGE\textsubscript{1})-\textsuperscript{14}C in rats is excreted as (14) CO\textsubscript{2} in expired air. Very recently, Pace-Asciak et al. (12) showed that prostaglandin F\textsubscript{1} is metabolized by homogenates of the rat stomach. The present study was undertaken to examine whether or not PGE\textsubscript{1} could be effectively inactivated by the stomach, jejunum and thoracic lymph, and to compare the rate of the metabolic degradation of PGE\textsubscript{1} by these tissues with that by kidneys, lungs and plasma of the dog and rat.

METHODS

Mongrel dogs (14-16 kg) of both sexes and male Holzman rats (200-250 g) were fed ad libitum with Rockland dog chow and Purina rat chow, respectively. The dogs were

* This work was supported in part by research grants from U.S. Public Health Service (HE 11848) and from the U.S. Navy (Contract No. N00014-68-A-0496).

** Trainee of the U.S. Public Health Service Cardiovascular Physiology and Pharmacology Training Grant (HE 05859).
anesthetized lightly with an i.v. injection of sodium pentobarbital (25 mg/kg). The rats were sacrificed by cervical dislocation. After mid-line laparotomy and thoracotomy, stomach, jejunum, lungs and kidneys of the dog and rat were immediately removed. In the dog, after left hemithoracotomy, the thoracic duct was cannulated toward the diaphragm and approx. 75 ml of chyle per dog was collected in a pre-cooled graduate cylinder. The lungs, kidneys, stomach and jejunum were excised and cooled immediately, and thereafter homogenized at 4°C in 4 volumes of Buercher medium (20 mM KH₂PO₄, 72 mM K₂HPO₄, 27.6 mM nicotinamide, 3.6 mM MgCl₂, pH 7.4) with a Potter-Elvehjem tissue grinder. The homogenates were centrifuged at 10,000 × g for 20 min using a Sorvall centrifuge (Model RC 2). The protein concentrations of the supernatant were determined by the method described by Lowry et al. (13) and usually ranged between 10 and 15 mg/ml. After 0.1 μg/ml of ³H-prostaglandin E₁ (³H-PGE₁) (28 c/mmole), 50 ng/ml of prostaglandin E₁ (PGE₁) and 2 μM of NAD⁺ were added, the supernatant, plasma or thoracic lymph was incubated at 37°C in a Research Specialties temperature-controlled water bath shaker (Model 2156). Before, and 20 and 60 min after the incubation was started, an aliquot (5 ml) of the samples was pipetted into tubes containing 0.5 ml of 2 N HCl solution to terminate the reaction and to acidify to pH 3.0. ³H-PGE₁ and its metabolites were extracted twice with ethyl acetate. The extract was filtered through a Whatman No. 3 paper and washed with distilled water. After evaporation under reduced pressure, the extract was separated with discontinuous silicic acid column chromatography using different ratios of the solvent mixture of ethyl acetate: toluene as described previously 14-16. PGE₁ was eluted with 70% ethyl acetate in toluene, whereas less polar metabolites, 11α-hydroxy-9, 15-diketo-13-prostaenoic acid (15-keto-PGE₁) and 11α-hydroxy-9, 15-diketo-prostanoic acid (15-keto-dihydro-PGE₁) were eluted with 40% ethyl acetate in toluene. An aliquot (4 ml) of each chromatography fraction was pipetted into a counting vial, and then 15 ml of the counting solution was added. Radioactivity of each sample was counted in a Packard Tri-Carb liquid scintillator counter (Model 3000 series). The data in this study were analyzed statistically using the t test 17.

PGE₁ and 15-keto-PGE₁ were obtained from Dr. J.E. Pike, Chemistry Dept., Upjohn Co., Kalamazoo, Mich. and from Dr. R.L. Bergström, G.D. Searle Co., Chicago, Ill., respectively. ³H-PGE₁ was purchased from New England Nuclear Corp. The purity of the PGE₁ and 15-keto-PGE₁ was ascertained by thin layer chromatography using the solvent system; ethyl acetate-isooctane-acetic acid-water (100: 20: 10: 110) and the spots were identified by spraying with 10% phosphomolybdic acid in ethanol.

RESULTS

As shown in Fig. 1, silicic acid chromatography of the extract of the rat plasma prior to incubation revealed a single peak of ³H-PGE₁, which was eluted in Fractions 11-14 (solvent system; ethyl acetate-toluene, 70: 30). Silicic acid chromatography of the extract of the dog plasma which was incubated at 37°C for one hr shows an almost identical pattern with a single peak corresponding to ³H-PGE₁. As reported previously from this
FIG. 1. Silicic acid column chromatography of $^3$H-PGE$_1$ and its metabolites in the rat plasma before (Upper) and after (Lower) 60 min after incubation at 37°. Column, 1 g silicic acid; fraction 10 ml. Fractions 1 to 5: ethyl acetate-toluene, 20:80; Fractions 6 to 10: ethyl acetate-toluene, 40:60; Fractions 11 to 15: ethyl acetate-toluene, 70:30; Fractions 16 to 20: ethyl acetate alone.

FIG. 2. Silicic acid column chromatography of $^3$H-PGE$_1$ and its metabolites in rat lung before (Upper) and after (Lower) 20 min incubation at 37°.
laboratory (16, 18), this appears to indicate that practically no metabolic degradation of PGE₁ occurred in the dog plasma. In contrast, as shown in Fig. 2, silicic acid chromatography of the extract of the rat lung homogenate, incubated for 20 min, showed that the ³H-PGE₁ peak in Fractions 11-14 practically disappeared. Instead, a prominent peak was observed in Fractions 6-9 which appeared to correspond to a less polar PGE₁ metabolite, Metabolite I. Thin layer chromatography showed that the Rf value, 0.63, of Metabolite I was identical with that of 15-keto-PGE₁.

Fig. 3 shows the kinetics of the metabolic degradation of ³H-PGE₁, which was incubated with the rat plasma and with the homogenates of the rat kidney, lung, stomach and jejunum. As shown in Fig. 3, very slow metabolic degradation of ³H-PGE₁ was observed in rat plasma, rat brain and heart homogenates. In contrast, the rate of the metabolic degradation of ³H-PGE₁ in the rat stomach, jejunum and lung was significantly greater than that in the rat plasma, but markedly slower than that in the rat kidney and plasma. The rat kidney and lung homogenates metabolized 95% of ³H-PGE₁ within 20 min, whereas the rat stomach and jejunum homogenate converted only 40% of ³H-PGE₁ within 20 min.

Fig. 4 shows the kinetics of the metabolic degradation of ³H-PGE₁ in the dog plasma, thoracic lymph, and the homogenates of the dog kidney and lung. As seen in the experiments with the rat, the dog plasma metabolized little PGE₁ during one hour's incubation. Likewise, very little PGE₁ was metabolized by the dog thoracic lymph. In contrast, both kidney and lung homogenates metabolized PGE₁ at significantly faster rates than either the dog plasma or dog thoracic lymph, but the rate was markedly slower than for the dog lung or kidney homogenates. The dog kidney and lung homogenates metabolized 80% of ³H-PGE₁ within 20 min, whereas the dog stomach and jejunum homogenates converted only 40% of ³H-PGE₁ within 20 min.
FIG. 4. Metabolic degradation of PGF₁ in dog plasma, and homogenates of dog kidney, lung, stomach, jejunum and thoracic lymph. Mean ± S.E.M.

DISCUSSION

From the present study, it is evident that rat and dog plasma as well as dog thoracic lymph metabolize very little of the released or injected PGF₁ in the body. In contrast, dog and rat stomach and jejunum metabolize PGF₁ rather rapidly although the rate of the metabolic degradation in the stomach and jejunum are significantly slower than that in the lung and kidney. The results of the present study on the metabolic degradation of PGF₁ in the homogenates of rat lung and kidney and of dog kidney are essentially in agreement with those made previously in this laboratory (16, 18) as well as those made by Ånggärd and Samuelsson (19, 20).

It has been well established that PGF₁ is converted into 15-keto-PGF₁ by oxidation of the secondary alcohol group at 15-C in swine lungs, whereas PGF₁ is converted into dihydro-PGF₁ and 15-keto-dihydro-PGF₁ by the reduction of a double bond in guinea-pig lungs. The microsomal enzyme which catalyzes the oxidation of PGF₁ was identified as NAD⁺-dependent 15-hydroxy-prostaglandin dehydrogenase (or NAD⁺-15-hydroxy-prostanoate oxidoreductase, EC 1.1.1.) and has been purified from swine lungs (20). Ånggärd and Samuelsson (20) and Nakano et al. (21) found that this enzyme is specific for prostaglandins (20, 21). Recently, Pace-Asciak et al. (12) showed that PGF₁α is converted into two metabolites, 15-keto-PGF₁α and dihydro-15-keto-PGF₁α, by a supernatant fraction of rat stomach. The present study showed that PGF₁ also is converted rather efficiently by the homogenate of both rat and dog stomach and jejunum, but not by the dog thoracic duct. This observation may prove that the oral administration of prostaglandins is not as effective in reducing the gastric acidity as compared with the i.v. route as demonstrated by Horton et al. (2). Parkinson et al. (11) found that approx. 58% of a dose of
PGE$_1$-$^{14}$C administered orally to rats was excreted as $^{14}$CO$_2$ in expired air in 24 hrs; only an additional 0.5-5.0% was found in thoracic duct lymph. Parkinson and Schneider (22) also found that, when segments of rat jejunum were perfused in vitro with PGE$_1$-$^{14}$C, approx. 15% of the radioactivity which disappeared from the mucosal fluid in one hr was trans-located in the serosal fluid, 34% was taken up by the tissue and 49% was lost from the tissue, presumably as $^{14}$CO$_2$. They concluded that the intestine may be a primary site of oxidative metabolism for orally administered PGE$_1$. Pace-Asciak et al. (12) stated that the metabolism of prostaglandins in the rat stomach occurs by the prostaglandin 15-hydroxy-dehydrogenase pathway rather than by beta-oxidation. Observations by Parkinson and Schneider (22) appear to confirm however, that prostaglandins are metabolized not only by specific enzymes such as 15-hydroxy-prostaglandin dehydrogenase and $^{12}D$ prostaglandin reductase, but also by non-specific beta-oxidation in the intestines as is also seen in the liver (23-25), lungs (26) and kidney (26). The major and initial inactivation of prostaglandins released or administered does however take place by oxidation of the secondary alcohol group of prostaglandins (21, 27), since 15-keto-prostaglandins are very poor substrates or no substrate at all for 15-hydroxy-prostaglandin dehydrogenase (21).

Acknowledgements: The authors are indebted to Dr. J.E. Pike of the Upjohn Co., Kalamazoo, Mich. and Dr. R.L. Bergström of the G.D. Searle Co., Chicago, Ill. for the generous supply of PGE$_1$ and 15-keto-PGE$_1$, respectively.

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