METABOLISM OF PROSTAGLANDIN E_1 IN STOMACH, JEJUNUM CHYLE AND PLASMA OF THE DOG AND THE RAT*

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Abstract—The metabolic degradation of PGE_1 was studied by the incubation of 3H - PGE_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_1 even when incubated for one hr. Very little metabolic degradation of PGE_1 was found by dog thoracic lymph. In contrast, rat and dog lung and kidney metabolize PGE_1 very rapidly, and approx. 95% of PGE_1 was converted to a less polar metabolite within 20 min of incubation. The metabolic degradation of PGE_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_1 (PGE_1)- $1-^{14}C$ in rats is excreted as $(14) CO_2$ in expired air. Very recently, Pace-Asciak et al. (12) showed that prostaglandin F_1 is metabolized by homogenates of the rat stomach. The present study was undertaken to examine whether or not PGE_1 could be effectively inactivated by the stomach, jejunum and thoracic lymph, and to compare the rate of the metabolic degradation of PGE_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

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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 Bücher medium (20 mM KH_2PO_4 , 72 mM K_2HPO_4 , 27.6 mM nicotinamide, 3.6 mM MgCl_2 , pH 7.4) with a Potter-Elvehjem tissue grinder. The homogenates were centrifuged at $10,000 \times g$ 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 $\mu\text{C}/\text{ml}$ of ^3H -prostaglandin E_1 ($^3\text{H}\text{-PGE}_1$) (28 c/mmole), 50 ng/ml of prostaglandin E_1 (PGE_1) and 2 mM 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, and 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. $^3\text{H}\text{-PGE}_1$ 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. PGE_1 was eluted with 70% ethyl acetate in toluene, whereas less polar metabolites, 11 α -hydroxy-9, 15-diketo-13-prostaenoic acid (15-keto- PGE_1) and 11 α -hydroxy-9, 15-diketo-prostanoic acid (15-keto-dihydro- PGE_1) 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.

PGE_1 and 15-keto- PGE_1 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. $^3\text{H}\text{-PGE}_1$ was purchased from New England Nuclear Corp. The purity of the PGE_1 and 15-keto- PGE_1 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 $^3\text{H}\text{-PGE}_1$, 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 $^3\text{H}\text{-PGE}_1$. As reported previously from this

FIG. 1. Silicic acid column chromatography of $^3\text{H}\text{-PGE}_1$ 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 $^3\text{H}\text{-PGE}_1$ and its metabolites in rat lung before (Upper) and after (Lower) 20 min incubation at 37° .

FIG. 3. Metabolic degradation of PGE_1 in rat plasma and in homogenates of rat kidney, lung, stomach and jejunum. Mean $\pm$ S.E.M.

laboratory (16, 18), this appears to indicate that practically no metabolic degradation of PGE_1 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 $^3\text{H-PGE}_1$ 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 PGE_1 metabolite, Metabolite I. Thin layer chromatography showed that the R_f value, 0.63, of Metabolite I was identical with that of 15-keto- PGE_1 .

Fig. 3 shows the kinetics of the metabolic degradation of $^3\text{H-PGE}_1$, 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 $^3\text{H-PGE}_1$ was observed in rat plasma, rat brain and heart homogenates. In contrast, the rate of the metabolic degradation of $^3\text{H-PGE}_1$ 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 $^3\text{H-PGE}_1$ within 20 min, whereas the rat stomach and jejunum homogenate converted only 40% of $^3\text{H-PGE}_1$ within 20 min.

Fig. 4 shows the kinetics of the metabolic degradation of $^3\text{H-PGE}_1$ 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 PGE_1 during one hour's incubation. Likewise, very little PGE_1 was metabolized by the dog thoracic lymph. In contrast, both kidney and lung homogenates metabolized PGE_1 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 $^3\text{H-PGE}_1$ within 20 min, whereas the dog stomach and jejunum homogenates converted only 40% of $^3\text{H-PGE}_1$ within 20 min.

FIG. 4. Metabolic degradation of PGE_1 in dog plasma, and homogenates of dog kidney, lung, stomach, jejunum and thoracic lymph. Mean $\pm$ 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 PGE_1 in the body. In contrast, dog and rat stomach and jejunum metabolize PGE_1 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 PGE_1 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 Änggård and Samuelsson (19, 20).

It has been well established that PGE_1 is converted into 15-keto- PGE_1 by oxidation of the secondary alcohol group at 15-C in swine lungs, whereas PGE_1 is converted into dihydro- PGE_1 and 15-keto-dihydro- PGE_1 by the reduction of Δ^{13} double bond in guinea-pig lungs. The microsomal enzyme which catalyzes the oxidation of PGE_1 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). Änggård 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 $\text{PGF}_{1\alpha}$ is converted into two metabolites, 15-keto- $\text{PGF}_{1\alpha}$ and dihydro-15-keto- $\text{PGF}_{1\alpha}$, by a supernatant fraction of rat stomach. The present study showed that PGE_1 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

PGE₁-1-¹⁴C administered orally to rats was excreted as ¹⁴CO₂ 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 PGE₁-1-¹⁴C, 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 ¹⁴CO₂. They concluded that the intestine may be a primary site of oxidative metabolism for orally administered PGE₁. 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 Δ^13 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).

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