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Metabolism of prostaglandin E_1 in human plasma, uterus and placenta, in swine ovary and in rat testicle

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ÄNGGÅRD and SAMUELSSON[1,2] showed that prostaglandins E_1 and E_2 are oxidized to 15-keto-prostaglandins E_1 and E_2 by 15-hydroxy-prostaglandin dehydrogenase (PGDH) in swine lungs. The recent observations from this laboratory also showed that similar metabolic degradation occurs in dog kidneys.[3,4] Practically no information is available on the metabolism of prostaglandins in tissues other than lungs and kidneys. Many workers[5] have found that prostaglandins exert potent biological actions in the reproductive organs. Recently, prostaglandins E_2 (PGE_2) and $F_{2\alpha}$ ($PGF_{2\alpha}$) were found to induce effectively natural labor[6] and therapeutic abortion in humans.[7] In addition, $PGF_{2\alpha}$ exerts a contraceptive action in some species of animals.[8] Obviously, the tissue concentration of prostaglandins sufficient to produce the biological and therapeutic actions is greatly influenced by their inactivation in the tissues. The present study was undertaken to investigate the metabolic degradation of PGE_1 in human uterus, placenta and plasma, in rat testicle and in swine ovary and to compare with that in rat kidney.

Male Holtzman rats (200–250 g) were fed *ad lib.* with Purina rat chow until they were sacrificed by cervical dislocation. Kidneys and testicles were removed immediately from several rats and pooled. Freshly frozen swine ovaries were purchased from Pel-Freez Biologicals, Inc., Rogers, Ark. Human uterus specimens were obtained from six patients who were subjected to hysterectomy for cervical neoplasm or for prolapse of uterus at the University of Oklahoma Hospital. A part of the resected specimens used for this study was macroscopically and microscopically within normal limits. Placentas were obtained from six women who had natural labor at full term in the same hospital. The tissues were homogenized at 4° with a Potter–Elvehjem tissue grinder after 4 vol. of ice-cold Bücher medium (20 mM KH_2PO_4, 72 mM K_2HPO_4, 27·6 mM nicotinamide, 316 mM $MgCl_2$, pH 7·4) was added. The homogenates were centrifuged at 10,000 *g* for 20 min with a Sorvall centrifuge (model RC 2). The protein concentrations of the supernatant were determined by the method described by Lowry *et al.*[9] and usually ranged between 10 and 15 mg/ml.

After 0·1 μc/ml of ^{3}H-PGE_1 (28 c/m-mole), 50 ng/ml of PGE and 2 mM NAD^+ were added, the supernatant was incubated at 37·5° in a Dubnoff bath shaker. Prior to and 2, 5, 10, 20, 40 and 60 min after the incubation was started, an aliquot (4 ml) of the samples was pipetted into tubes containing 0·5 ml of a 1 N HCl solution to terminate the reaction and to acidify to pH 3·0. ^{3}H-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 the different ratio of the solvent mixture of ethyl acetate–toluene as described previously.[3] As reported previously, PGE_1 was eluted with 70% ethyl acetate in toluene, whereas the less polar metabolite, 15-keto-PGE_1, was 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[3] was added. Radioactivity of each sample was counted in a Packard Tri-Carb liquid scintillator counter (model 3000 series).

Crystalline powder of PGE_1 was obtained from Dr. J. E. Pike, Chemistry Department, Upjohn Company, Kalamazoo, Mich. ^{3}H-PGE_1 was purchased from the New England Nuclear Corp., Boston, Mass. ^{3}H-PGE_1 was purified and ascertained by silicic acid column chromatography and thin-layer chromatography using Silica gel G with the following solvent system: ethyl acetate–water–isoctane–acetic acid (110:100:20:10; by vol.). The spots were identified by heating at 110° after spraying 10% phosphomolybdic acid solution in 96% ethanol.[3,10]

As shown in the top part of Fig. 1, the silicic acid column chromatography of the extract of the rat testicle homogenate prior to incubation showed a single peak of ^{3}H-PGE_1, which was eluted in Fractions 11–14 (solvent system; ethyl acetate–toluene, 70:30). The silicic acid chromatography (middle) of the extract of the rat testicle homogenate, which was incubated for 10 min, showed that the ^{3}H-PGE_1 peak decreased in its height as another peak appeared in Fractions 6–9 (solvent system; ethyl acetate–toluene, 40:60). The chromatography of the extract of the homogenate, which was incubated for 20 min, showed that the PGE_1 peak practically disappeared as the peak (Metabolite I) in Fractions 6–9 became more prominent. From the previous studies, this Metabolite I is most likely 15-keto-PGE_1, since it has the same R_f value (0·63) as the standard 15-keto-PGE_1, by thin-layer chromatography.[1–3,10]

In Fig. 2, the kinetics of the metabolic degradation of ^{3}H-PGE_1, which was incubated with the human plasma and the homogenates of the human uterus and placenta, and of the swine ovary are

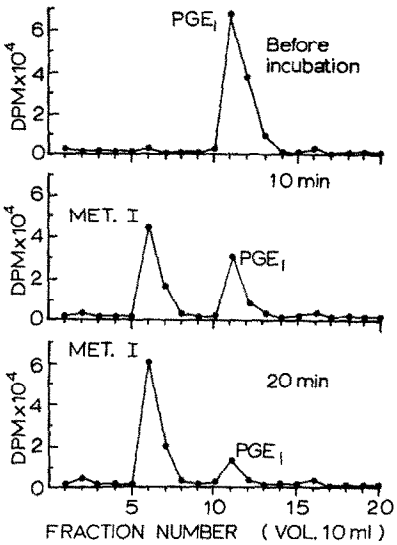

FIG. 1. Silicic acid column chromatography of ^{3}H-PGE$_1$ and its metabolite in the rat testicle homogenate prior to (top) and in the homogenate 10 min (middle) and 20 min (bottom) after incubation at 37°. Column, 1 g silicic acid; fraction volume, 10 ml. Fractions 1 to 5: ethyl acetate–toluene, 20:80; 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.

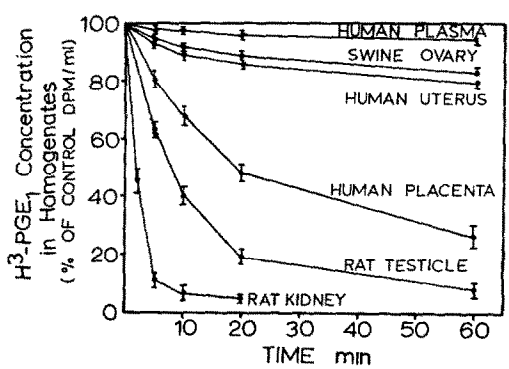

FIG. 2. Metabolic degradation of PGE$_1$ in human plasma and in the homogenates of human uterus and placenta, or rat testicle and kidney, and of swine ovary.

compared with those of the rat testicle and kidney. Very little ^{3}H-PGE$_1$ was metabolized by incubating in the human plasma. Likewise, only slight metabolic degradation of ^{3}H-PGE$_1$ occurred during incubation with the swine ovary and human uterus. In contrast, the human placenta metabolized 50 and 70 per cent of ^{3}H-PGE$_1$ within 20 and 60 min of the incubation. However, as compared with that in the rat testicle and kidney homogenates, the velocity of the metabolic degradation of ^{3}H-PGE$_1$ in the human placenta was slower.

The results of the present study of the metabolic degradation of PGE$_1$ in rat kidney homogenate are essentially in agreement with those in dog kidney made previously in this laboratory[3,4] as well as those in swine and guinea pig lungs made by Änggård and Samuelsson.[1,2] PGE$_1$ was metabolized into a less polar metabolite, 15-keto-PGE$_1$, by the oxidation of the secondary alcohol group at 15-C in the microsomal fraction of lung and kidney homogenates. The specific enzyme which catalyzes the oxidation of PGE$_1$ has been identified as NAD$^+$-dependent PGDH.[2,11] The observations made by

Änggård[1,2] and in this laboratory (J. Nakano and J. Kessinger, unpublished data) showed that the vasodilator and ileum-stimulating actions of 15-keto-PGE_1 in dogs and guinea pig were considerably feeble as compared with those of PGE_1. This indicates that the enzymatic degradation with PGDH is most likely one of the major mechanisms responsible for inactivation of PGE_1 and PGE_2 *in vivo*. However, thereafter, 15-keto-PGE_1 has been found to undergo further degradation by *beta-* or *omega*-oxidation, or by both, in the liver.[13,14]

It has been shown that PGE_1 is effectively metabolized in the homogenates of swine and guinea pig lungs[1,2] as well as in the perfused guinea pig lung[4] and perfused dog kidney[3,4] where prostaglandins are known to be synthesized from the precursor fatty acids.[5] The results of the present observations clearly showed that PGE_1 is also rather effectively metabolized in the homogenates of both rat testicle and human placenta, suggesting the similar inactivation in these tissues *in vivo*. In contrast, swine ovary and human plasma and uterus appear to be very ineffective in metabolizing PGE_1. Recently, Karim *et al.*[6,7] reported that both PGE_2 and $PGE_{2\alpha}$ are very effective in inducing normal labor in women at full term and therapeutic abortion in women at early pregnancy. Duncan and Pharriss[8] found that $PGF_{2\alpha}$ exerts a contraceptive action in monkeys, the mode of the action of which entirely differs from that of widely used progestin- and estrogen-type contraceptive agents. From the present study, it seems that any PGE_1 (and presumably PGE_2 and $PGF_{2\alpha}$) present in circulating blood would not be appreciably inactivated in the ovary and uterus, although greatly inactivated in the lungs by a single circulation[15,16]. Since the K_m and V_{max} of PGE_2 for PGDH are almost identical with those of PGE_1[11], it is reasonable to assume that the similar magnitude of the metabolic degradation would take place in the tissues studied in the present experiment. It is conceivable that the rapid metabolic degradation in the human placenta may play an improtant role in the modulation or the maintenance of natural labor. Recently, Carpenter and Wiseman[17] found that various prostaglandins are biosynthesized in the rat testicle from linoleic acid. However, the biological significance of the efficient metabolic degradation of PGE_1 in rat testicle remains unclear.

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