Metabolic degradation of prostaglandin E_1 in the rat plasma and in rat brain, heart, lung, kidney and testicle homogenates

Änggård & Samuelsson (1964, 1966) showed that prostaglandins are metabolized by oxidation of the secondary alcohol group at C-15 in swine lungs. This reaction is catalysed by NAD^+-dependent 15-hydroxy-prostaglandin dehydrogenase (Änggård & Samuelsson, 1966). Recently Nakano (Nakano, 1970a, b, c) showed that prostaglandin E_1 (PGE_1) was converted to less polar metabolites in dog isolated kidneys. Little information is available on the distribution of this enzyme or on the rate of the prostaglandin degradation in tissues other than lungs and kidneys. The present study was made to compare the rate of metabolism of PGE_1 in rat plasma, brain, heart, kidney and testicle with that in rat lung.

Male Holzman rats (200–250 g) were fed freely with Purina rat chow. They were killed by cervical dislocation and the lungs, kidneys, heart, brain and testicles were removed immediately from several rats and pooled. The tissues were homogenized at 4° 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 *g* for 20 min and protein in the supernatant was determined (Lowry, Rosenbrough & others, 1951); concentrations usually ranged from 10 to 15 mg/ml. The supernatant was shaken with 0·1 μCi/ml of ^{3}H-PGE_1 (28 Ci/mmol), 50 ng/ml of PGE_1 and 2 mmol of NAD^+ at 37·5°. Before 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 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 separated with discontinuous silicic acid column chromatography using different ratios of ethyl acetate–toluene as described previously (Nakano, 1970a, b). PGE_1 was eluted with 70% ethyl acetate in toluene, whereas a less polar metabolite, thought to be 15-keto PGE_1, was eluted with 40% ethyl acetate in toluene (Nakano, 1970a, b). An aliquot (4 ml) of each chromatography fraction was pipetted into a counting vial, 15 ml of the scintillation fluid added, and the radioactivity of each sample was counted.

Crystalline PGE_1 was donated by Dr. J. E. Pike, Upjohn Co. Ltd, Kalamazoo, U.S.A. and ^{3}H-PGE_1 was obtained from New England Nuclear Corp. 11-α-Hydroxy-9,15-diketo-13-prostanoic acid (15-keto-PGE_1) was synthesized by MnO_2 oxidation of PGE_1 according to the method described by Attenburrow, Cameron & others (1952). The purity of the PGE_1, and 15-keto-PGE_1 was ascertained by thin-layer chromatography (Gréen & Samuelsson, 1964).

The silicic acid chromatography of the extracted rat plasma before incubation revealed 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 of the extract of the rat plasma which was incubated at 37° for 1 h showed an almost identical pattern with a single peak corresponding to ^{3}H-PGE_1. Thus practically no metabolic degradation of PGE_1 was detected in the rat plasma. This agrees with the findings of Vane (1969) that the biological activity of PGE_1 is not altered by incubation with rat plasma. In contrast, the silicic acid chromatography of the extract of the rat lung homogenate showed that after 20 min incubation the ^{3}H-PGE_1 peak in fractions 11–14 had practically disappeared. Instead, a prominent peak appeared in fractions 6–9 which was apparently due to a less polar PGE_1 metabolite (which we called Metabolite 1). Thin-layer chromatography showed that the R_F value, 0·63, of Metabolite 1 was identical with that of 15-keto-PGE_1 (Änggård & Samuelsson, 1964).

Very slow metabolic degradation of ^{3}H-PGE_1 was observed in the rat plasma and in the brain and heart homogenates. In contrast, the metabolic degradation of

^{3}H-PGE_1 occurred in the rat testicle, kidney and lung. The kidney and lung homogenates metabolized 95% of ^{3}H-PGE_1 within 20 min, whereas the rat testicle homogenate converted 80% of ^{3}H-PGE_1 within 20 min.

From the present study, it is evident that little metabolism of any released or injected PGE_1 occurs in rat plasma or in homogenized brain or heart. In contrast, rat homogenized lung, kidney and testicle metabolize PGE_1 quite quickly as do guinea-pig, cat, rabbit and dog lung, cat liver and dog kidney (Änggård & Samuelsson, 1964; Ferreira & Vane, 1967; Nakano, 1970a, b). PGE_1 is converted into 15-keto-PGE_1 by the oxidation of the secondary alcohol group at 15-C in swine lung and this appears to be the mechanism in rat lung. PGE_1 in guinea-pig lung is converted into dihydro-PGE_1 and 15-keto-dihydro-PGE_1 by the reduction of the Δ^{13}-double bond. The enzyme that catalyses the oxidation of PGE_1 has been identified as NAD^+-dependent 15-hydroxy-prostaglandin dehydrogenase which is specific for PG (Änggård & Samuelsson, 1966; Nakano, Änggård & Samuelsson, 1969). Further evidence for the inactivation of PG in rat, dog and human lung is that the hypotensive effect of PGE_1 or $PGF_{2\alpha}$ injected intra-arterially or into the left atrium is greater than with intravenous injection (Bennett, Eley & Scholes, 1968; Bergström, Carlson & others, 1965; Nakano & Cole, 1969; Nakano & Kessinger, unpublished data). It is not known whether this is due to uptake of PG by the lung, but if the metabolites are present in the blood they presumably have little effect; the vasodilator activities of the PGE_1 metabolites, 15-keto-PGE_1 and 15-keto-dihydro-PGE_1 are approximately 1/100 to 1/150 of that of PGE_1 in the dog hind-limb preparation (Nakano & Kessinger, unpublished data). In summary, rat homogenized lung, liver and testicle inactivate PGE_1. The product formed by the lung appears to be 15-keto PGE_1. The plasma and homogenized brain or heart have little ability to metabolize PGE_1.

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