The Catabolism of Prostaglandins by Rat Skin

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(Received 8 May 1979)

The activities of NAD⁺-dependent 15-hydroxy prostaglandin dehydrogenase in soluble fractions of rat skin and lung were compared by using a radiochemical assay method. Tritiated prostaglandin F_{2α} was incubated with NAD⁺ and 120000g supernatant of tissue homogenate. Extracted prostaglandin substrate and reaction products were separated by t.l.c. and quantitatively determined by liquid-scintillation counting. With skin 120000g supernatant, 10mM-NAD⁺ and an incubation time of 15 min, the mean $V_{\max}$ was 5.5 nmol of prostaglandin F_{2α} converted/s per litre of reaction mixture. With lung 120000g supernatant, 60mM-NAD⁺ and an incubation time of 5 min, the mean $V_{\max}$ was 26.9 nmol/s per litre, demonstrating 5-fold greater dehydrogenase activity in lung per unit wet weight of tissue. However, the total wet weight of skin was about 23 times that of lung, on dissection of individual rats, indicating that the entire skin may contain 4.5 times the total 15-hydroxy prostaglandin dehydrogenase activity of the lungs. Skin may thus be an important organ of prostaglandin catabolism.

The prostaglandins are a group of biologically active acidic lipids that are widely distributed in mammalian tissues (Piper, 1973a). They have a wide range of biological actions (Nakano, 1973), many of which are considerably diminished by oxidation of the 15(*S*)-hydroxy group (Anggard & Samuelsson, 1966; Nakano, 1972; Crutchley & Piper, 1975; Wasserman, 1975). This oxidation is catalysed by NAD⁺-dependent 15-hydroxy prostaglandin dehydrogenase (11α,15α-dihydroxy-9-oxoprost-13-enoate-NAD⁺ 15-oxidoreductase, EC 1.1.1.141), and represents the first step in the catabolism of the parent prostaglandins (Anggard & Larsson, 1971; Anggard *et al.*, 1971; Hamberg & Samuelsson, 1971). This enzyme is active in particle-free fractions of many mammalian tissues (Hansen, 1976), but there have been few studies directly comparing its activities

in different tissues of the same species. One such study (Anggard *et al.*, 1971) demonstrated that its activity was highest in soluble fractions of kidney, spleen and lung of the pig, but skin was not assayed. Preparations of kidney, placenta and lung of various species have high activity, and are the usual sources of purified enzyme (Hansen, 1976). The importance of lung as an organ of prostaglandin catabolism is borne out by studies made *in vivo* and by experiments involving perfusion of isolated organs (Piper, 1973b). Activity of NAD⁺-dependent 15-hydroxy prostaglandin dehydrogenase has been demonstrated in human (Jonsson & Anggard, 1972), newborn mouse (Wilkinson & Rabinowitz, 1976) and rat (Camp *et al.*, 1978) skin. As skin is by weight a very large organ, it was decided to compare the properties of the enzyme in rat skin and lung, in order to assess the relevance of skin as an organ of prostaglandin catabolism.

Experimental

Materials

Unlabelled prostaglandins were a gift from Dr. J. Pike (The Upjohn Co., Kalamazoo, MI, U.S.A.). NaB³H₄ (555–661 mCi/mmol) was from The Radiochemical Centre (Amersham, Bucks., U.K.). NAD⁺ (free acid, grade 1) and pig heart lactate dehydrogenase [specific activity approx. 300 units (μmol of

Abbreviations used: prostaglandin F_{2α}, 9α,11α,15α-trihydroxyprosta-5-*cis*,13-*trans*-dienoic acid; prostaglandin E₂, 11α,15α-dihydroxy-9-oxoprost-5-*cis*,13-*trans*-dienoic acid; prostaglandin D₂, 9α,15α-dihydroxy-11-oxoprost-5-*cis*,13-*trans*-dienoic acid; 15-oxoprostaglandin F_{2α}, 9α,11α-dihydroxy-15-oxoprost-5-*cis*,13-*trans*-dienoic acid; 15-oxo-13,14-dihydroprostaglandin F_{2α}, 9α,11α-dihydroxy-15-oxoprost-5-*cis*-enoic acid; 13,14-dihydroprostaglandin F_{2α}, 9α,11α,15α-trihydroxyprost-5-*cis*-enoic acid; 15-oxoprostaglandin E₂, 11α-hydroxy-9,15-dioxoprost-5-*cis*,13-*trans*-dienoic acid; 15-oxo-13,14-dihydroprostaglandin E₂, 11α-hydroxy-9,15-dioxoprost-5-*cis*-enoic acid; prostaglandin F_{2β}, 9β,11α,15α-trihydroxyprosta-5-*cis*,13-*trans*-dienoic acid.

lactate transformed/min)/mg] were from Boehringer Corp. (London) (Lewes, East Sussex, U.K.). Sigma (London) Chemical Co. (Poole, Dorset, U.K.) supplied 2-mercaptoethanol. Sephadex LH-20 was from Pharmacia (Great Britain) (London W.5, U.K.) and Lipidex 5000 from Packard Instrument Co. (Caversham, Berks., U.K.). Silica gel G1500 t.l.c. plates (0.25 mm thickness) were manufactured by Schleicher und Schüll (Dassel, West Germany) and were supplied by Anderman and Co. (East Molesey, Surrey, U.K.). Organic solvents used in extraction and chromatographic procedures were from BDH Chemicals (Poole, Dorset, U.K.) and were distilled before use. Chloroform was distilled over anhydrous CaCl_2 and stabilized with 2% (v/v) ethanol. Liquid-scintillant chemicals included 2-ethoxyethanol, sulphur-free toluene, naphthalene (from BDH Chemicals) and 2,5-diphenyloxazole (from Intertechnique, Uxbridge, Middlesex, U.K.). Materials used in g.l.c.-mass spectrometry have been described previously (Black *et al.*, 1978). All other chemicals used were obtained from BDH Chemicals and were of AnalaR grade or of the highest purity available. Water was distilled and de-ionized before use.

Laboratory animals

Albino C.F.Y. rats were purchased from Anglia Laboratory Animals (Alconbury, Huntingdon, U.K.) and a colony was maintained at the Institute of Dermatology, London. Males weighing 300–400 g were used in all experiments.

Total skin and lung weights were determined in four animals. Each animal was killed by a blow to the head and cervical dislocation, and was shaved with Oster clippers from the base of the skull to the tail. The entire skin was removed excluding that of head and tail, subcutaneous fat was excised and the skin was weighed. Lungs from the same animals were removed, blotted dry and weighed.

Preparation of subcellular fractions

Animals were killed as described above, and the abdominal skin was shaved with Oster clippers, excised and freed of subcutaneous fat. Thereafter all procedures were carried out at 0–4°C. The skin was chopped with scissors and homogenized in 0.11 M-sodium phosphate buffer (0.09 M- Na_2HPO_4 /0.02 M- NaH_2PO_4), pH 7.3, containing 0.06% (v/v) 2-mercaptoethanol. A fixed ratio of 2 g of tissue to 5 ml of buffer was always used. The homogenizer (type X1020; Internationale Laboratoriums-Apparate G.m.b.H., Ballrichen-Dottingen, West Germany) was fitted with a 1 cm-diameter shaft and was operated at half-maximum speed for four 15 s periods, each period being interrupted by an interval of 15 s to avoid overheating. In experiments to deter-

mine subcellular enzyme distribution, the homogenate was centrifuged at 600 *g* for 10 min at 3°C in an MSE Mistral 6L centrifuge (r_{av} 23 cm). In all other experiments the homogenate was centrifuged at 2500 *g* under otherwise similar conditions. The supernatant was re-centrifuged at 120 000 *g* for 60 min at 3°C in an MSE Superspeed 75 ultracentrifuge with a 10 × 10 ml titanium angle rotor (r_{av} 6.435 cm), and the supernatant was used in subsequent experiments. In the experiments to determine subcellular enzyme distribution, the 120 000 *g* pellet derived from 3 ml of 600 *g* supernatant was suspended in buffer and re-centrifuged at 120 000 *g* for a further 60 min at 3°C. The supernatant was discarded and the pellet resuspended in 1 ml of the above buffer, in a hand-held glass homogenizer. This suspension was used in subsequent experiments.

When lung was to be processed, rats that had no overt signs of respiratory disease were selected. The resected lungs were briefly rinsed with distilled de-ionized water and dried with filter paper. Those showing prominent inflammatory changes were discarded. The lungs were then processed exactly as described above, with the same tissue/buffer ratio on homogenization. Subcellular fractions were freshly prepared for each experiment, and incubations were carried out immediately after centrifugation.

Preparation of substrate

[9 β - ^3H]Prostaglandin $\text{F}_{2\alpha}$ was prepared by reduction of prostaglandin E_2 with NaB^3H_4 , by using the method described by Samuelsson (1964). The epimeric reduction products, [9 β - ^3H]prostaglandin $\text{F}_{2\alpha}$ and [9 α - ^3H]prostaglandin $\text{F}_{2\beta}$, were converted into methyl esters with ethereal methanolic diazomethane and separated by Lipidex 5000 straight-phase gel partition chromatography as described by Hensby (1975). Radioactivity in the column effluent fractions was determined by liquid-scintillation counting. The fractions containing [9 β - ^3H]prostaglandin $\text{F}_{2\alpha}$ methyl ester were pooled and evaporated *in vacuo*, and the residue was demethylated by reaction with 30 ml of methanolic 1.0 M-KOH for approx. 15 h at room temperature (approx. 24°C). This mixture was then diluted to 300 ml with distilled de-ionized water, acidified to pH 3 with 0.5 M-HCl and extracted with 3 equal volumes of ethyl acetate. The pooled organic phases were evaporated *in vacuo* and vacuum-desiccated, and the residue was subjected to reversed-phase gel partition chromatography with the Sephadex LH-20 system previously described (Hensby, 1975). This chromatographic step yielded [9 β - ^3H]prostaglandin $\text{F}_{2\alpha}$ of at least 98% radiochemical purity as determined by the three t.l.c. systems described below. The [9 β - ^3H]prostaglandin $\text{F}_{2\alpha}$ was added to unlabelled prostaglandin $\text{F}_{2\alpha}$ to give the desired specific radioactivity.

[11 β -³H]Prostaglandin F_{2 α} was similarly prepared by reduction of prostaglandin D₂ with NaB³H₄. The [11 β -³H]prostaglandin was separated from its [11 α -³H]prostaglandin epimer and purified as described above, yielding [11 β -³H]prostaglandin F_{2 α} with radiochemical purity of 99% on t.l.c. system A described below. It was added to unlabelled prostaglandin F_{2 α} to give the appropriate specific radioactivity. Both substrates were stored at -20°C in methanol at known concentrations.

Reaction conditions

Initially incubation volumes of 1.5 and 0.75 ml were used, but these were later scaled down to 187.5 μ l without altering the relative proportions of reactants. The procedures adopted for the small incubation volume are described below. [9 β -³H]- and [11 β -³H]-Prostaglandin F_{2 α} substrates were prepared to give specific radioactivities of 4.33 and 4.27 Ci/mol respectively. [11 β -³H]Prostaglandin F_{2 α} was required in a few experiments only, [9 β -³H]prostaglandin F_{2 α} being otherwise routinely used. Exact quantities of the methanolic stock solutions were added to test tubes, and the solvent was evaporated *in vacuo*. To each was added 62.5 μ l of 0.11 M-sodium phosphate buffer, pH 7.3, containing 0.06% (v/v) 2-mercaptoethanol and the desired amount of NAD⁺. Reactions were initiated by the addition of 125 μ l of subcellular fraction, the test tubes were incubated in a Grant shaking water bath, and reactions were terminated by acidification to approx. pH 3 with 37.5 μ l of 0.5 M-HCl. In some experiments lactate dehydrogenase and pyruvic acid were added to the reaction mixture. In determining blank rates at a range of [9 β -³H]prostaglandin F_{2 α} concentrations, acidification was carried out before the addition of high-speed supernatant, and extraction was performed immediately.

Extraction

Saturating amounts of solid NaCl were added to the acidified reaction mixtures. This simplified subsequent extraction by diminishing protein emulsification. After the addition of 0.8 ml of ethyl acetate, each tube was vortex-mixed and briefly centrifuged at maximum speed in an MSE Minor centrifuge. The ethyl acetate was removed, the extraction was repeated and the pooled organic phases were evaporated under a stream of N₂ at 42°C. After vacuum-desiccation for 20 min the samples were either subjected to t.l.c. or stored at -20°C for 1-2 days before chromatography.

T.l.c.

Sample residues were dissolved in 40 μ l of methanol and applied to 10 cm $\times$ 20 cm t.l.c. plates as a narrow streak of approx. 2.5 cm length by using a microsyringe. A further application of sample in

40 μ l of methanol was made. Two different samples were applied to each plate, and plates were developed to a distance of 15 cm. Three t.l.c. systems were used. In system A, the solvent mixture contained ethyl acetate/acetone/acetic acid (90:10:1, by vol.) as described by Andersen (1969), and plates were each developed twice. In system B (Sun & Armour, 1974), plates were subjected to a single development in chloroform/methanol/acetic acid (18:1:1, by vol.). In system C, plates were treated by brief immersion in 3% (w/v) AgNO₃ in methanol/water (9:1, v/v), followed by heating at 85-90°C for approx. 4 min; after application of the sample, they were developed once in chloroform/methanol/acetic acid (18:3:1, by vol.). In initial experiments, 0.5 cm sections of the developed plates were scraped into scintillation vials. The central section of each plate, where reference prostaglandins had been spotted, was left intact and the positions of the standards were detected with I₂ vapour. In later experiments, unlabelled standard prostaglandin F_{2 α} , 15-oxoprostaglandin F_{2 α} and 15-oxo-13,14-dihydroprostaglandin F_{2 α} were spotted directly on to the applied residues. After development in solvent system B, the plates were briefly exposed to I₂ vapour and the positions of the three standards were marked. The I₂ staining was allowed to fade under a stream of air, and the three sections of the plate corresponding to the positions of the three reference standards were scraped into scintillation vials.

Scintillation counting

Liquid scintillant was prepared in the proportions 1500 ml of toluene, 900 ml of 2-ethoxyethanol, 112.5 g of naphthalene and 10.5 g of 2,5-diphenyloxazole, the final mixture containing 2% (v/v) methanol. An LKB Ultrabeta 1210 scintillation counter was used, d.p.m. being calculated by the external-standard channels-ratio method. The radioactivities of the 0.5 cm t.l.c. scrapings were counted in 10 ml of scintillant for 5 min each, or in the later method the radioactivities of the three sections corresponding to reference prostaglandins were each counted in 14 ml of scintillant to a 2 σ standard error of 2.5%. In both cases counting of the radioactivity of the external standard was for 1 min.

Quantitative determination of endogenous prostaglandins in skin 120 000 g supernatant

Portions (50 μ l) of 120 000 g supernatant were equilibrated with mixtures of 100 ng each of tetra-deuterated prostaglandins E₂ and F_{2 α} in 1 ml of ethanol/water (1:19, v/v) saturated with indomethacin. The mixtures were acidified to pH 4 with 0.5 M-HCl, extracted and subjected to t.l.c. as described above, with a single development with solvent system A. Sections of the plates corresponding to the

positions of reference prostaglandins E_2 and $F_{2\alpha}$, developed in parallel, were eluted with methanol. This was evaporated and the endogenous prostaglandins E_2 and $F_{2\alpha}$ in the residues were measured quantitatively by multiple-ion-detection g.l.c.-mass spectrometry. Details of this method have been described previously (Hensby *et al.*, 1977; Black *et al.*, 1978).

Determination of protein

This was carried out by the method of Lowry *et al.* (1951), with human plasma albumin as standard. This was supplied in aqueous solution by the Blood Products Laboratory, Lister Institute, Elstree, Herts., U.K.

Results and Discussion

Representative R_F values obtained for the relevant prostaglandin standards, with the three described t.l.c. systems, are listed in Table 1.

Enzyme activity in 120 000 g supernatant of skin homogenate

Under various conditions (0.5–20 mM-NAD⁺, 1.9–136 μ M-prostaglandin $F_{2\alpha}$ and incubation times from 5 to 60 min at 32–50°C), [9 β -³H]prostaglandin $F_{2\alpha}$ was converted into two less-polar metabolites, which, on t.l.c. system B, had R_F values corresponding to those of authentic 15-oxoprostaglandin $F_{2\alpha}$ and 15-oxo-13,14-dihydroprostaglandin $F_{2\alpha}$. As indicated in Table 1, t.l.c. system B does not separate adequately prostaglandins E_2 and D_2 from these two metabolites, nor 13,14-dihydroprostaglandin $F_{2\alpha}$ from prostaglandin $F_{2\alpha}$. T.l.c. systems A and C were thus used to exclude the possibility of concurrent oxidation of 9 α - and 11 α -hydroxy groups, and the further conversion of 15-oxo-13,14-dihydroprostaglandin $F_{2\alpha}$ into 13,14-dihydroprostaglandin $F_{2\alpha}$. In these experiments an incubation time of 15 min and NAD⁺ and prostaglandin $F_{2\alpha}$ concentrations of 10 mM and 56.5 μ M respectively were used. With t.l.c. system A, concurrent oxidation of the 11 α -hydroxy group was excluded, as there was no evidence of

conversion of [9 β -³H]prostaglandin $F_{2\alpha}$ into prostaglandin D_2 , which is clearly separated from the 15-oxo and 15-oxo-13,14-dihydro metabolites of prostaglandin $F_{2\alpha}$ (Table 1). To exclude concurrent oxidation of the 9-hydroxy group of prostaglandin $F_{2\alpha}$, substrate tritiated in the 11 β -position was required. Such oxidation of [9 β -³H]prostaglandin $F_{2\alpha}$ would result in loss of the radiolabel and consequent inability to detect the resulting 9-oxo compound(s) on t.l.c. By using [11 β -³H]prostaglandin $F_{2\alpha}$ and t.l.c. system A, it could be shown that no prostaglandin E_2 or its 15-oxo or 15-oxo-13,14-dihydro metabolites were being formed. With t.l.c. system C, which separates 13,14-dihydroprostaglandin $F_{2\alpha}$ from prostaglandin $F_{2\alpha}$ and its other two metabolites (Table 1), it was evident that no dihydro metabolite was being formed.

These results indicate that, under the conditions used, two prostaglandin-catabolizing enzymes were active in skin 120 000 g supernatant, a 15-hydroxy prostaglandin dehydrogenase and a prostaglandin Δ^{13} -reductase. These two enzymes commonly co-exist in other tissues (Hansen, 1976), and oxidation of the 15-hydroxy group has been shown to precede saturation of the 13,14-double bond (Anggard & Larsson, 1971; Anggard *et al.*, 1971; Hamberg & Samuelsson, 1971). It was possible to confirm this enzymic sequence in the present reactions. When incubations were carried out with 82.8 μ M-[9 β -³H]-prostaglandin $F_{2\alpha}$ and 10 mM-NAD⁺ for 15 min at 37°C, t.l.c. system B, which separates the two metabolites adequately, showed that the ratio of radioactivity corresponding to 15-oxoprostaglandin $F_{2\alpha}$ and to 15-oxo-13,14-dihydroprostaglandin $F_{2\alpha}$ respectively was approximately 3:7. Addition of 50 mM-pyruvic acid and 0.94 unit of lactate dehydrogenase to incubation mixtures that were otherwise identical resulted in a reverse of this product ratio to approximately 2:1, without significantly affecting the total mass of products formed. These results suggest that NADH, formed by the action of 15-hydroxy prostaglandin dehydrogenase, is being bound in the enzymic conversion of pyruvate into lactate, reducing available NADH and inhibiting

Table 1. R_F values of standard prostaglandins
Details of each t.l.c. system are described in the Experimental section.

Prostaglandin	R_F		
	T.l.c. system A	T.l.c. system B	T.l.c. system C
$F_{2\alpha}$	0.15	0.18	0.19
13,14-Dihydro- $F_{2\alpha}$	N.D.	0.23	0.33
E_2	0.31	0.33	N.D.
15-Oxo- $F_{2\alpha}$	0.41	0.39	0.46
15-Oxo-13,14-dihydro- $F_{2\alpha}$	0.48	0.52	0.53
D_2	0.57	0.41	N.D.
15-Oxo- E_2	0.69	0.60	N.D.
15-Oxo-13,14-dihydro- E_2	0.71	0.65	N.D.

prostaglandin Δ^1 -reductase activity. The resulting accumulation of 15-oxoprostaglandin $F_{2\alpha}$ and diminished 15-oxo-13,14-dihydroprostoglandin production, as demonstrated, could occur only if oxidation of the 15-hydroxy group was the first step. Activity of the 15-hydroxy prostaglandin dehydrogenase could thus be determined by calculating the percentage conversion of prostaglandin $F_{2\alpha}$ into total products, and velocities were expressed as nmol of prostaglandin $F_{2\alpha}$ converted/s per litre of reaction mixture, after subtraction of blank rates. Velocities were not expressed in terms of the protein content of the reaction mixtures, as the intention was to refer enzyme activity to wet weight of tissue. The total protein concentration of lung 120000g supernatant was 22.2 ± 5.2 mg/ml and that of skin 120000g supernatant was 9.2 ± 1.0 mg/ml (means $\pm$ s.d., $n = 11$). Expression of velocities in terms of protein would thus bias results.

The effects of temperature, NAD^+ concentration and incubation time were studied, with $[9\beta\text{-}^3\text{H}]$ prostaglandin $F_{2\alpha}$ and t.l.c. system B (Figs. 1–3). Incubations were performed in duplicate, as shown, and repeat experiments gave similar results. Fig. 1 demonstrates increases in enzyme activity up to 50°C , with decrease in rate above this temperature. Fig. 2 illustrates an experiment to determine the NAD^+ concentration required for enzyme saturation. The NAD^+ concentrations used were not in the range required for satisfactory determination of a K_m value for NAD^+ , but nevertheless it was established that a concentration of 10 mM approaches enzyme saturation. Fig. 3 demonstrates that prostaglandin $F_{2\alpha}$ conversion increased in a near-linear fashion up to 15 min. A lower prostaglandin $F_{2\alpha}$ concentration ($15\mu\text{M}$) was used in the illustrated time-course experiments because the rate-limiting effects of prostaglandin $F_{2\alpha}$ depletion would be most

evident. With a higher prostaglandin $F_{2\alpha}$ concentration of $60.3\mu\text{M}$, similar linearity was obtained. A 10 mM- NAD^+ concentration and incubation time of 15 min were thus used in subsequent experiments. Shorter incubation times resulted in smaller differences between background and product radioactivity on t.l.c., especially at higher $[9\beta\text{-}^3\text{H}]$ prostaglandin $F_{2\alpha}$ concentrations, and were thus avoided. Probable lack of absolute linearity would result in slight under-estimations of V_{max} , which is therefore referred to as 'apparent'. A representative prostaglandin $F_{2\alpha}$ saturation curve and corresponding

Fig. 2. Dependence of skin 15-hydroxy prostaglandin dehydrogenase activity on NAD^+ concentration. Prostaglandin $F_{2\alpha}$ ($60.3\mu\text{M}$) was incubated with 120000g skin supernatant and different concentrations of NAD^+ for 60 min at 37°C . Experimental details are given in the text. Reaction velocity (v) is expressed as in Fig. 1.

Fig. 1. Dependence of skin 15-hydroxy prostaglandin dehydrogenase activity on incubation temperature. Prostaglandin $F_{2\alpha}$ ($60.3\mu\text{M}$) was incubated with skin 120000g supernatant and 10 mM- NAD^+ for 60 min at different temperatures. Experimental details are given in the text. Reaction velocity (v) is expressed as nmol of prostaglandin $F_{2\alpha}$ converted/s per litre of reaction mixture.

Fig. 3. Dependence of 15-hydroxy prostaglandin dehydrogenase activity on incubation time. Prostaglandin $F_{2\alpha}$ ($15\mu\text{M}$) was incubated for different time periods at 37°C . Experimental details are given in the text. Enzyme activity is expressed as μmol of prostaglandin $F_{2\alpha}$ converted per litre of reaction mixture. (a) Incubation mixtures included skin 120000g supernatant and 10 mM- NAD^+ ; (b) incubation mixtures included lung 120000g supernatant and 60 mM- NAD^+ .

Hanes plot are shown in Figs. 4 and 5, and demonstrate Michaelis–Menten kinetics. In five such experiments, the apparent K_m for prostaglandin $F_{2\alpha}$ at 10mM- NAD^+ was $38.0 \pm 10.2 \mu M$ (mean $\pm$ s.d.), and the apparent V_{max} was 5.5 ± 1.3 nmol/s per litre of reaction mixture (mean $\pm$ s.d.). Straight lines were constructed by simple linear regression, and the mean r value was 0.9824 ± 0.0069 (s.d.). Slight deviations from linearity were occasionally noted at lower prostaglandin $F_{2\alpha}$ concentrations, and were investigated. A range of seven different [9β - 3H]prostaglandin $F_{2\alpha}$ concentrations (1.9 – $13.2 \mu M$) were incubated with 10mM- NAD^+ and skin 120000g supernatant for 15 min at $37^\circ C$. Inconsistent minor deviations from linearity were noted, and were assumed to be of methodological origin.

Enzyme activity in particulate fractions of skin homogenate

Suspensions of 120000g pellet were prepared as described, and incubated with $56.5 \mu M$ -[9β - 3H]prostaglandin $F_{2\alpha}$ and 10mM- NAD^+ for 60 min at $37^\circ C$. T.l.c. revealed that no conversion of substrate had occurred, confirming the soluble nature of the enzyme.

Enzyme activity in 120000g supernatant of lung homogenate

By using the three t.l.c. systems it was shown that, in the presence of NAD^+ , [3H]prostaglandin $F_{2\alpha}$ was again converted into two less-polar metabolites with

Fig. 4. Dependence of 15-hydroxy prostaglandin dehydrogenase activity on prostaglandin $F_{2\alpha}$ concentration. Different concentrations of prostaglandin $F_{2\alpha}$ were incubated at $37^\circ C$. Experimental details are given in the text. Reaction velocity (v) is expressed as in Fig. 1. (a) Incubations of mixtures containing skin 120000g supernatant and 10mM- NAD^+ were carried out for 15 min; (b) incubations of mixtures containing lung 120000g supernatant and 60mM- NAD^+ were carried out for 5 min.

R_F values corresponding to those of 15-oxoprostaglandin $F_{2\alpha}$ and 15-oxo-13,14-dihydroprostaglandin $F_{2\alpha}$. In these experiments an incubation time of 5 min and NAD^+ and prostaglandin $F_{2\alpha}$ concentrations of 60mM and $56.5 \mu M$ respectively were used. By using the same combination of t.l.c. systems it was possible to exclude concurrent oxidation of 9- or 11-hydroxy groups and further conversion of 15-oxo-13,14-dihydroprostaglandin $F_{2\alpha}$ into 13,14-dihydroprostaglandin $F_{2\alpha}$. T.l.c. radio-profiles showed that, of the two metabolites, radioactivity with the same R_F value as reference 15-oxoprostaglandin $F_{2\alpha}$ formed the major peak. Activity of 15-hydroxy prostaglandin dehydrogenase was calculated and expressed as described above.

The effects of incubation time and NAD^+ concentration were studied, with [9β - 3H]prostaglandin $F_{2\alpha}$ and t.l.c. system B (Figs. 3 and 6). Incubations were performed in duplicate and repeat experiments gave similar results. As shown in Fig. 6, a higher concentration of NAD^+ of 60mM was required to approach enzyme saturation, but increasing the concentration to 100mM resulted in a large decrease in the rate. Again, the NAD^+ concentrations used were not in the range required for satisfactory K_m determination, but determination of an adequate saturating concentration was possible. An incubation time of 5 min provided near-linear reaction rates (Fig. 3), but a shorter time than this was not adopted

Fig. 5. Linear plot of the effect of prostaglandin $F_{2\alpha}$ concentration on reaction velocity. Experimental details are as described in Fig. 4. Plots of [9β - 3H]prostaglandin $F_{2\alpha}$]/ v versus [9β - 3H]prostaglandin $F_{2\alpha}$] are demonstrated for skin (a) and lung (b) 15-hydroxy prostaglandin dehydrogenase. The mean kinetic constants obtained in a number of such experiments are stated in the Results and Discussion section.

Fig. 6. Dependence of lung 15-hydroxy prostaglandin dehydrogenase activity on NAD^+ concentration

Prostaglandin $\text{F}_{2\alpha}$ ($97.9\mu\text{M}$) was incubated with lung 120000g supernatant and different concentrations of NAD^+ for 15 min at 37°C . Experimental details are given in the text. Reaction velocity (v) is expressed as in Fig. 1.

for the reasons discussed above. A representative prostaglandin $\text{F}_{2\alpha}$ saturation curve and corresponding Hanes plot are shown in Figs. 4 and 5, and again demonstrate Michaelis-Menten kinetics. In three such experiments the apparent K_m for prostaglandin $\text{F}_{2\alpha}$ at 60mM- NAD^+ was $44.7 \pm 10.2\mu\text{M}$ (mean $\pm$ s.d.) and the apparent $V_{\max}$ $26.9 \pm 3.0\text{nmol/s per litre}$ of reaction mixture (mean $\pm$ s.d.). Straight lines were constructed by simple linear regression and the mean r value was 0.9824 ± 0.0103 (s.d.). Slight deviations from linearity were again noted at lower prostaglandin $\text{F}_{2\alpha}$ concentrations and were again investigated. The same range of prostaglandin $\text{F}_{2\alpha}$ concentrations (1.9 – $13.2\mu\text{M}$) were incubated with 60mM- NAD^+ and lung 120000g supernatant for 5 min at 37°C . Inconsistent minor deviations from linearity again occurred, and were assumed to be of methodological origin.

Recovery of radioactivity in all the described experiments was between 80 and 98%.

Conclusion

The purpose of this study was to compare maximal 15-hydroxy prostaglandin dehydrogenase activities in skin and lung. Crude enzyme preparations were used, containing associated prostaglandin Δ^{13} -reductase activity. This activity is particularly significant in skin 120000g supernatant, and would interfere with 15-hydroxy prostaglandin dehydrogenase assays based on measurement of NADH or 15-oxo-prostaglandin production. A series of purification steps was required to separate prostaglandin Δ^{13} -reductase from 15-hydroxy prostaglandin dehydro-

genase activity in the tissues studied (Hansen, 1976). During this purification, considerable decrease in total recoverable enzyme activity occurs (Hansen, 1976), complicating accurate determination of enzyme activity in terms of original wet weight of tissue.

In the present radiochemical assay method, with unpurified enzyme, K_m values were obtained that correspond to those obtained for prostaglandin $\text{F}_{2\alpha}$ with purified 15-hydroxy prostaglandin dehydrogenase from various sources (Hansen, 1976). The variation in the K_m values in the present experiments may be a reflection both of the number of steps involved in the assay and of variation in the concentration of endogenous prostaglandins in the crude enzyme preparations. G.l.c.-mass-spectrometric analysis of five different skin 120000g supernatants showed concentrations of $142 \pm 21\text{nm}$ and $168 \pm 53\text{nm}$ (means $\pm$ s.d.) for endogenous prostaglandins $\text{F}_{2\alpha}$ and E_2 respectively, compared with added [9β - ^3H]prostaglandin $\text{F}_{2\alpha}$ concentrations of 1.9 – $136\mu\text{M}$. The NAD^+ concentrations required for enzyme saturation were high, especially with the lung preparations. Possible explanations include the presence of other NAD^+ -binding activities in the crude enzyme preparations.

Comparison of the mean apparent $V_{\max}$ values obtained indicates that rat lung contains approximately 5-fold more 15-hydroxy prostaglandin dehydrogenase activity than skin, on the basis of unit wet weight of tissue. However, on dissection of four individual rats, the total wet weight of skin ($41.3 \pm 2.5\text{g}$, mean $\pm$ s.d.) was 23-fold that of lung ($1.8 \pm 0.5\text{g}$, mean $\pm$ s.d.). This suggests that the entire skin contains approximately 4.5 times the enzyme activity of whole lungs, assuming no regional variation of enzyme activity in skin. The brief report (Wilkinson & Rabinowitz, 1976) that most 15-hydroxy prostaglandin dehydrogenase activity in newborn mouse skin was located in the dermis requires confirmation, but suggests that the enzyme may be in close association with skin vasculature. Early radioautographic work has shown that there is a significant accumulation of radioactivity in the subcutaneous tissue of mice after intravenous injection of tritiated prostaglandins $\text{F}_{2\alpha}$ and E_1 (Hansson & Samuelsson, 1965; Green *et al.*, 1967). Skin may thus be an important organ of prostaglandin catabolism, especially where 15-hydroxy prostaglandins survive pulmonary passage, are released from lung or are formed locally in skin.

We are indebted to Dr. C. N. Hensby for his continued interest and advice, and for carrying out g.l.c.-mass spectrometry, and to Dr. M. Thompson for helpful discussion. R. C. was supported by grants from Stiefel Laboratories (U.K.) and The Sir Herbert Dunhill Trust.

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