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DISTRIBUTION OF FOUR PROSTAGLANDIN-METABOLISING ENZYMES IN ORGANS OF THE RABBIT

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Abstract—The activities of four prostaglandin-metabolising enzymes, prostaglandin 15-hydroxydehydrogenase (PGDH), prostaglandin Δ13 reductase (Δ13-R), prostaglandin 9-hydroxydehydrogenase (9-HDH) and 'enzyme X' were measured in 100,000 *g* supernatants prepared from 11 organs of the rabbit. Enzyme activity was determined using a radiochemical method with $PGF_{2\alpha}$ or $PGF_{2\alpha}$ metabolites as substrates. Although useful qualitative information concerning PGDH, Δ13-R, 9-HDH and 'enzyme X' activity in supernatants may all be derived from incubations using $PGF_{2\alpha}$, it was found preferable to utilise radiolabelled 15-keto $PGF_{2\alpha}$ for Δ13-R determination and 13,14-dihydro-15-keto $PGF_{2\alpha}$ for 9-HDH and 'enzyme X'. 'Enzyme X' converts 13,14-dihydro-15-keto $PGF_{2\alpha}$ to 13,14-dihydro $PGF_{2\alpha}$ and is found in appreciable amounts only in rabbit liver. PGDH and Δ13-R are present in high concentrations in kidney, lung, stomach and ileum; and levels are very low in heart, brain, skeletal muscle and aorta, with spleen and liver intermediate. Rabbit colon contains large amounts of PGDH but no Δ13-R, and is therefore useful for biological preparation of 15-keto prostaglandins and for PGDH purification studies. 9-HDH has restricted distribution, with largest concentrations in kidney, stomach, ileum and colon. The advantages of these microradiochemical methods over other techniques for prostaglandin metabolism studies are discussed.

The biological actions of classical prostaglandins (PG), e.g. PGE_2 and $PGF_{2\alpha}$, are generally short lasting and localised to sites close to their synthesis and release. This is largely due to rapid breakdown by enzymes which are localised intracellularly; thus PG inactivation is probably preceded by active uptake. This has been verified in the lung which very effectively removes and inactivates circulating PGs [1, 2]. The brief actions of the chemically unstable PG endoperoxides [3, 4], thromboxane A_2 [5] and prostacyclin (PGI_2) [6, 7] may also be influenced by metabolic degradation or enzymatic transformation, although spontaneous breakdown could be the crucial factor.

The most important initial steps common to the metabolism of all classical PGs are sequential oxidation of the 15-hydroxyl group to a ketone and reduction of the Δ13,14 double bond by the enzymes prostaglandin 15-hydroxydehydrogenase (PGDH) and prostaglandin Δ13 reductase (Δ13-R) [8]. The metabolites formed by these reactions, 15-keto and 13,14-dihydro-15-keto PGs, are much less potent than their parent molecules when tested on a wide variety of preparations [9, 10] (although there is some doubt about the lack of potency of 15-keto $PGF_{2\alpha}$ [11]), and so this pathway is effective for terminating PG actions. Both enzymes appear to be widely distributed in mammalian tissues [12]. $PGF_{2\alpha}$ metabolites may also be converted to their E-series counterparts following oxidation at C-9 by the enzyme prostaglandin 9-hydroxydehydrogenase (8-HDH) which was recently identified in rat kidney homogenates [13]. We have also found this enzyme in rabbit kidney, and shown that in this organ 9-HDH is capable *in vitro* of the direct conversion of $PGF_{2\alpha}$ to PGE_2 [14]. A further enzyme ('enzyme X'), found in pig and guinea pig kidney, converts 13,14-dihydro-15-keto $PGF_{2\alpha}$ to 13,14-dihydro $PGF_{2\alpha}$ [15], thereby enhancing its biological activity, since 13,14-dihydro compounds have more potent actions than the 13,14-dihydro-15-keto and 15-keto metabolites [16].

In this paper, we describe the organ-specific distribution in the rabbit of these four prostaglandin metabolising enzymes measured radiochemically. Previously, we have used radiolabelled $PGF_{2\alpha}$ as the sole substrate for these enzymes [14, 15]; here we show that the activities of Δ13-R, 9-HDH and 'enzyme X' are better measured by employing radiolabelled 15-keto $PGF_{2\alpha}$ or 13,14-dihydro-15-keto $PGF_{2\alpha}$ as substrates.

METHODS AND MATERIALS

Animals. Male New Zealand White rabbits (2–4 kg) were killed by a blow on the neck and exsanguinated. The following organs were quickly removed, cleaned and placed on ice: lungs, heart, kidneys, ileum, colon, stomach (separated into fundus and antrum), brain, liver, spleen, a portion of skeletal muscle (psoas muscle), abdominal aorta.

Chemicals. [9β-^{3}H]-prostaglandin $F_{2\alpha}$ (specific activity 81.6 Ci/mole) was synthesised by Dr. A. R. Brash, and [9β-^{3}H]-15-keto prostaglandin $F_{2\alpha}$ prepared from it as described below. [6-^{3}H]-13,14-dihydro-15-keto prostaglandin $F_{2\alpha}$ (specific activity 75 Ci/mole) was purchased from the Radiochemical Centre, Amersham. These radiolabelled prostaglandins were 95 per cent pure or better as measured by thin layer radiochromatography. NAD^+ and NADH were purchased from Sigma London Ltd. Reference standards of prostaglandins and prostaglandin metabolities were generous gifts from the Upjohn Company, U.S.A.

Preparation of homogenates and high-speed supernatants. The weighed organs were homogenised in 4 vols ice-cold pH 7.5 50 mM potassium phosphate buffer containing EDTA and cysteine (both 1 mM) by forcing them past the blades of an Ultra-Turrax homogeniser, type 18/2 N, in 3 or 4 strokes (about 5 sec). The homogenates were centrifuged twice at 4°, first at 3000 *g* for 10 min and the supernatant again at 100,000 *g* for 45 min, to give cell-free high-speed supernatants which were stored at −15°. There was no appreciable loss of enzymatic activity of the supernatants stored up to 10 weeks, provided that they were not thawed and refrozen.

Incubation of supernatants with prostaglandins; radiochemical assays for enzyme activity. All incubations contained 10 μg/ml prostaglandin $F_{2\alpha}$ (or metabolites), labelled with 0.05–0.2 μCi of the appropriate radiolabelled prostaglandin and 5 mM NAD^+ or NADH, as appropriate. The tubes were prepared on ice, and 0.2 ml samples removed before transfer to a 37° waterbath (zero-time samples) and after 60 min incubation. Samples were extracted twice with 0.8 ml ethyl acetate after addition of 0.2 ml ethanol and acidification with formic acid to pH 3.0. The organic phase was removed and evaporated in an air stream at 35°, and the residue resuspended in 200 μl methanol. In experiments using [9β-^{3}H]-labelled $PGF_{2\alpha}$ and 15-keto $PGF_{2\alpha}$, 20 μl of this resuspended extract was transferred to scintillation vials for counting. The counts obtained for each 60 min sample were subtracted from the zero-time sample, and the difference taken as the amount of $PGF_{2\alpha}$ or 15-keto $PGF_{2\alpha}$ converted by 9-HDH to equivalent E-series prostaglandin due to loss of the 9-β tritium as described previously [14, 15].

The remainder of the methanol extract (or the entire amount in the case of the [6-^{3}H]-13,14-dihydro-15-keto $PGF_{2\alpha}$ experiments) was dried down again, resuspended in 20 μl methanol, and 5 to 10 μl as appropriate applied in 1 cm bands to plastic-backed t.l.c. sheet coated with silica gel (Kodak, chromagram, type 13181). Authentic reference prostaglandins and prostaglandin metabolites were applied to separate channels. The sheets were developed for 70 min in ethyl acetate/acetone/glacial acetic acid (90:10:1, v/v), and the position of the standards visualised in an iodine chamber. Sections of the chromatograms corresponding to the different metabolites were cut out, and the proportion of each metabolite obtained after scintillation counting, making corrections for background and quenching as appropriate. In our hands, the metabolites of $PGF_{2\alpha}$ are separated effectively using this solvent with R_F values as follows: $PGF_{2\alpha}$-0.21, PGE_2-0.32, 15-keto $PGF_{2\alpha}$-0.33, 13,14-dihydro-15-keto $PGF_{2\alpha}$-0.46, 13,14-dihydro $PGF_{2\alpha}$-0.26.

In incubations using $PGF_{2\alpha}$ as substrate, PGDH activity was calculated from the sum of 15-keto $PGF_{2\alpha}$, 13,14-dihydro-15-keto $PGF_{2\alpha}$, 13,14-dihydro $PGF_{2\alpha}$ and E-series compounds since the PGDH step is common to the formation of all these metabolites. Δ13-R activity was calculated from the sum of the latter three metabolites, and 9-HDH activity from the amount of E-series compounds. 'Enzyme X' activity was calculated from the amount of 13,14-dihydro $PGF_{2\alpha}$. The same procedures were adopted for calculation of these enzyme activities using 15-keto $PGF_{2\alpha}$ or 13,14-dihydro-15-keto $PGF_{2\alpha}$ as substrates. NAD^+ was generally used as cofactor, except when assaying Δ13-R with 15-keto $PGF_{2\alpha}$ when NADH was utilised. However, in control experiments using lung and kidney it was found that NAD^+ or NADH stimulated 15-keto $PGF_{2\alpha}$ metabolism equally compared to controls to which an equal volume of distilled water was added.

Soluble protein was measured using the Folin reagent with bovine serum albumen as standard. Calculated values are the mean ±S.E.M.

Preparation of [9β-^{3}H]-15-keto $PGF_{2\alpha}$. Duplicate tubes containing 0.4 ml rabbit colon high-speed supernatant, 10 μg/ml $PGF_{2\alpha}$ labelled with 3.2 μCi [9β-^{3}H]-$PGF_{2\alpha}$ and 5 mM NAD^+ were incubated at 37° for 30 min, acidified to pH 3.0 and extracted with ethyl acetate. The organic phase and washings were combined, evaporated to dryness, and the residue (extraction efficiency = 80 per cent) was spotted quantitatively on plastic-backed t.l.c. sheet and chromatographed as above. A narrow band was cut corresponding to the position of authentic 15-keto $PGF_{2\alpha}$ (comprising 73 per cent of total radioactivity; 21 per cent was unchanged $PGF_{2\alpha}$), and the purity of this material (95 per cent) checked by further t.l.c. after elution from the plastic sheet into methanol.

RESULTS

Figure 1 shows the distribution in 12 organs of the rabbit of four enzymes involved in the biological inactivation of the classical prostaglandins. The activity of each enzyme was measured in cell-free 100,000 *g* supernatants using the appropriate radiolabelled substrate, viz. $PGF_{2\alpha}$ for PGDH, 15-keto $PGF_{2\alpha}$ for Δ13-R and 13,14-dihydro-15-keto $PGF_{2\alpha}$ for 9-HDH and 'enzyme X'. The results are expressed as rate of removal of prostaglandin substrate per mg soluble protein; as shown on the figure, there were large variations in soluble protein content of the supernatants between organs. The correction for protein concentration exaggerates the enzyme activity observed in the aorta, which in fact was very low: in common with the heart, muscle and brain, there was less than 5 per cent conversion in 60 min of the 10 μg/ml substrates by any of the four enzymes. By contrast, PGDH of lung, kidney and colon broke down more than 90 per cent of added $PGF_{2\alpha}$ in this time.

Lung, kidney and the organs of the gastrointestinal tract contain large amounts of PGDH and Δ13-R, with the notable exception of the colon which is deficient in Δ13-R. These organs also contain 9-HDH, although activity is low in the lung. Significant amounts of 'enzyme X' are found only in the liver.

In previous studies, estimates of the amounts of Δ13-R, 9-HDH and 'enzyme X' in homogenates were made indirectly by measuring the quantity of metabolites formed later in the pathway in incubations using radiolabelled $PGF_{2\alpha}$ as substrate, i.e. the reactions were not carried out with the specific substrates for these enzymes. Although

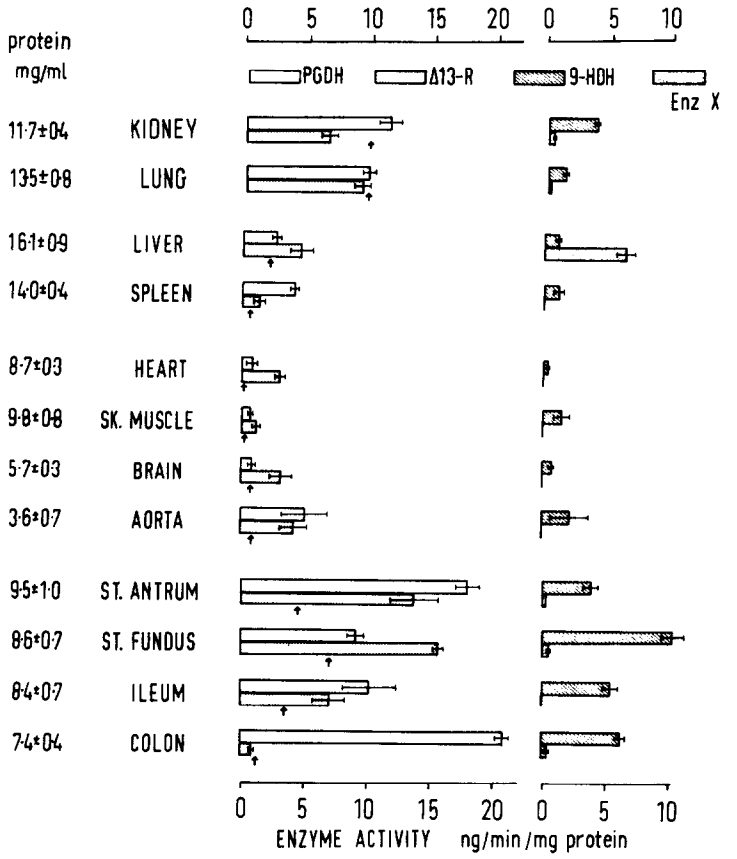

Fig. 1. Distribution of four prostaglandin-metabolising enzymes in organs of the rabbit. The activities of PGDH, Δ13-R, 9-HDH and 'Enzyme X' were measured using appropriate substrates ($PGF_{2\alpha}$, 15-keto $PGF_{2\alpha}$ and 13,14-dihydro-15-keto $PGF_{2\alpha}$) as described in the text. 10 μg/ml prostaglandin was incubated for 60 min at 37°; enzyme activity is expressed in terms of rate of prostaglandin breakdown per mg soluble protein and results show mean ±S.E.M. for 6–15 experiments (PGDH), 5–8 experiments (Δ13-R), 4–6 experiments (9-HDH and 'Enzyme X'). The arrows show Δ13-R activity in experiments using $PGF_{2\alpha}$ as substrate (see Results). Protein concentrations measured using supernatants prepared from 7 animals. SK = skeletal, ST = stomach.

this method generally gives a clear qualitative indication of the types of enzymes present, the errors in measuring enzyme activity in this way may be considerable if the rate of formation of enzyme substrate by a previous step in the pathway is slow in relation to the amount of enzyme present. Our results show that this indirect method underestimates enzyme activity in most of the rabbit organs: the arrows in Fig. 1 show the apparent Δ13-R activity as measured by conversion of $PGF_{2\alpha}$ beyond the 15-keto $PGF_{2\alpha}$ stage to the 13,14-dihydro-15-keto $PGF_{2\alpha}$ and E-series metabolites. Similarly, 9-HDH activity in all organs was underestimated when measured as the amount of E-series metabolites formed from $PGF_{2\alpha}$ or 15-keto $PGF_{2\alpha}$ substrates. The most extreme example of this problem occurred in the colon, since $PGF_{2\alpha}$ was only broken down as far as 15-keto $PGF_{2\alpha}$ and E-series metabolites were not formed at all. However, the colon contains plentiful 9-HDH which was detected when 13,14-dihydro-15-keto $PGF_{2\alpha}$ was used as substrate.

DISCUSSION

The results show that there are marked differences in the content of prostaglandin metabolising enzymes in organs of the rabbit. High concentrations of PGDH and Δ13-R are found in the kidney, lung and gastrointestinal tract, and this may reflect the importance of PGs in these organs. Many physiological roles for PGs have been suggested [17, 18, 19], but it is worth noting that these tissues all possess very active prostaglandin-sensitive epithelial transport systems. The very low enzyme levels detected in brain, aorta, skeletal muscle and heart do not preclude physiological roles for PGs in these tissues, since PG synthesis and metabolism may occur in a relatively few discrete parts of the tissue, with the enzymes effectively diluted out by gross homogenisation of the whole organ, as used here.

There are many similarities in organ-specific distribution of PGDH and Δ13-R between rabbit and pig [20]. The principal differences in the pig are spleen (much more PGDH and Δ13-R), stomach and intestine (much less PGDH and Δ13-R) and brain (much more Δ13-R). However, in the latter study [20], the enzymes were assayed spectrophotometrically, and high substrate concentrations of PGE_1 were used. Furthermore, only three experiments on each organ were performed and the variation between results was large, the tissues were not matched for age or sex and were homogenised after being stored frozen.

The tissue distribution of 9-HDH and 'enzyme X' has not previously been studied. 9-HDH occurs in substantial amounts in the kidney and gastrointestinal tract of the rabbit, but small amounts were detected in most tissues when 13,14-dihydro-15-keto $PGF_{2\alpha}$ was used as substrate. These results confirm that 13,14-dihydro-15-keto $PGF_{2\alpha}$ is the preferred substrate for 9-HDH as suggested previously [21]. Apart from kidney, which contains a unique 9-HDH [14], there is scant evidence that $PGF_{2\alpha}$ can be converted directly to PGE_2 by the 9-HDH of rabbit organs. 'Enzyme X' occurs only in the liver, and reduces the 15-keto group of 13,14-dihydro-15-keto $PGF_{2\alpha}$, thereby producing 13,14-dihydro $PGF_{2\alpha}$. We do not know whether this enzyme can reduce 15-keto $PGF_{2\alpha}$ to $PGF_{2\alpha}$ or act on the equivalent E-series prostaglandins, but it is evident from its specialised distribution and the absence of detectable amounts of 13,14-dihydro $PGF_{2\alpha}$ in the lung and kidney experiments that this enzymatic conversion cannot simply be due to PGDH acting in the reverse direction. Hamberg and co-workers have found a similar enzyme in guinea-pig liver [22], and showed that the 13,14-dihydro metabolite of PGE_2 was not formed directly by the action of Δ13-R on PGE_2, but arose by enzymatic reduction of the 13,14-dihydro-15-keto metabolite as suggested here for rabbit liver. We also doubt whether 'enzyme X' action could reflect a hitherto unrecognised facet of prostaglandin 9-keto reductase action [23, 24], i.e. the reduction to hydroxyl of the ketone group at C-15 rather than at C-9. 9-keto reductase is found in high concentrations in rabbit kidney [25], but we found that this organ contained little 'enzyme X'. Furthermore, it is dependent upon NADH or NADPH cofactor, whereas in our experiments 5 mM NAD^+ was added to incubations.

The rabbit colon is unusual in that it contains large amounts of PGDH but Δ13-R activity is absent. This enzyme distribution is peculiar to the rabbit because the colons of other species contain large amounts of both enzymes (unpublished data). Rabbit colon homogenates are therefore very useful for the biological preparation of 15-keto prostaglandins, as they are formed rapidly in high yield with relatively little contamination by other metabolites. The rabbit colon might also be the best organ to choose for PGDH purification studies.

We believe that the radiochemical techniques used in these experiments are the methods of choice for *in vitro* analysis of prostaglandin-metabolising enzymes. They afford the identification and measurement of the different metabolites formed, and can be performed on small volumes of supernatant without further purification or dilution, and using low substrate concentrations. The ease, speed and economy of the method allow the analysis of many replicated samples, and permit the direct identification of the pathways of prostaglandin metabolism. Spectrophotometric methods have also been used to measure prostaglandin metabolism: by contrast, these methods are much less sensitive, generally require high substrate concentration, dilution of enzyme supernatants (to remove background absorption), and may involve complicated or laborious manipulations following metabolite extraction. Most important, each assay permits only the measurement of a single reaction. This latter shortcoming is also the major failing of radioimmunoassay methods, unless each incubation is worked up separately using antibody specific for each metabolite (if available). Nevertheless, radioimmunoassay is the most accurate and sensitive method available for the detection of individual non-radioactive prostaglandin metabolites, e.g. in those studies of PG synthesis and release when radioactive tracers cannot be used.

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