Biochemical Pharmacology, Vol. 33, No. 8, pp. 1277–1284, 1984.
Printed in Great Britain.

0006-2952/84 $3.00 + 0.00
Pergamon Press Ltd.

ENZYMATIC INACTIVATION OF 6-KETO-PROSTAGLANDIN E_1 *IN VITRO*: COMPARISON WITH PROSTAGLANDIN E_1

C. N. BERRY, J. R. S. HOULT, *R. J. GRIFFITHS and *P. K. MOORE

Department of Pharmacology, King's College, Strand, London WC2R 2LS, U.K. and *Department of Pharmacology, Chelsea College, Manresa Road, London SW3 6LX, U.K.

(*Received* 31 *March* 1983; *accepted* 4 *November* 1983)

Abstract—The inactivation of 6-keto PGE_1, a biologically active and stable metabolite of prostacyclin, was studied in 100,000 *g* cytosolic supernatants by bioassay on rat stomach strip (contraction) and human platelets (inhibition of ADP-induced aggregation). PGE_1 was used as a reference compound. Both PGs were inactivated in supernatants from colon, kidney and liver of rat, rabbit and guinea-pig. Inactivation was time- and NAD^+ -dependent and was generally greater for PGE_1 than 6-keto-PGE_1. The enzyme responsible for 6-keto-PGE_1 inactivation in cytosolic supernatants is distinct from prostaglandin 15-hydroxydehydrogenase and 9-keto reductase, is not inhibitable by sulphasalazine-like drugs and its activity is recoverable after precipitation by ammonium sulphate. We conclude that 6-keto-PGE_1 can be inactivated by enzymes with wide tissue distribution, but further studies are needed for identification of these novel enzymes and the products formed as well as to assess their significance in the intact animal.

6-Keto prostaglandin E_1 (6-keto-PGE_1) has attracted recent interest because of its formation *in vitro* from prostacyclin (PGI_2) and 6-keto prostaglandin $F_{1\alpha}$ in human platelets [1–3] and in rabbit liver [4] and kidney [5]. In the case of human platelets, the conversion is effected by a NAD^+ -dependent 9-hydroxyprostaglandin dehydrogenase which has recently been isolated [1]. 6-Keto-PGE_1 also occurs as one of several transient products of prostacyclin metabolism in the intact dog [6], and has been detected in human plasma by a gas-chromatographic–mass-spectrometric assay at levels of 9–23 pg/ml [7], i.e. at concentrations considerably higher than those prevailing for the stable prostacyclin hydrolysis product, 6-keto prostaglandin $F_{1\alpha}$ [8], but nevertheless below biological threshold. Taken together, these observations suggest that 6-keto-PGE_1 might be a physiologically significant endogenous metabolite of prostacyclin.

Furthermore, 6-keto-PGE_1 shares many of the biological properties of prostacyclin and, importantly, is chemically stable under physiological conditions. Briefly summarised, it inhibits platelet aggregation [4, 9–11] and is a vasodilator, causing overall reductions in blood pressure and decreased resistance in many vascular beds [12–18]. Like prostacyclin, it also causes bronchodilatation [19], inhibits gastric acid secretion [20], is cytoprotective [20], and stimulates renin release [21]. In isolated preparations, 6-keto-PGE_1 relaxes or contracts the superfused rabbit coeliac artery and rat stomach fundus strip preparations, respectively, [11] and contracts other isolated gastrointestinal smooth muscle preparations [19] but unlike prostacyclin, also contracts strips of bovine coronary artery [3]. In many of these tests, 6-keto-PGE_1 is at least as potent as prostacyclin.

Little is yet known about the biological inactivation of 6-keto-PGE_1, but—again like prostacyclin—it is not extensively inactivated in the rat isolated lung preparation [11] and survives passage through the lungs of anaesthetised rats [12] and cats [13]. However, PGI_2 is a good substrate for prostaglandin 15-hydroxydehydrogenase (PGDH, ref. 22), whereas 6-keto-PGE_1 was found to be very poorly oxidised [11].

In the light of these findings we have examined the metabolic inactivation of 6-keto-PGE_1 in various cytosolic organ supernatants and have attempted to define whether these processes are dependent upon the enzymatic actions of PGDH or prostaglandin 9-keto reductase (9-KR), both of which are involved in the inactivation of prostaglandins E_1 and E_2. These prostaglandins were therefore used as reference standards for the present experiments.

MATERIALS AND METHODS

Drugs. NAD^+, $NADP^+$, NADH, NADPH, glucose-6-phosphate, glucose-6-phosphate dehydrogenase, dithiothreitol and indomethacin were purchased from Sigma (London) Ltd, Poole, Dorset, U.K. Radiochemicals were obtained from Amersham International, Amersham, U.K., and Ph CK 47A (4-carboxy-3′-carboxymethyl-4′-hydroxyazobenzene) was a kind gift from Pharmacia AB, Uppsala, Sweden. Bovine lung PGDH (75 mU/ml) was purchased from BDH, Dagenham, U.K., and human placental PGDH (200 mU/ml) was generously donated by Dr. J. Jeffery, Department of

Biochemistry, University of Aberdeen, U.K. One mU is defined as that amount of enzyme which oxidises 1 nanomole prostaglandin E_1 per min at 37°, pH 7.4. Unlabelled prostaglandins were gifts from Dr. J. E. Pike, Upjohn Company, Kalamazoo, Michigan, U.S.A.

Prostaglandin inactivation in 100,000 g supernatants [23]. All organs tested were homogenised in 4 vol ice-cold 50 mM phosphate buffer pH 7.4 (containing 1 mM EDTA and cysteine) and centrifuged at 3000 *g* at 4° for 15 min. The resulting supernatants were centrifuged at 100,000 *g* for 45 min at 4°. The microsomal pellets were discarded and the 100,000 *g* cytosolic supernatants stored at −20° until required. They were used within 14 days, and loss of activity on storage and thawing is less than 20%.

Samples containing 180 μl cytosolic supernatant, 2 μl PGE_1 or 6-keto-PGE_1 (final concentration 10 μg/ml), 10 μl NAD^+ (final concentration 5 mM), and 10 μl Ph CK 47A (final concentration 50 μM) or vehicle, were incubated without shaking for 60 min at 37°. In some instances aliquots were removed at timed intervals and assayed for their ability to inhibit ADP-induced aggregation of human platelets suspended in platelet rich plasma, using a Payton 2-channel aggregometer [24]. In most experiments, the reactions were stopped with 0.2 ml absolute ethanol, acidified with 0.2 ml 1 M formic acid and the prostaglandins extracted twice into ethyl acetate.

After removing solvent and resuspending the dried extracts in 0.5 ml Krebs' solution, prostaglandins were assayed in terms of PGE_1 or 6-keto-PGE_1 on the isolated rat stomach fundus strip preparation [23].

PGE 9-keto reductase assay. Rabbit kidney cortex and duck heart 9-keto reductase activity was determined using the method of Stone and Hart [25]. Organs were homogenised in 4 vol ice-cold 0.1 M potassium phosphate buffer containing magnesium chloride (4 mM) and dithiothreitol (0.1 mM) and 100,000 *g* supernatants prepared as above. The 200 μl samples contained 100 μl supernatant, 2 μl of PGE_2 or 6-keto-PGE_1 (final concentration 10 μg/ml), and either NADPH alone (0.1 or 1.0 mM final concentration) or 0.1 mM NADPH plus a cofactor regenerating system (0.5 units glucose-6-phosphate dehydrogenase and 2 μmoles glucose-6-phosphate). The prostaglandins were extracted as above and the dried extracts resuspended in 1.0 ml distilled water and radioimmunoassayed for $PGF_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$ [26].

Ammonium sulphate fractionation. In order to obtain a less contaminated form of the metabolising enzymes than that present in the 100,000 *g* supernatants (to enable the study of cofactor requirements), organs were homogenised in ice-cold 0.1 M phosphate buffer containing 0.1 mM dithiothreitol and 100,000 *g* cytosolic supernatants were prepared. All procedures were at 4°. Solid ammonium sulphate was added with stirring to the supernatant to 30% saturation. After 30 min, the precipitate was removed by centrifugation at 10,000 *g* for 20 min. The supernatant fluid was then brought to 60% saturation and equilibrated for another 30 min. The precipitate (insoluble in 60% saturated ammonium sulphate) was collected by centrifugation (10,000 *g* 20 min), dissolved in phosphate buffer and dialysed overnight against 100 vol phosphate buffer. The dialysate was assayed for enzyme activity by incubation in the presence of NAD^+, $NADP^+$ (both 5 mM), NADH or NADPH (both 1 mM), followed by extraction and bioassay on the rat fundus strip as described above.

RESULTS

It was established that 100,000 *g* cytosolic supernatants prepared from various sources efficiently inactivated 6-keto-PGE_1 when incubated in the presence of NAD^+. Figure 1 shows results obtained using colon, kidney and liver supernatants from 3 species as well as rat lung. In this experiment PGE_1 was used as a reference standard since it is good substrate for PGDH. There are clearly large differences in the efficiency with which the various supernatants inactivate both PGE_1 and 6-keto-PGE_1; moreover there is no correlation between the apparent PGDH content (i.e. ability to inactivate PGE_1) and the extent to which 6-keto-PGE_1 is broken down (Fig. 1, inset). It is of interest to note that in all three species (guinea-pig, rat and rabbit) colon is rich in PGDH, whereas kidney activity varies (guinea-pig > rabbit > rat, cf. [23]). Rabbit and guinea-pig liver both contain PGDH-like activity, whereas it is absent from rat liver. By contrast rat liver homogenates inactivate 6-keto-PGE_1 to a similar extent as those from rabbit and guinea-pig.

These results were obtained by assaying timed extracts of the incubations against authentic standards using the isolated rat stomach fundus strip preparation. It is possible that when inactivation is extensive the method may underestimate the true extent of metabolic transformation of both PGE_1 and, especially, 6-keto-PGE_1 if the products themselves have biological activity or interfere with the assay. In the case of 6-keto-PGE_1 the products are not known, whereas those of PGE_1 (15-keto-PGE_1 and 13,14-dihydro-15-keto-PGE_1) possess little if any biological activity [27]. For this reason we validated the method by assaying samples from incubations of the three guinea-pig organs against ADP-induced aggregation of human platelets (PGE_1 and 6-keto-PGE_1 both inhibit aggregation). Table 1 shows that the two bioassays give identical results.

The agreement between the two assay methods is further illustrated in Fig. 2 for experiments on supernatants prepared from guinea-pig kidney and rabbit kidney cortex. This graph also shows that inactivation is time-dependent. The experiments of Figs. 1, 2 and Table 1 all included NAD^+ in the incubation mixtures as this is the cofactor required for type I PGDH. Figure 3 shows that NAD^+ significantly enhances the inactivation of 6-keto-PGE_1 in rat colon, guinea-pig colon and guinea-pig kidney supernatants but not in rabbit kidney cortex. However, in the latter case addition of NAD^+ did not enhance inactivation of PGE_1 either, suggesting that endogenous levels are sufficient for full enzyme activity [28].

Two possible candidate enzymes responsible for inactivating 6-keto-PGE_1 are type I NAD^+-dependent PGDH and NADPH-dependent prostaglandin

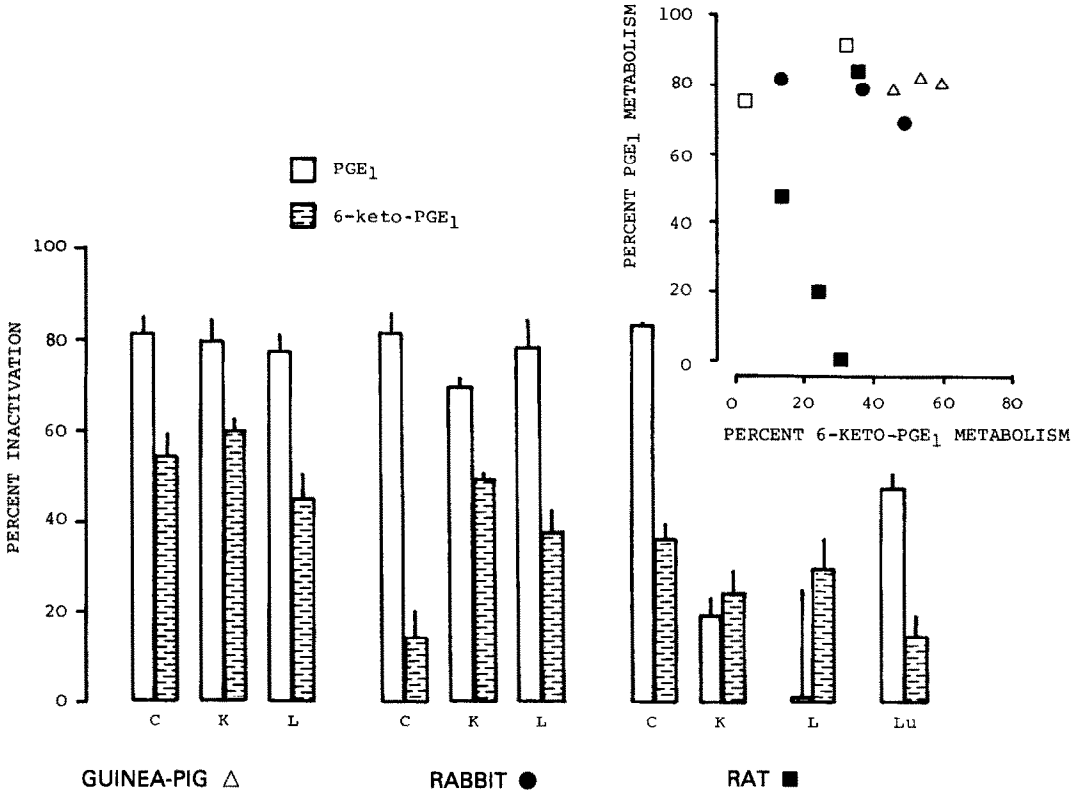

Fig. 1. Biological inactivation of PGE_1 and 6-keto PGE_1 in cytosolic supernatants of guinea-pig, rabbit and rat organs (C, colon; K, kidney; L, liver; Lu, lung). Tubes containing 10 μg/ml substrate ($\approx$ 28 μM) and 5 mM NAD^+ were incubated for 60 min (rat lung 90 min) and the extent of inactivation measured after bioassay of extracts on the rat stomach fundus strip preparation by comparison with control samples extracted at time zero. Results show mean values ± S.E.M. (vertical bars), N = 4. To convert percentage inactivation to metabolism as pmoles/mg wet weight tissue/hr multiply values by 112 (rat lung by 74). The inset shows that there is no correlation between the extent of PGE_1 and 6-keto PGE_1 inactivation in each type of supernatant ($r = 0.26$, $P > 0.1$). This graph includes 2 data points (□) for results using purified PGDH preparations (see text).

9-keto-reductase (9-KR, shown recently to be identical to the type II $NADP^+$-dependent PGDH, ref. [29]). However, it was found that 6-keto-PGE_1 was a poor substrate for partially purified bovine lung PGDH (inactivation of 32.0 ± 8.0% compared to 91.0 ± 2.0% for PGE_1 under identical conditions, 90 min incubations containing 5 mM NAD^+ and 75 μU enzyme in 0.2 ml, N = 4). Similar results were obtained using 100 μU purified human placental NAD^+ dependent PGDH (2.7 ± 8.9% inactivation of 6-keto-PGE_1 compared to 75.0 ± 4.0% for PGE_1, N = 4).

Further evidence that PGDH activity cannot explain the biological inactivation of 6-keto-PGE_1 observed in these experiments on supernatants was obtained using the sulphasalazine analogue Ph CK 47A. This substance is a powerful inhibitor of PGDH [30] with an $ID_{50} \leq 0.5$ μM on the two purified enzyme preparations described in the preceding paragraph. Figure 4 shows that Ph CK 47A at 50 μM significantly inhibits inactivation of prostaglandins E_1, $F_{2\alpha}$ and 6-keto-$F_{1\alpha}$ (measured by bioassay or radioimmunoassay techniques) but did not affect breakdown of 6-keto-PGE_1 by the guinea-pig kidney supernatant.

Table 1. Comparison of PGE_1 and 6-keto PGE_1 inactivation in supernatants from guinea-pig organs using two bioassay methods

	Inactivation of PGE_1 (%)		Inactivation of 6-keto PGE_1 (%)	
	Fundus strip	Platelets	Fundus strip	Platelets
Kidney	96.0 ± 1.0	96.5 ± 3.0	53.8 ± 5.0	59.0 ± 3.0
Colon	97.0 ± 1.0	96.4 ± 2.0	80.0 ± 3.0	84.0 ± 3.0
Liver	97.0 ± 1.5	97.5 ± 1.3	80.0 ± 2.5	80.0 ± 3.0

Incubations contained 5 mM NAD^+ and 10 μg/ml substrate, 60 min at 37°; results show mean values ± S.E.M., N = 8.

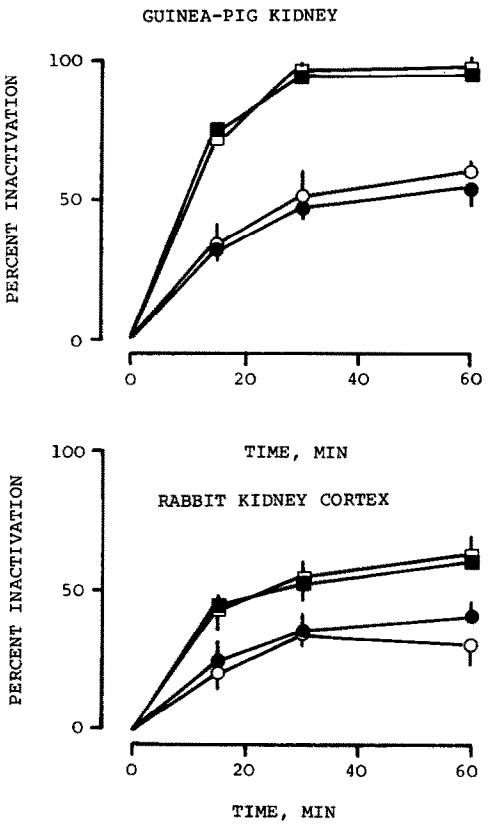

Fig. 2. Time course of biological inactivation of PGE_1 (squares) and 6-keto PGE_1 (circles) by 100,000 *g* supernatants prepared from rabbit kidney cortex (upper graph) and guinea-pig kidney (lower graph) as assayed by bioassay on human platelets (open symbols) or the isolated rat stomach strip preparation (solid symbols). Conditions as in Fig. 1, results show mean ± S.E.M., N = 4.

Similar conclusions were obtained in further experiments on guinea-pig colon, rat colon and rabbit kidney cortex supernatants (Table 2): 50 μM sulphasalazine analogue strongly inhibited the inactivation of PGE_1 (although the effect was much less marked on rabbit kidney cortex), but did not inhibit inactivation of 6-keto-PGE_1. At the same dose the analogue completely prevented the inactivation of PGE_1 by the purified placental PGDH.

Duck heart and rabbit kidney cortex 100,000 *g* supernatants were selected as test systems for assay of 9-keto reductase activity because the avian heart and rabbit kidney are known sources of this enzyme [25, 31]. It converts prostaglandin E_2 to $PGF_{2\alpha}$ [32] and would be expected to generate 6-keto-prostaglandin $F_{1\alpha}$ from 6-keto-PGE_1. In these experiments the putative products were measured by specific radioimmunoassays performed on incubation extracts. With the addition of increasing amounts of NADPH or, better, addition of both NADPH and an enzymatic regenerating system, there was extensive conversion of PGE_2 to $PGF_{2\alpha}$ by both duck heart and rabbit kidney cortex supernatants (Table 3). However, under these optimal conditions there was negligible conversion of 6-keto-PGE_1 to 6-keto-$PGF_{1\alpha}$, although some of the small increases achieved statistical significance (Table 3). Because 6-keto-PGE_1 possesses significant cross-reactivity with the antibody used for the 6-keto-$PGF_{1\alpha}$ assay (7.2 ± 1.9%) it was verified in separate experiments that the method could clearly identify small proportions of 6-keto-$PGF_{1\alpha}$ in mixtures containing excess quantities of 6-keto-PGE_1. The sulphasalazine analogue Ph CK 47A did not inhibit conversion of PGE_2 to $PGF_{2\alpha}$ by 9-KR (in fact in rabbit kidney cortex conversion was enhanced by 44.0 ± 14.5%, presumably because of inhibition of the competing PGDH pathway), and had no effect on the 6-keto-PGE_1 results. The ability of the duck heart and rabbit

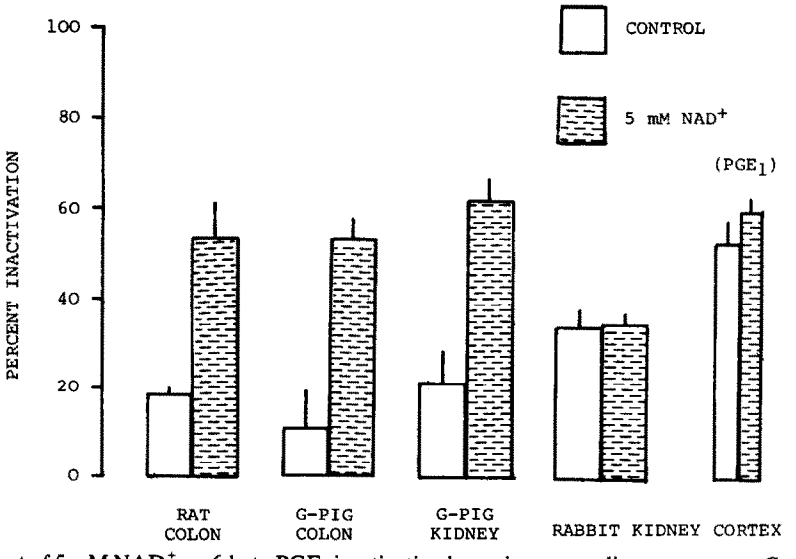

Fig. 3. Effect of 5 mM NAD^+ on 6-keto PGE_1 inactivation by various cytosolic supernatants. Conditions as in Fig. 1. PGE_1 was also tested in the rabbit kidney cortex experiments. Results show inactivation as mean values ± S.E.M., N = 4.

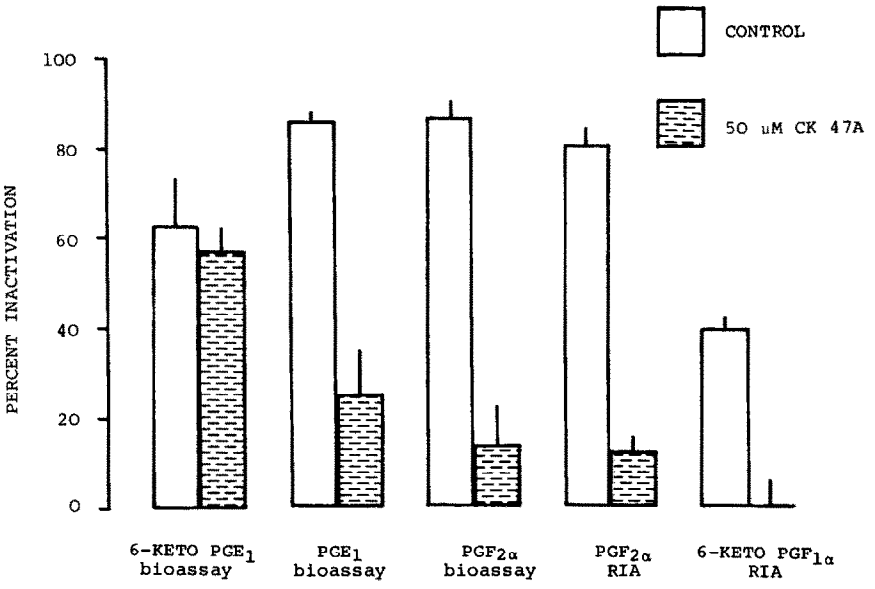

Fig. 4. Inactivation of four prostaglandins by 100,000 *g* supernatant from guinea-pig kidney (open columns) and its inhibition by 50 μM sulphasalazine analogue Ph CK 47A (stippled columns). Substrates were 10 μg/ml ($\approx$ 28 μM), incubated for 20 min and the extent of inactivation was measured by smooth muscle bioassay or radioimmunoassay of extracts as indicated. Results show mean values and bars represent S.E.M., N = 4.

Table 2. Sulphasalazine analogue Ph CK 47A inhibits PGE$_1$ but not 6-keto PGE$_1$ inactivation in cytosolic supernatants

Source of supernatant	Inhibition by 50 μM Ph CK 47A (%) PGE$_1$ inactivation	6-Keto PGE$_1$ inactivation
Guinea-pig colon	73.1 ± 11.6***	25.8 ± 13.8
Rat colon	82.4 ± 26.8*	10.3 ± 13.8
Rabbit kidney cortex	39.3 ± 8.9**	11.6 ± 14.5

Incubations contained 10 μg/ml substrate and 5 mM NAD^+ and inactivation (60 min, 37°) was measured by assay of extracts on the isolated rat fundus strip preparation. Results show inhibition in drug-treated samples compared to controls (mean ± S.E.M., N = 4) and asterisks indicate significances of differences with respect to drug-free controls by Student's unpaired *t*-test.

* $P < 0.05$, ** $P < 0.01$, ***$P < 0.002$.

Table 3. Prostaglandin 9-ketoreductase activity in rabbit kidney cortex and duck heart supernatants using PGE_2 and 6-keto PGE_1 as substrates

Experimental condition	Percent conversion to *F*-series prostaglandin: Rabbit kidney cortex PGE_2	Rabbit kidney cortex 6-Keto PGE_1	Duck heart PGE_2	Duck heart 6-Keto PGE_1
Zero time control	0.5 ± 0.1	3.2 ± 0.2	3.7 ± 0.4	1.9 ± 0.3
No cofactor	1.2 ± 0.2	2.4 ± 0.1	0.9 ± 2.8	1.6 ± 0.2
0.1 mM NADPH	3.7 ± 0.6**	2.4 ± 0.2	20.4 ± 2.8**	3.2 ± 0.3**
1.0 mM NADPH	55.7 ± 21.9*	4.7 ± 0.2***	26.8 ± 4.0***	3.6 ± 0.5**
0.1 mM NADPH + regenerating system	74.7 ± 11.3***	8.3 ± 0.9***	74.7 ± 2.3***	3.2 ± 0.2***

Incubations contained 10 μg/ml substrate for 30 min (rabbit kidney cortex) or 60 min (duck heart) at 37°. Results show mean values ± S.E.M., N = 4, and were obtained by RIA of extracts. Asterisks indicate significances of differences with respect to 'no cofactor' control by Student's unpaired *t*-test.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.002$.

Table 4. Effect of different cofactors on the biological inactivation of 6-keto PGE_1 by precipitated and dialysed guinea-pig supernatant enzymes

Source of supernatant	No cofactor	Percent inactivation NAD^+	$NADP^+$	NADH	NADPH
Kidney	36.6 ± 12.0	77.2 ± 3.5**	57.8 ± 5.8	1.5 ± 5.3*	17.2 ± 3.2
Colon	0 ± 2.0	35.4 ± 6.1***	15.5 ± 4.8*	3.8 ± 4.5	0 ± 11.7
Liver	14.2 ± 6.0	49.8 ± 2.4***	15.7 ± 5.1	19.6 ± 4.9	18.3 ± 4.9

Substrate concentration was 10 μg/ml, incubations 60 min at 37° and assayed on rat stomach strip preparation. Values show inactivation, mean ± S.E.M., N = 4, relative to control taken at time zero. Cofactors were added at 5 mM (NAD^+, $NADP^+$) or 1 mM (NADH, NADPH). The statistical significance of differences of cofactor-treated incubations relative to controls lacking cofactors were analysed by Student's *t*-test.

* $P < 0.05$, ** $P < 0.02$, *** $P < 0.002$.

kidney cortex supernatants to metabolise 6-keto-PGE_1 was checked by bioassay: under the conditions shown in Table 3, the rabbit kidney preparation was active (inactivation at 30 min was 29–46%), whereas duck heart was essentially inactive (< 15% at 60 min). The latter preparation was not tested using either NAD^+ as cofactor with 6-keto-PGE_1 or PGE_1 or PGE_2 as substrate for bioassay determination, so the presence of an NAD^+-dependent 6-keto-PGE_1 metabolising enzyme or PGDH cannot be excluded.

The cofactor requirements for 6-keto-PGE_1 metabolising activity were tested in further experiments by precipitating the enzyme(s) with ammonium sulphate and subjecting the redissolved preparation to extensive dialysis. This was done for guinea-pig kidney, colon and liver (Table 4). The results show that addition of NAD^+ considerably enhanced 6-keto-PGE_1 inactivation as determined by bioassay. $NADP^+$ at the same concentration had a smaller effect except in liver. NADH and NADPH were not active. Identical experiments were performed using PGE_1 as substrate as a positive control (data not shown); the results were similar in that NAD^+ was the preferred cofactor and the reduced forms were inactive or inhibitory.

In contrast to the ability of various cytosolic supernatants to inactivate 6-keto-PGE_1, human red blood cells were inactive. After preparing platelet rich plasma from citrated human blood, the packed red cells from the 200 *g* centrifugation were incubated with 5 mM NAD^+ and 10 μg/ml 6-keto-PGE_1 and timed extracts prepared for bioassay as spasmogens on the rat stomach strip and as inhibitor of ADP-induced platelet aggregation. Over 60 min there was no decrease in biological activity according to either method.

DISCUSSION

Our experiments provide a detailed survey of the capacity of cytosolic supernatants to inactivate 6-keto-PGE_1, a biologically active and chemically stable metabolite of prostacyclin. Inactivation of 6-keto-PGE_1 was time-dependent and occurred in supernatants prepared from the colon, kidney and liver of guinea-pig, rat and rabbit, although there was considerable quantitative variation (Fig. 1). Clearly it would be desirable to extend the range of species and organs tested so as to validate the generality of these findings. However, we have also found that other organs including different parts of the gastrointestinal tract, including human colonic and stomach mucosa, inactivate 6-keto-PGE_1 by a time- and NAD^+-dependent enzymatic mechanism which is thermolabile, i.e. abolished by 15 min pre-incubation at 55° (C. N. Berry and J. R. S. Hoult, unpublished experiments).

Three questions follow from these studies: What is the enzyme responsible? What metabolite is formed? Does this occur *in vivo*?

Although 6-keto-PGE_1 inactivation *in vitro* appears to be NAD^+-dependent (Fig. 3, Table 4), a major contribution from PGDH (NAD^+-dependent prostaglandin 15-hydroxydehydrogenase, EC 1.1.1.141) can be excluded for several reasons. Firstly, a highly purified human placental PGDH preparation failed to inactivate 6-keto-PGE_1 under conditions favourable for PGE_1 breakdown; likewise semi-purified bovine lung PGDH only slightly inactivated 6-keto-PGE_1 (and this itself could conceivably have been due to contamination by other enzymes). Moreover, in previous spectrophotofluorometric experiments we found that 6-keto-PGE_1 is oxidised by placental PGDH at only 9% of the rate of PGE_1 [11]. Secondly, Fig. 1 also shows clearly that there is no correlation in various systems between cytosolic PGE_1 inactivation—presumed to reflect PGDH activity—and 6-keto-PGE_1 breakdown. Finally, the sulphasalazine analogue Ph CK 47A failed to inhibit 6-keto-PGE_1 inactivation in all test systems that we have so far examined (Fig. 4, Table 2), whereas as predicted the PGDH-dependent inactivation of PGE_1, $PGF_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$ was markedly inhibited. This analogue was selected because it is the most potent of a large series of novel homosalazine analogues which inhibit PGDH [30, 33]; the present results also show that Ph CK 47A does not inhibit prostaglandin 9-keto reductase.

Our experiments also ruled out any role for prostaglandin 9-keto reductase as the enzyme responsible for 6-keto-PGE_1 degradation (the observed NAD^+-dependency would in any case make this unlikely as 9-ketoreductase is NADPH-dependent, refs. [25, 31, 32] and Table 3). In brief, 6-keto-$PGF_{1\alpha}$ was not detectable in incubations in which 6-keto-PGE_1 was demonstrably inactivated (rabbit kidney cortex, Table 3) and the duck heart preparation did not inactivate 6-keto-PGE_1 or form 6-keto-$PGF_{1\alpha}$,

despite very substantial conversion of PGE_2 to $PGF_{2\alpha}$ (Table 3).

There remain two general possibilities: either the enzyme responsible represents a novel 6-keto-PGE_1-specific metabolising enzyme which is widely distributed, or 6-keto-PGE_1 degradation is catalysed by enzymes (? dehydrogenases) which fulfil other catalytic functions and which in a non-specific manner will accept this prostaglandin as substrate. Clearly, enzyme purification is needed to resolve these possibilities.

As yet we do not know what are the products of 6-keto-PGE_1 inactivation *in vitro*, except that they have little rat stomach strip spasmogenic and platelet anti-aggregatory activity. The delineation of these metabolic pathways would clearly be greatly facilitated by the availability of radiolabelled 6-keto-PGE_1 as the alternative strategy (GC/MS separation and identification) is not available to us. A reasonable hypothesis based on the present experiments showing NAD^+-dependency is that 6-keto-PGE_1 is inactivated by an oxidative process occurring at the 11-hydroxyl position rather than at C-15. There is a precedent in that Roberts *et al.* [34] have shown in guinea-pig liver homogenates that thromboxane B_2 is efficiently dehydrogenated at the C-11 hemiacetal alcohol group by a soluble NAD^+-dependent enzyme. This process also occurs in humans [34]. They did not test other organ supernatants, but it is notable in our experiments (Tables 1 and 4) that guinea-pig liver efficiently inactivates 6-keto-PGE_1 and that this is strictly NAD^+-dependent (Table 4). However, in drawing this analogy it should be noted that the 11-hydroxyl groups of 6-keto-PGE_1 (secondary alcohol with beta-keto function) and thromboxane B_2 (hemiacetal alcohol) differ considerably in their chemical properties.

The final question concerns whether these processes occur *in vivo*. Pulmonary degradation of 6-keto-PGE_1 has been measured directly in anaesthetised rats and cats [12, 13] and in the isolated perfused rat lung [11] and is not extensive. Our data on rat lung homogenate (Fig. 1) suggests that in this species poor pulmonary degradation of 6-keto-PGE_1 is primarily due to lack of appropriate enzyme (whereas prostacyclin appears to be resistant to inactivation in the lung because it is not taken up by the cellular carrier mechanism [35, 36]. In contrast to this, in anaesthetised dogs [18], 6-keto-PGE_1 is quite extensively inactivated after infusion through the kidney ($66 \pm 5\%$) and liver ($51 \pm 14\%$), but less so in the lung ($40 \pm 13\%$). Again this is compatible with our data which show that kidney and liver of the three species tested contain 6-keto-PGE_1 degrading activity.

Therefore, at the present time it seems reasonable to suppose that *in vivo* 6-keto-PGE_1 is vulnerable to metabolism, especially since several other studies [12–15, 19, 37, 38] showing pharmacological responses to 6-keto-PGE_1 *in vivo* do not contain any evidence or comment that the actions of this compound are any more prolonged than those of reference prostaglandins known to be subjected to rapid enzymatic inactivation *in vivo*.

Indeed, van Dam *et al.* [15] suggest that "6-keto-PGE_1 may be inactivated in the systemic circulation in a manner similar to prostacyclin", i.e. in liver and peripheral vascular beds. Thus in conclusion we suggest that like other biologically relevant prostaglandins 6-keto-PGE_1 is subjected to rapid metabolic inactivation by cytosolic enzymes akin to those whose activity is demonstrated in this paper.

Acknowledgements—C. N. B. and R. J. G. were supported by the British Heart Foundation and Medical Research Council, respectively. We thank Dr J. Jeffery (University of Aberdeen), Dr J. E. Pike (Upjohn Company, U.S.A.) and Dr H. Agback (Pharmacia AB, Sweden) for gifts of PGDH, prostaglandins and sulphasalazine analogues.

REFERENCES

1. P. Y-K. Wong, W. H. Lee, P. H-W. Chao, R. F. Reiss and J. C. McGiff, *J. biol. Chem.* **255**, 9021 (1980).
2. J. R. S. Hoult, F. J. Lofts and P. K. Moore, *Br. J. Pharmac.* **73**, 218P (1981).
3. M. F. Gimeno, L. Sterin-Borda, E. S. Borda, M. A. Lazzari and A. L. Gimeno, *Prostaglandins* **19**, 907 (1980).
4. P. Y-K. Wong, K. U. Malik, D. M. Desiderio, J. C. McGiff and F. F. Sun, *Biochem. biophys. Res. Commun.* **93**, 486 (1980).
5. R. J. Griffiths and P. K. Moore, *Br. J. Pharmac.* **79**, 149 (1983).
6. B. M. Taylor, R. J. Shebuski and F. F. Sun, *J. Pharmac. exp. ther.* **224**, 692 (1983).
7. E. K. Jackson and R. P. Goodman, *New Engl. J. Med.* **305**, 287 (1981).
8. I. A. Blair, S. E. Barrow, K. A. Waddell, P. J. Lewis and C. T. Dollery, *Prostaglandins* **23**, 579 (1982).
9. P. Y-K. Wong, J. C. McGiff, F. F. Sun and W. H. Lee, *Eur. J. Pharmac.* **60**, 245 (1979).
10. O. V. Miller, J. W. Aiken, R. J. Shebuski and R. R. Gorman, *Prostaglandins* **20**, 391 (1980).
11. C. N. Berry and J. R. S. Hoult, *Pharmacology* **26**, 324 (1983).
12. C. P. Quilley, P. Y-K. Wong and J. C. McGiff, *Eur. J. Pharmac.* **57**, 273 (1979).
13. A. L. Hyman and P. J. Kadowitz, *J. Pharmac. exp. ther.* **213**, 468 (1980).
14. M. L. Tod and S. Cassin, *Proc. Soc. exp. Biol. Med.* **166**, 148 (1981).
15. J. van Dam, T. M. Fitzpatrick, L. S. Friedman, P. W. Ramwell, J. C. Rose and P. A. Kot, *Proc. Soc. exp. Biol. Med.* **166**, 76 (1981).
16. P. Nandiwada, A. L. Hyman, L. P. Feigen and P. J. Kadowitz, *Archs int. Pharmacodyn. Ther.* **245**, 118 (1980).
17. H. L. Lippton, B. M. Chapnick, A. L, Hyman and P. J. Kadowitz, *Prostaglandins* **19**, 299 (1980).
18. S. Tsunoda, E. K. Jackson, R. A. Branch and J. F. Gerkens, *Eur. J. Pharmac.* **77**, 147 (1982).
19. E. W. Spannhake, J. L. Levin, A. L. Hyman and P. J. Kadowitz, *Prostaglandins* **21**, 267 (1981).
20. B. J. R. Whittle, S. Moncada and J. R. Vane, in *Medicinal Chemistry Advances* (Eds. F. G. de las Heras and S. Vega), p. 141. Pergamon Press, Oxford (1981)
21. E. K. Jackson, W. A. Herzer, J. B. Zimmerman, R. A. Branch, J. A. Oates and J. F. Gerkens, *J. Pharmac. exp. ther.* **216**, 24 (1981).
22. J. C. McGuire and F. F. Sun, *Archs. Biochem. biophys.* **189**, 92 (1978).
23. J. R. S. Hoult and P. K. Moore, *Br. J. Pharmac.* **61**, 615 (1977).
24. F. J. Lofts and P. K. Moore, *Clin. Sci.* **64**, 63 (1983).
25. K. J. Stone and M. Hart, *Prostaglandins* **10**, 273 (1975).

26. J. R. S. Hoult and H. Page, *Lancet* **ii**, 255 (1981).
27. D. J. Crutchley and P. J. Piper, *Br. J. Pharmac.* **54**, 397 (1975).
28. P. K. Moore and J. R. S. Hoult, *Biochim. biophys. Acta* **528**, 276 (1978).
29. D. G-B. Chang, M. Sun and H-H. Tai, *Biochem. biophys. Res. Commun.* **99**, 745 (1981).
30. C. N. Berry, J. R. S. Hoult, S. H. Peers and H. Agback, *Biochem. Pharmac.* **32**, 2863 (1983).
31. S-C. Lee and L. Levine, *J. biol. Chem.* **250**, 4549 (1975).
32. C. A. Leslie and L. Levine, *Biochem. biophys. Res. Commun.* **53**, 717 (1973).
33. C. N. Berry, J. R. S. Hoult, J. Jeffery, O. T. Mak and S. H. Peers, *Br. J. Pharmac.* **75**, 7P (1982).
34. L. J. Roberts II, B. J. Sweetman and J. A. Oates, *J. biol. Chem.* **256**, 8384 (1981).
35. J. M. Armstrong, N. Lattimer, S. Moncada and J. R. Vane, *Br. J. Pharmac.* **62**, 125 (1978).
36. C. Robinson and J. R. S. Hoult, *Biochem. Pharmac.* **31**, 633 (1982).
37. L. P. Feigen, B. M. Chapnick, A. L. Hyman, L. King, B. Marascalco and P. J. Kadowitz, *J. Pharmac. exp. ther.* **214**, 528 (1980).
38. E. K. Jackson, H. T. Heidemann, R. A. Branch and J. F. Gerkens, *Circ. Res.* **51**, 67 (1981).