EVIDENCE FOR THE PRESENCE OF A PROSTAGLANDIN E_2-9-KETO REDUCTASE IN RAT ORGANS*

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Summary - Antibodies directed toward $PGF_{2\alpha}$ which cross react with PGE_2 only slightly were used to detect conversion of PGE_2 to $PGF_{2\alpha}$ by homogenates of several rat tissues. This conversion by rat heart homogenates was demonstrated to be reversible, lost after trypsin digestion, and inhibited by several sulfhydryl blocking agents. The activity of the rat heart homogenate was precipitable by ammonium sulphate, was not dialyzable, and was 50% destroyed when the homogenate was incubated at 50° for 5 min. In the rat, the heart had the highest activity, followed by the kidney, brain, and liver. Negligible activity was found in smooth muscle, skeletal muscle, and whole blood of rat.

Of all the known prostaglandins (PGs), the PGEs and PGFs have been most frequently implicated in the diverse and often very potent effects of these substances in many physiological systems (1-3). For example, contraction of circular muscle is increased by PGF compounds and inhibited by PGE compounds in isolated human and guinea pig ileum and colon (4). PGE_1 and PGE_2 in certain blood vessels of most species are powerful vasodilators and in all species reported so far lower systemic arterial blood pressure. On the contrary, PGFs are vasoconstrictors and in some species $PGF_{2\alpha}$ augments arterial blood pressure (1-3). These opposing effects in some systems together with the structural similarity between PGE and PGF compounds make an enzyme that might convert one to the other of potential interest

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in the regulation of these systems.

Radioimmunoassays for the PGs whereby a particular PG can be identified without prior purification and in the presence of excess of other materials, including other PGs, have been developed (5). An antiserum to $PGF_{2\alpha}$ has been produced with which nanogram levels of $PGF_{2\alpha}$ can be detected in the presence of excess PGE_2. Similarly, using an antiserum to PGB_1, picogram levels of PGB_2 can be detected after alkali treatment even in the presence of a relatively high concentration of $PGF_{2\alpha}$ (5).

We have used the $PGF_{2\alpha}$ antiserum to detect conversion of PGE_2 to $PGF_{2\alpha}$ in homogenates of organs of rat and other species and the PGB_1 antiserum to detect conversion of $PGF_{2\alpha}$ to PGE_2 in rat heart homogeantes.

Materials and Methods - Tissues were homogenized in 3 volumes of phosphate buffer of the following composition: 0.02 M KH_2PO_4; 0.072 M K_2HPO_4; 0.028 M nicotinamide; 0.004 M $MgCl_2$, pH 7.3 (6). The homogenate was centrifuged at 10,000 g for 15 min and the resulting supernatant fluid centrifuged at 100,000 g for 60 min. This second supernatant fraction was used in these studies.

Radioimmunoassays were carried out according to Levine et al. (5). Protein was determined by the method of Lowry (7). The PGs used were obtained from Ono Pharmaceutical Co., Ltd., Osaka, Japan. The same results were obtained when PGE_2, obtained from Dr. J. Pike of the Upjohn Company, Kalamazoo, Michigan was used.

Results and Discussion - The specificity of the $PGF_{2\alpha}$ antiserum used in this study is shown in Fig. 1. The 9-hydroxyl group is immunodominant and PGE_2 with a keto group in the 9-position inhibits much less effectively. Theoretically it should be possible to detect even less than 2% conversion of PGE_2

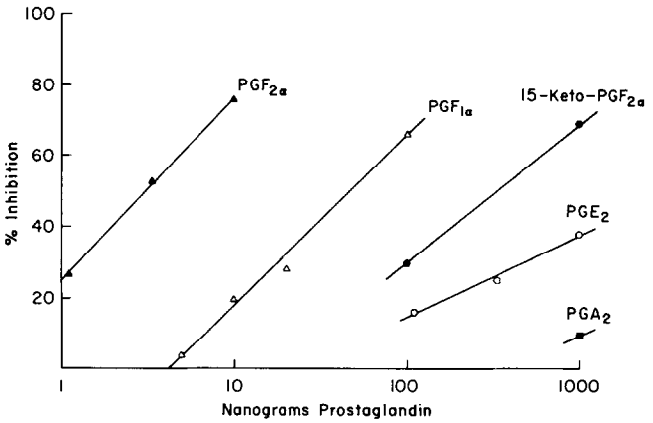
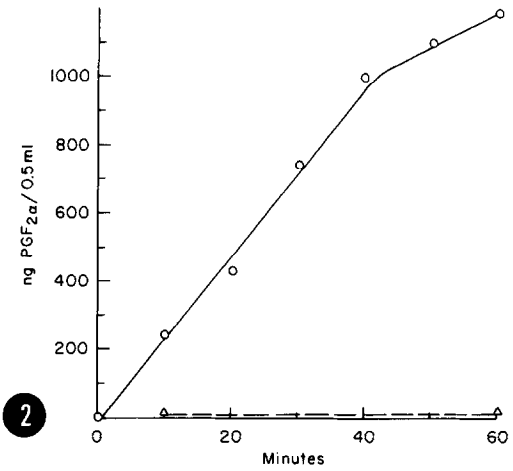


Fig. 1. Inhibition of the binding of [^{3}H]$PGF_{2\alpha}$ to anti-$PGF_{2\alpha}$ by (▲) $PGF_{2\alpha}$, (△) $PGF_{1\alpha}$, (●) 15-keto-$PGF_{2\alpha}$, (○) PGE_2, and (■) PGA_2.

to $PGF_{2\alpha}$. With rat kidney (and other homogenates showing this conversion) as the time of incubation with PGE_2 and NADH increased, so did the inhibition of [^{3}H]$PGF_{2\alpha}$ anti-$PGF_{2\alpha}$ binding, indicating the accumulation of $PGF_{2\alpha}$. In the absence of NADH the inhibition still increased with time but more slowly. It was assumed that this slower increase was due to the presence of an endogenous source of reducing agent. In the absence of substrate and when the enzyme preparation was boiled, there was no increase in inhibition. A typical experiment using a rat heart preparation shows that about 10% of the the total PGE_2 added was converted to $PGF_{2\alpha}$ with little increase after 1 hr of incubation (Fig. 2). The simplest explanation for conversion of PGE_2 to $PGF_{2\alpha}$ is a one-step reduction of the keto group of PGE_2, although conversion of PGE_2 to other PGFs, such as $PGF_{1\alpha}$, cannot be excluded by radioimmunoassay with our $PGF_{2\alpha}$ antiserum. Of course, further metabolism of the generated $PGF_{2\alpha}$ to 15-keto-$PGF_{2\alpha}$ could theoretically be partly contributing to the inhibition (Fig. 1).



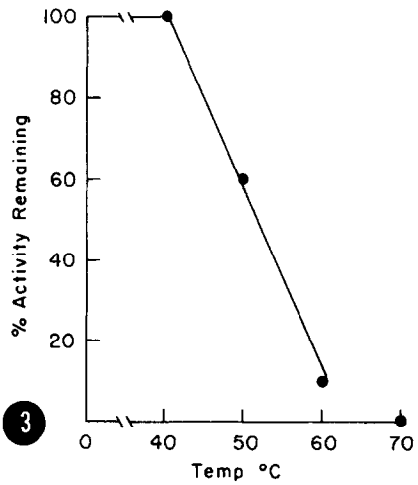


Fig. 2. The production of $PGF_{2\alpha}$ with time, on incubating rat heart homogenate with NADH with (0) and without (Δ) PGE_2. 0.5 ml of homogenate was incubated at 37° with 0.4 μmoles NADH and 10 μg PGE_2. At various times 0.1 ml samples were removed and diluted 1:10 in cold tris-saline buffer, pH 7.4, containing 0.1% gelatin. These diluted samples were placed in boiling water for 3 min and centrifuged to spin down denatured protein; 0.1 ml of this supernatant fluid was assayed for inhibition of $[^3H]PGF_{2\alpha}$ anti-$PGF_{2\alpha}$ binding. Preliminary experiments established that boiling the diluted sample prior to the assay did not affect the serologic activity of those PGs estimated in the reaction mixture but eliminated interference of immune binding by excess protein in the homogenate. To test for the serologic activity of substrate alone and/or its stability during the incubation an equal volume of buffer replaced the tissue preparation in the presence of the same amount of PGE_2 and NADH. Some inhibition (about 25%) was observed in these controls which did not change with time of incubation and which was due to substrate. The amount of $PGF_{2\alpha}$ was calculated from the inhibtion of the binding obtained in the presence of buffer, substrate, and NADH.

Fig. 3. The conversion of PGE_2 to $PGF_{2\alpha}$ by rat heart homogenate as a function of temperature. The homogenate was kept at the given temperature for 5 min and immediately chilled to 0° before addition of NADH and PGE_2. After 60 min, the mixture was assayed for $PGF_{2\alpha}$, as described in the legend of Fig. 2. One hundred per cent activity was defined as the amount of product obtained after 60 min incubation from a homogenate not heated before addition of substrate.

When rat heart homogenate was incubated with various concentrations of $PGF_{2\alpha}$ and NAD, small amounts of PGB_2 were detected after alkali treatment, indicating that the conversion of PGE_2 to $PGF_{2\alpha}$ was reversible (Table I). In the absence of substrate or in the absence of NAD there was no increase in the amount of PGE_2 formed between 1 and 60 min (Table I).

Table I. The Production of PGE_2 on Incubating Rat Heart Homogenate with $PGF_{2\alpha}$

$PGF_{2\alpha}$ added (μg)	NAD added (μmoles)	Total PGE_2 detected (ng)	
		1 min	60 min
100	2	15	175
100	-	9	10
50	2	10	125
10	2	5	25
--	2	0	0

The $PGF_{2\alpha}$ and/or NAD were added to 0.5 ml of heart preparation. After incubation, 0.1 ml samples were diluted 1:10 in cold tris-saline buffer, pH 7.4, containing 0.1% gelatin. The diluted sample was brought to pH 12.5 with alkali and boiled for 5 min. This alkaline treatment converts almost quantitatively only PGs of the E and A type to PGs of the B series. After neutralization, 0.1 ml was assayed for PGB_2 by inhibition of the binding of [^{3}H]PGB_1 to the PGB_1 antiserum.

Treatment with trypsin completely prevented the conversion of PGE_2 to $PGF_{2\alpha}$ in rat heart homogenate. The enzyme preparation was incubated with 1% (by weight of total protein) of trypsin for 1 hr at 37° before addition of NADH and substrate. Trypsin alone did not interfere with the radioimmunoassay. The activity in the rat heart homogenate was precipitated by ammonium sulphate and was not dialyzable. When the rat heart preparation was heated to 60° for 5 min, all activity was completely destroyed (Fig. 3). Even after incubation at 50° for 5 min half the activity was lost. The activity in rat heart homogenates was inhibited by sulfhydryl blocking agents (Table II). Except for N-ethylmaleimide all inhibitors tested at 10^{-3} M effectively blocked the activity. Neither removal of $MgCl_2$ from the buffer nor its replacement by $CaCl_2$ or $CaSO_4$ affected the activity.

The relative activities of this enzyme in all rat organs

Table II. Effect of Sulfhydryl Blocking Agents on the Conversion of PGE_2 to $PGF_{2\alpha}$ by Rat Heart Homogenate

Inhibitor	Concentration (M)	Inhibition (%)
Iodoacetamide	1×10^{-3}	100
	10^{-4}	23
	10^{-5}	19
	10^{-6}	<10
Iodoacetate	4×10^{-3}	100
	1×10^{-3}	88
	10^{-4}	31
	10^{-5}	<10
p-Chloromercuri-benzoate	5×10^{-4}	96
	1×10^{-4}	56
	10^{-5}	<10
Ellman's reagent	1×10^{-3}	87
	10^{-4}	49
	10^{-5}	31
N-ethylmaleimide	1×10^{-3}	57
	10^{-4}	<10

The homogenate was preincubated at 37° for 1 hr with the inhibitor before the addition of NADH and PGE_2. After 60 min, the mixture was assayed for $PGF_{2\alpha}$, as described in the legend of Fig. 1. Inhibition was calculated as a percentage of the amount of $PGF_{2\alpha}$ produced with preincubation without inhibitor. The inhibitor alone did not interfere with the radioimmunoassay.

so far tested are summarized in Table III. The heart has the most activity. Preliminary experiments have shown activity to be present in heart homogenates of chicken, rabbit, cat, beef, and guinea pig and in rabbit kidney and guinea pig liver homogenates. Activity was found neither in guinea pig spleen, lung, striated muscle, and kidney nor in cat kidney and beef kidney extracts.

Hamberg and Israelsson (6), after incubating tritium

Table III. Activity of PGE_2-9-Keto-Reductase in Rat Organs

Rat	Activity units/mg protein*
Heart	49 (10)
Kidney	27 (6)
Brain	22 (2)
Liver	7 (2)
Adrenals	2 (1)
Uterus	1 (2)
Whole blood	1 (2)
Lung	1 (2)
Striated muscle	0 (2)
Spleen	0 (2)
Ileum	0 (1)

* A unit of activity was arbitrarily defined as the amount of $PGF_{2\alpha}$ generated after incubation for 60 min with PGE_2 and NADH. In most tissues there was a small amount of endogenous $PGF_{2\alpha}$ present. This contribution to the total amount of $PGF_{2\alpha}$ produced at 60 min was <3% in the active tissues and was not subtracted.

Nos. in parantheses are the number of times a particular organ from a different rat was checked for activity.

labeled PGE_2 with guinea pig liver homogenate, identified by chemical techniques, as a minor portion of the recovered radioactivity, three compounds belonging to the F type of PG. However, the indications to date have been that the PGEs and PGFs are not interconvertible.

We have demonstrated such a conversion in several rat tissue homogenates with evidence that it is catalyzed by an enzyme, a PGE_2-9-keto reductase. The specificity of this enzyme and its role in regulation of PGE and PGF levels in physiological processes is presently being investigated.

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