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# On the Characterization of Prostaglandin $E_2$ 9-Keto Reductase from Aorta and Regional Differences in Its Activity (40510)

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Numerous reports (1–3) have noted the diversity in the action of E and F groups of prostaglandins (PGs) in physiological systems. Recent studies from our laboratory (4, 5) noted that while $PGE_1$ inhibited the activity of the enzyme that hydrolyzes cholesteryl esters in the aorta, $PGF_{1\alpha}$ seems to stimulate this reaction. The balance between the synthesis and hydrolysis of cholesteryl esters is very important in determining the accumulation of cholesteryl esters during atherosclerosis in the aorta (5). Therefore, it was of interest to investigate the enzyme system involved in the interconversion of E and F groups of PGs in the aorta and surprisingly very little is known (6) about this aspect. The occurrence of $PGE_2$ 9-keto reductase, an enzyme catalyzing the transformation of $PGE_2$ into $PGF_{2\alpha}$, has been reported in a variety of animal tissues (7–10). The activity of this enzyme was found to be dependent on the availability of NADPH in tissues (7–10), except for the monkey liver enzyme which preferred NADH instead of NADPH for reaction (7). In this communication we provide evidence for its occurrence in the aorta, study its properties, and found differences in its activity between normal and atherosclerosis portions of the aorta.

*Methods.* White Carneau pigeons (3 years old) were obtained from Palmetto Pigeon Plant, Sumter, SC, and kept on pigeon chow diet during the course of these experiments. The pigeons were sacrificed and the aortas were dissected out quickly. The area of the coeliac branch of the aorta (where spontaneous atherosclerotic lesions occur) (11) and the lesion free thoracic aorta were used for the studies.

[$^{14}$C]$PGE_2$ (sp act 40 mC/m*M*) was purchased from New England Nuclear, Boston, MA, and its purity was checked by thin-layer chromatography and radiochromatographic scanning (12) using a solvent system consisting of the organic phase (9) of ethyl acetate: acetic acid:isooctane:water (110:20:30:100, vol/vol/vol/vol).

The enzyme activity was assayed according to the procedure of Wong *et al.* (6) and modified to suit the requirements of the pigeon aortic enzyme. The aortas were homogenized with 0.1 *M* $KH_2PO4$ buffer pH 7.4 containing 0.1 m*M* dithiothreitol and the homogenate was sequentially centrifuged at 1000*g* for 20 min, 12,000*g* for 15 min, and then 100,000*g* for 30 min. The supernatant obtained at 100,000*g* centrifugation was used as the source of the enzyme. In selected instances, this soluble fraction was further centrifuged at 107,000*g* for 1 h and the supernatant fraction was used as the enzyme source. Preliminary studies indicated that the enzyme activity is located exclusively in the high-speed supernatant fraction and the maximum conversion occurred in 5 min. The soluble fraction of 0.5–1 ml (equivalent to 1–2 mg protein) was incubated with 50,000 dpm [$^{14}$C] $PGE_2$ (sp act 40 mC/m*M*) in K phosphate buffer pH 7.4 with $NADPH^+$ (2 m*M*) for 2 min. The reaction was terminated by the addition of 1 M citric acid to bring the solution to pH 4.0 and 10 ml of chloroform: methanol (2:1). The organic phase was collected, concentrated, and subjected to thin-layer chromatography using solvent system chloroform:methanol:acetic acid:water (90:9:1.0:0.65; vol/vol/vol/vol), using authentic $PGE_2$ and $PGF_{2\alpha}$ as markers. This solvent system clearly separates $PGE_2$, $PGF_{2\alpha}$ from both $PGA_2$ and $PGB_{2\alpha}$ (13). The identity of $PGF_{2\alpha}$ was further established by its elution from gel using chloroform: methanol (1:1) and subjecting it to chromatography using phase of ethyl acetate: acetic acid:isooctane: water (110:20:30:100, v/v/v/v) mixture (9). The area of the gel corresponding to $PGF_{2\alpha}$ was scraped and its radioactivity determined. Enzyme activity is expressed as dpm of [$^{14}$C]$PGF_{2\alpha}$ formed/mg/protein/2 min.

*Results.* Figure 1 shows the time course of



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the enzymatic reaction in the aorta. The reaction reached a maximum of 5 min and remained fairly constant thereafter. Figure 2 shows the increase in the enzyme activity with an increase in protein concentration. Table I shows the subcellular distribution of $PGE_2$ 9-keto reductase activity. As can be seen from the table, most of the enzyme activity was associated with the soluble fraction of the aorta.

Figure 3A shows the effect of adding NADPH to the reaction mixture. The enzyme activity surprisingly showed a decrease with the increase in NADPH concentration. However, when the supernatant obtained by centrifuging the original soluble fraction at 107,000*g* for 1 h was used, the addition of NADPH increased the rate of reaction (Fig. 3B). Figure 4 shows the effect of pH on this enzymatic reaction. Surprisingly, in addition to the enzyme activity with a pH at 7.4 (similar to that noted in other tissues), there was an additional enzyme activity with an optimum pH of 4. This enzyme activity was also stimulated by NADPH as shown in Table II. $PGE_2$ 9-keto reductase activities were then measured in the normal and atherosclerotic portions of the pigeon aorta both at pH 7.4 and pH 4 (Table III). It was found that the enzyme activity at pH 7.4 was decreased in the atherosclerotic lesions, while the enzyme activity at pH 4 was enhanced in the atherosclerotic aorta.

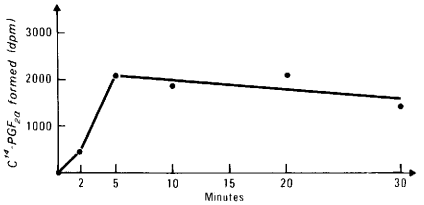


FIG. 1. Time course of the $PGE_2$ 9-keto reductase activity in pigeon aorta. Enzyme activity is expressed as dpm in [$^{14}$C]-$PGF_2$ $\alpha$ formed/1 mg protein.

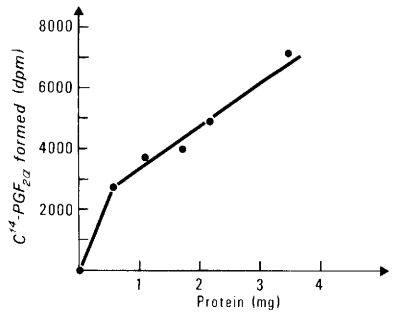


FIG. 2. Effect of protein concentration on the activity of $PGE_2$ 9-keto reductase in pigeon aorta. Conditions as described in Fig. 1. Incubation time: 5 min.

TABLE I. SUB-CELLULAR DISTRIBUTION OF AORTIC $PGE_2$-9 KETO REDUCTASE ACTIVITY.[a]

| Cell fraction | [$^{14}$C] $PGF_{2\alpha}$ formed (dpm/mg) |
|---|---|
| 1,000*g* pellet | 369 |
| 10,000*g* pellet | 475 |
| 100,000*g* pellet | 533 |
| 100,000*g* supernatant | 2665 |

[a] [$^{14}$C] $PGE_2$ (50,000 dpm) was incubated with various sub-cellular fraction for 5 min. Other conditions as described in this text.

*Discussion.* This study has characterized $PGE_2$ 9-keto reductase activity in the pigeon aorta. The subcellular distribution and cofactor requirements are very similar to the properties of the enzymes reported for other tissues (7–9). The inhibitory effect of NADPH on the enzyme reaction when added to the 100,000*g* supernatant is similar to the findings of Lee and Levine (8). This is believed to be due to the formation of endogenous inhibitors (perhaps $NAD^+$). These inhibitors could be removed by dialysis (8), or by sephadex chromatography (9). Following these purifications, addition of NADPH had a stimulatory effect on the enzyme activity. Our experiments have shown that centrifugation at 107,000*g* for 1 h also removes the inhibitors. This suggest that the inhibitor could also be either a protein (perhaps which binds $NAD^+$) which sediments at 107,000*g* centrifugation. Our preliminary studies with the dialysed supernatant fraction confirmed previous reports (8) that following dialysis $NADPH^+$ stimulated enzyme activity. A surprising finding in this report is the presence of two optimum pHs for this enzyme activity. An optimum pH of 7.4 has been found for this enzyme in other tissues (7–9). However, no reports on a $PGE_2$ 9-keto reductase activity in pH 4 have appeared thus far. Whether these activities are due to different enzymes or represent two forms of the same enzyme remains to be determined.

A comparison of the $PGE_2$ 9-keto reductase activities (at pH 4 and pH 7.4) indicate that there was a decrease in the pH 7.4 en-

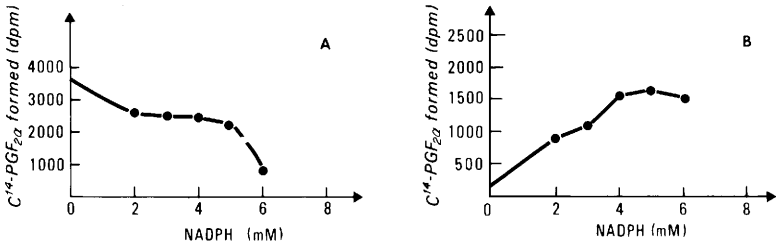


FIG. 3. Effect of NADPH on the activity of $PGE_2$ 9-keto reductase in pigeon aorta. A: Supernatant from centrifugation at 100,000*g* for 30 min; B: Supernatant from additional centrifugation at 107,000*g* for 1 hr. Other conditions as described in Fig. 1 except that in Fig. 4B 0.4 mg of protein was used instead of 1 mg protein.

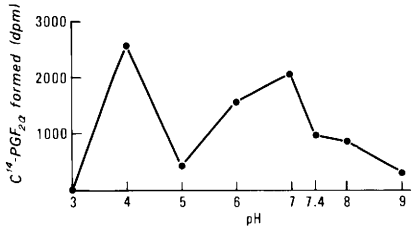


FIG. 4. Effect on pH on the activity of $PGE_2$ 9-keto reductase in pigeon aorta. Incubation conditions as described in Fig. 1

TABLE II. EFFECT OF NADPH ON $PGE_2$ 9-KETO REDUCTASE ACTIVITY (pH 4) IN AORTA.[a]

| NADPH m*M* | [$^{14}$C] $PGF_{2\alpha}$ formed (dpm) |
|---|---|
| 0 | 1700 |
| 1 | 2500 |
| 2 | 2940 |
| 3 | 3240 |
| 4 | 3450 |

[a] 107,000*g* supernatant fraction (1 mg protein) was incubated with [$^{14}$C] $PGE_2$ (50,000 dpm) for 5 min and its conversion to [$^{14}$C]-$PGE_{2\alpha}$ recorded.

TABLE III. $PGE_2$ 9-KETO REDUCTASE ACTIVITIES IN NORMAL AND ATHEROSCLEROTIC PORTIONS OF PIGEON AORTA.[a]

| Aorta | pH | Enzyme activity (Mean ± SEM) [$^{14}$C]-$PGF_{2\alpha}$ formed/mg/protein/5 min |
|---|---|---|
| Normal | 4 | 1830 ± 34[b] |
| | 7.4 | 1902 ± 19[c] |
| Atherosclerotic | 4 | 3075 ± 356[b] |
| | 7.4 | 767 ± 78[c] |

[a] Incubation conditions as described in Table II.

[b] $p < 0.05$ for differences between normal and atherosclerotic tissues.

[c] $p < 0.01$ for differences between normal and atherosclerotic tissues.

zyme activity in atherosclerotic aorta when compared to normal aorta. However, the enzyme activity at pH 4 was enhanced in the atherosclerotic aorta. If one assumes that the enzyme active at pH 7.4 (which is found in all other tissues) is the major one, then during atherosclerosis there seems to be a decrease in the conversion of $PGE_2$ into $PGF_{2\alpha}$. Further studies on (a) sequential changes in the enzyme activity during various stages of atherogenesis and (b) significance of enzyme activity at pH 4 are needed. The enzymes that are active in pH of acidic range are generally confined to lysosomes (14). Whether such is the case for $PGE_2$ 9-keto reductase (active at pH 4) in pigeon aorta remains to be seen. At this point it is interesting to note that Hassid and Levine (15) noted multiple molecular forms of chicken kidney PG-9-keto reductase as evidence by isoelectric focussing criteria. These enzyme forms also differed in their response to inhibitors. It seems likely that the PG 9-keto reductase activity noted at pH 4.0 and pH 7.4 might also represent two molecular forms of the enzyme. Further work in this aspect is currently in progress in our laboratory.

*Summary.* Evidence is presented for the presence of a prostaglandin $E_2$ 9-keto reductase activity in the soluble fraction of the pigeon aorta. The enzyme reaction was stimulated by NADPH when 107,000*g* (1 h) supernatant was used as the enzyme source. Surprisingly, the enzyme activity exhibited two pH optimums (pH 4 and 7.4, respectively), in contrast to other tissues where only an optimum pH 7.4 was found. In the atherosclerotic portion of the aorta, the enzyme activity at pH 7.4 was reduced while the activity at pH 4 was enhanced.

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