Regulation of Myocardial Prostaglandin Dehydrogenase Activity. The Role of Cyclic 3',5'-AMP and Calcium Ions (37214)

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(Introduced by E. D. Freis)

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Prostaglandins are a class of biologically active lipids widely distributed in animal tissues (1). Because of their potency and diversity of action their *in vivo* metabolism has been the subject of intense investigation. Although prostaglandins are circulated in the blood without being destroyed by its constituents, it is most likely that they are synthesized and destroyed in the same tissue where their physiological activity occurs (2). In order to achieve rapid and efficient regulation of the tissue levels of prostaglandins, enzyme systems for their biosynthesis and degradation are present in the cells of the tissues where these substances are thought to play a physiological role.

Recently, we isolated and characterized an active prostaglandin synthetase from the myocardial microsomal fraction which could synthesize prostaglandins from precursor fatty acids in the presence of glutathione and certain aromatic compounds (3). Since the early steps in the metabolic degradation of prostaglandins involve dehydrogenation at C-15 to relatively inactive keto compounds (4) we undertook the characterization of the responsible enzyme from the heart. Änggård and Samuelsson (5) first isolated prostaglandin dehydrogenase from the swine lung; the enzyme was later demonstrated in a variety of tissues (6) where, in conjunction with prostaglandin synthetase and other prostaglandin-degrading enzymes, it regulates the levels of prostaglandins within the cells.

Methods. Canine myocardium was processed for prostaglandin dehydrogenase (PGDH) according to the method of Saeed and Roy (7). Twenty grams of left ventricular tissue, freed of epicardial fat and vessels, were homogenized in a Waring blender for 2 min in a solution containing 0.05 *M* sodium phosphate (pH 7.4), 1 m*M* EDTA, 1 m*M* β-mercaptoethanol and 1.4 μM NAD and the homogenate was blended with 25 ml of the same solution for an additional 10 min. The resulting suspension was clarified by first straining through cheesecloth and then by centrifugation. A red supernatant was obtained. To this, solid ammonium sulfate was added in small amounts each time to 25% saturation. The precipitate was removed by centrifugation at 37,000*g* and discarded. Solid ammonium sulfate was then added to the supernatant to give a 65% saturation. Stirring continued during and for 1 hr after the last addition of the salt. After standing overnight at 4° the precipitate was centrifuged as before and dissolved in the homogenizing buffer. The dissolved precipitate was then dialyzed exhaustively against the same buffer followed by centrifugation at 37,000*g* to remove particulate material.

The supernatant was diluted to a protein concentration of 15 mg/ml. Acetone precooled to −15° was added slowly with efficient stirring to the diluted enzyme solution until an acetone to buffer ratio of 1:3 (v/v) was reached. After standing at 0° for 10–15 min the resulting inactive precipitate was removed by centrifugation and discarded. Acetone (−15°) was again added dropwise to the supernatant until a final acetone to buffer ratio of 1:1 (v/v) was obtained. The suspension was allowed to remain at 0° for 10 min and then centrifuged and the supernatant solution was discarded. The precipitate

was dissolved in a small volume of the homogenizing buffer and dialyzed exhaustively against the same buffer. The enzyme was stored frozen at —20° until use.

The reaction mixture for the assay of PGDH activity contained 1.4 μM (500 μl) NAD and 100 μl enzyme solution in a total volume of 2.9 ml sodium pyrophosphate–semicarbazide buffer (pH 8.6) which contained 0.074 M $Na_2P_2O_7$, 0.075 M semicarbazide and 0.22 M glycine. The reaction was started by the addition of 0.34 μM prostaglandin in 100 μl ethanol. The blank contained, except prostaglandin substrate, 100 μl ethanol and all other reagents. The formation of NADH was monitored at 340 nm with a Beckman DU-2 spectrophotometer. Protein was estimated from the ultraviolet absorption at 280 nm. Activity of the enzyme was expressed in units: 1 unit was the amount of PGDH which caused a change in optical density of 0.001/min (equiv to the oxidation of 0.16 nmoles NAD/min). Specific activity was defined in units per milligram of protein.

The effects of changes in substrate concentration on the enzymatic activity was examined by using prostaglandin concentrations varying from 10^{-10} to 10^{-4} M. The influence of pH was studied within the range of 4.0–9.5. Cyclic 3′,5′-AMP was added in a separate series of experiments in concentrations of 10^{-10} to 10^{-4} M; Ca^{2+} was added as 0.1 ml of a $CaCl_2$ solution (10^{-8} to 10^{-3} M) both in the presence and in the absence of cyclic 3′,5′-AMP (0.1 ml of a 10^{-7} M solution). The PGDH activity in the presence of *p*-chloromercuribenzoate (CMB) was used to evaluate the importance of sulfhydryl groups. *p*-Chloromercuribenzoate was purchased from Sigma and was purified in acid as described by Boyer (8). Solutions of CMB were made by dissolving the solid in 1 ml of 1 N NaOH, bringing the volume to 20 ml with potassium dimethylglutarate (pH 7.0) and readjusting to pH 7.0 with 2 N HCl. The concentration of the solution was calculated from the absorbance at 232 nm with an ϵ of 7.69×10^4 cm^{-1} M^{-1}. Ten microliters of 1.3×10^{-3} M solution of CMB were added to the assay medium.

Results. Canine myocardium contained a prostaglandin dehydrogenase with a specific activity of 15 units/mg protein. The rate of oxidation of NAD was directly proportional to the concentration of prostaglandins within the range used in the present study. The substrate specificity of the enzyme is shown in Table I where the relative ability of various prostaglandins to act as substrates is compared to that of PGE_1 taken as 100. As shown, PGE_2, PGE_3, $PGF_{1\alpha}$, $PGF_{2\alpha}$ and PGA_1 are oxidized by the enzyme at roughly comparable rates, while PGA_2 and the PGBs are poor substrates. NAD was required for PGDH activity and could not be substituted for by NADP; K_m for NAD was 0.6 μmole. Intact sulfhydryl groups in the enzyme molecule is also a prerequisite for activity since total loss of PGDH followed addition of *p*-chloromercuribenzoate in the incubation medium. Requirements for pH were rather wide as evidenced by the stability of enzymatic activity between pH 4.8 and 8.2. At higher pH values both the substrate and the enzyme become unstable.

The effect of cyclic 3′,5′-AMP is shown in

TABLE I. Effectiveness of Various Prostaglandins as Substrates for PGDH.

Compound	Relative PGDH activity
11α,15(S)-Dihydroxy-9-ketoprost-13-enoic acid (PGE_1)	100
11α,15(S)-Dihydroxy-9-ketoprost-5,13-dienoic acid (PGE_2)	98
11α,15(S)-Dihydroxy-9-ketoprost-5,13,17-trienoic acid (PGE_3)	93
9α,11α,15(S)-Trihydroxy-9-prost-13-enoic acid ($PGF_{1\alpha}$)	82
9α,11α,15(S)-Trihydroxy-prost-5,13-dienoic acid ($PGF_{2\alpha}$)	85
15(S)-Hydroxy-9-keto-prost-10,13-dienoic acid (PGA_1)	86
15(S)-Hydroxy-9-keto-prost-5,10,13-trienoic acid (PGA_2)	40
15(S)-Hydroxy-9-keto-prost-8(12),13-dienoic acid (PGB_1)	(—)
15(S)-Hydroxy-9-keto-prost-5,8(12),13-trienoic acid (PGB_2)	(—)

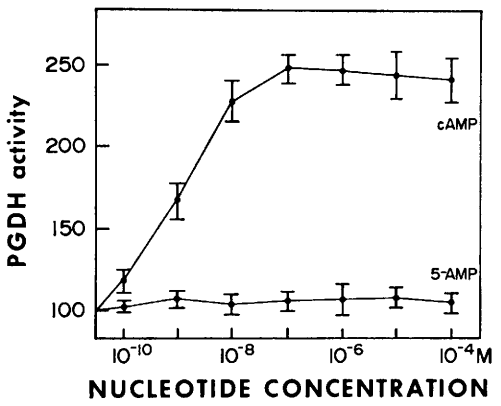

FIG. 1. Effects of cyclic 3′,5′-AMP and 5′-AMP on PGDH activity. Incubation mixture contained 500 μl NAD (1.4 μ*M*), 100 μl PGDH and 100 μl PGE_1 (0.34 μ*M* in ethanol) in 2.9 ml sodium pyrophosphate–semicarbazide buffer; nucleotides were added as 0.1 ml of solutions of different concentrations. Results are expressed in comparison to the basal (without nucleotides) PGDH activity taken as 100%.

Fig. 1. In concentrations between 10^{-10} and 10^{-7} *M* cyclic AMP stimulated PGDH activity; further increments in its concentration had little effect on the enzyme. In contrast to cyclic AMP, addition of $CaCl_2$ to the incubation medium resulted in a decrease of PGDH activity most evident at concentrations 10^{-7} *M* and above. Cyclic AMP could still restore enzymatic activity following inhibition by $CaCl_2$ (Table II), whereas 5′-AMP had no effect on PGDH either in the presence or in the absence of $CaCl_2$ (Fig. 2).

TABLE II. Effects of Cyclic AMP and Stimulators of Adenylate Cyclase on PGDH Activity.[a]

Additions	PGDH activity (units/mg protein)
None (control)	17 ± 6
Cyclic AMP (10^{-6} *M*)	37 ± 4
$CaCl_2$ (10^{-6} *M*)	8 ± 3
$CaCl_2$ (10^{-6} *M*) + cyclic AMP (10^{-6} *M*)	30 ± 5
Theophylline (10^{-4} *M*)	20 ± 7
Glucagon (10^{-3} *M*)	18 ± 5
Epinephrine (10^{-4} *M*)	18 ± 4

[a] Results from a total of eight experiments for each substance are given as the mean and SE. Composition of the incubation mixture is described in the text.

Theophylline, glucagon and epinephrine did not significantly affect the enzymatic activity (Table II) perhaps because of the relative purity of the preparation which did not include significant amounts of particulate adenylate cyclase.

Discussion. Recent recognition of the important biological functions of prostaglandins has fostered an interest in their metabolism and degradation. Although prostaglandins are ubiquitous and are not destroyed by blood elements, it is probable that tissues equipped with enzymes for both the biosynthesis and degradation of prostaglandins regulate the levels of these substances locally.

Recently, the prostaglandin dehydrogenase from swine lung was purified and studied with respect to substrate specificity (9). PGE_1 and PGE_2 were found to be the best substrates, PGE_3 and members of the F series were about 60% as effective and lower rates were noted for dihydro-PGE_1, PGA_1, PGA_2 and the 19-hydroxy-PGAs. In all of these compounds it could be shown that the enzyme specifically oxidized the hydroxyl group at C-15. Prostaglandins where the planarity of

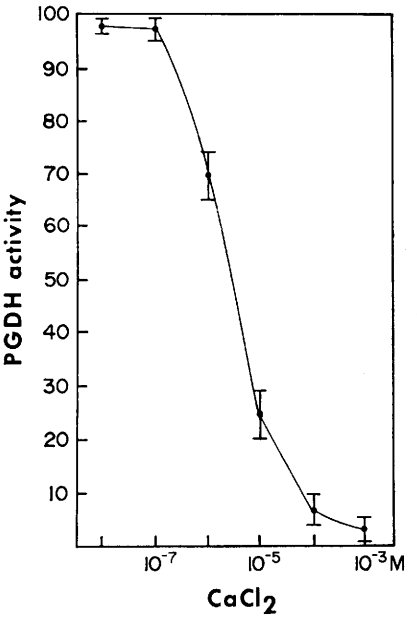

FIG. 2. Effects of $CaCl_2$ on PGDH activity. Incubation as in Fig. 1 with the exception that 0.1 ml of the $CaCl_2$ solutions are added instead of nucleotides.

the configuration of the carboxyl side chain relative to the ring has been changed (for example, PGBs, 8-iso-PGE_1) are poor substrates for the enzyme (10).

The stereospecificity of the dehydrogenase is also markedly dependent upon the stereochemistry of the prostaglandin substrate (11). A 15-α-hydroxyl group seems to be a major requirement since *rac*-15-epi-PGE_1 is not dehydrogenated by the enzyme but the conversion is not prevented by a change in configuration at C-11. Racemic mixtures are only dehydrogenated at half-molar amounts of the natural isomers; with excess enzyme the reaction does not proceed beyond 50% indicating that only one antipode is used and thus the *ent* forms are not affected.

The function of the myocardial prostaglandin dehydrogenase is probably best visualized in conjunction with the effects of its biosynthetic counterpart, prostaglandin synthetase. The presence of both enzymes in the myocardial cell (PGDH in the cytosol, prostaglandin synthetase in the microsomal fraction) suggests that the effects of prostaglandins on the heart are direct from prostaglandins synthesized *in situ* rather than the consequence of action of circulating prostaglandins. This is supported by recent evidence that, normally, tissue levels of these substances are extremely low and only rise as a result of *de novo* synthesis in response to appropriate stimuli (12).

If basal myocardial levels of prostaglandins are to be kept low, a rapid route for their inactivation should be available in close proximity to their site of biosynthesis. This requirement seems to be met, at least in part, by prostaglandin dehydrogenase. On the other hand, newly synthesized prostaglandins should be protected from the action of the enzyme at least until their levels increase sufficiently for their biological actions to be initiated.

The effects of cyclic 3′,5′-AMP and Ca^{2+} ions on the PGDH provide a framework for the following hypothesis on the control of the enzyme: Following an appropriate stimulus prostaglandins are rapidly synthesized from precursor fatty acids in the cardiac cell membrane. As a direct consequence of prostaglandin synthesis, Ca^{2+} ions enter the cell. The capacity of prostaglandins to facilitate calcium movements across biological membranes has long been recognized (13) and it was recently suggested that PGE_1 may even act as a "calcium ionophore" (14). The increase in Ca^{2+} concentration in the myoplasm results in inhibition of PGDH activity. This ensures the unencumbered increase of the levels of intramyocardial prostaglandins. One of the immediate effects of prostaglandins is to activate adenylate cyclase (15) and, as a result, increase the levels of cyclic 3′,5′-AMP. The latter does not affect prostaglandin synthesis (3) but reverses the inhibition of PGDH by Ca^{2+} and assures the return of myocardial prostaglandin levels to the low basal values. By this time, the sequence of events that leads to the expression of the physiological effect of prostaglandins has been initiated and does not require their continued presence in the cell.

It is evident that the outlined mechanism of PGDH control in the heart rests on the assumption that Ca^{2+} entry into the cell precedes temporally the activation of adenylate cyclase and initiation of other physiological events by prostaglandins. Thus far, there is no direct evidence bearing on this assumption. Moreover, the cyclic AMP–Ca^{2+} interaction is probably only one of the factors regulating PGDH activity and intramyocardial levels of prostaglandins. Further work is required to elucidate the early steps in the metabolism of cardiac prostaglandins.

Summary. A prostaglandin dehydrogenase (sp act, 15 units/mg protein) was isolated from the canine myocardium. The enzyme oxidized the 15-hydroxy group of most prostaglandins tested except for PGBs and PGA_2 which were poor substrates. It required NAD and intact sulfhydryl groups. Its activity was enhanced by cyclic 3′,5′-AMP at low concentrations (10^{-10} to 10^{-7} *M*) and inhibited by $CaCl_2$. The significance of these findings for the control of myocardial prostaglandin levels is discussed.

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