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Inhibition of 15-Hydroxyprostaglandin Dehydrogenase by Papaverine

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SUMMARY —— Papaverine was found to inhibit NAD^+-linked 15-hydroxyprostaglandin dehydrogenase partially purified from guinea pig lung. The inhibition was noncompetitive with prostaglandin E_2, uncompetitive with NAD^+, and reversible. The Ki was calculated to be 26 μM. Papaverine also inhibited the enzyme from swine lung, chicken and dog heart, and rat and dog kidney. The inhibitory effects of papaverine on the 15-hydroxyprostaglandin dehydrogenase were compared with those on cyclic AMP phosphodiesterases in these tissues.

Papaverine exhibits relaxant effect on the smooth muscle. Kukovetz and Pöch (1) found that papaverine is a potent inhibitor of cyclic AMP (cAMP) phosphodiesterase* and suggested that the following increase in cAMP level in the tissue is responsible for the pharmacological properties of the drug.

Recently, attention has been drawn to the role of prostaglandins, which have versatile actions on the smooth muscle (2). The close relationship between cAMP and prostaglandins has been recognized. For example, it has been indicated that PGE_1* and PGE_2* stimulate the cAMP synthesis in lymphocytes (3), platelets (4), and various tissues (5,6). 15-Hydroxyprostaglandin dehydrogenase*, the first step enzyme in the prostaglandin catabolism, oxidizes 15-hydroxyprostaglandins to 15-ketoprostaglandins, resulting in 10-fold loss of prostaglandin activity (7). Two types of 15-OH-PGDH have been reported (8) : NAD^+-linked 15-OH-PGDH shows higher affinity for PGE-compounds than PGF-compounds,

* Abbreviations: cAMP-PDE, adenosine 3':5'-cyclic phosphate phosphodiesterase; PGE_1, prostaglandin E_1; PGE_2, prostaglandin E_2; 15-OH-PGDH, 15-hydroxyprostaglandin dehydrogenase.

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whereas the $NADP^+$-linked enzyme exhibits higher affinity for PGF-compounds.

The present communication describes the finding that papaverine inhibited partially purified NAD^+-linked 15-OH-PGDH from the lung, heart, and kidney. The inhibitory effect of papaverine on the 15-OH-PGDH was compared with its effect on cAMP-PDE in the same tissue, and a possible biological significance of this finding is discussed.

MATERIALS AND METHODS

The drugs and chemicals were obtained from the following sources: PGE_2, Fuji Chemical Industries (Tokyo, Japan); Papaverine and theophylline, Wako Chemicals (Osaka, Japan); Dipyridamole, Tanabe Pharmaceutical Co. (Osaka, Japan); Nucleotides and cyclohexylaminopropane sulfonic acid, Sigma (U.S.A.). Other reagents were of the highest quality generally available.

Partial purification of 15-OH-PGDH from different sources: (a) Guinea pig lung 15-OH-PGDH was partially purified from the 100,000 g supernatant fraction of homogenates by ammonium sulfate fractionation (20 to 40% saturation), and chromatography on TEAE-cellulose (9). In the TEAE-cellulose chromatography, the column was washed with 10 mM phosphate buffer (pH 7.3) containing 0.1 M NaCl and the enzyme was eluted from the column with the phosphate buffer containing 0.2 M NaCl. (b) Swine lung 15-OH-PGDH was partially purified by essentially the same method as described by Änggård and Samuelsson (10). Chromatographies on hydroxylapatite and DEAE-Sephadex were omitted. (c) Chicken heart 15-OH-PGDH was partially purified by the method as described by Lee and Levine (8), but hydroxylapatite chromatography was not used. (d) Rat kidney 15-OH-PGDH was partially purified by the method as described by Wright, Corder, and Taylor (11). (e) Dog heart 15-OH-PGDH was partially purified as follows: All the procedures were carried out at 0-4° and the buffers used contained 1 mM EDTA, 0.2 mM dithiothreitol and 1.4 μM NAD^+. Fresh ventricular tissue (110 g), freed of epicardial fat and vessels, was homogenized in a Waring blendor for 2 min with 220 ml of 0.05 M sodium phosphate buffer (pH 7.4). The suspension was further homogenized with additional 110 ml of the same buffer for 8 min. The resulting suspension was centrifuged at 100,000 g for 60 min, and the precipitate was discarded. To the supernatant fluid, solid ammonium sulfate was added to 25% saturation. The precipitate was removed by centrifugation and discarded. Solid ammonium sulfate was further added to the supernatant fluid to give 65% saturation. The precipitate was collected by centrifugation and suspended in the small volume of 10 mM sodium phosphate buffer (pH 7.4) and was dialyzed against the same buffer for about 3 hours. The clear, but viscous solution was then applied to a Sephadex G-100 column (4 x 90 cm) equilibrated with 10 mM sodium phosphate buffer (pH 7.4) and eluted with the same buffer. The fractions eluted at

1.58-2.00 of Ve/Vo were combined and applied to a DE-52 column (2.2 x 20 cm) equilibrated with 10 mM sodium phosphate buffer (pH 7.4). 15-OH-PGDH activity was eluted from the column with a linear gradient consisting of 10 mM to 400 mM phosphate buffer (pH 7.4). The major PGDH activity was eluted at the concentration of 100 mM of sodium phosphate. The active fractions were pooled and stored at -15°. Although Lima and Cohn (12) reported that the dog heart 15-OH-PGDH activity was enhanced by cAMP and inhibited by Ca^{++}, no such effect of cAMP or Ca^{++} was found with our preparation of dog heart 15-OH-PGDH. (f) Dog kidney 15-OH-PGDH was partially purified by essentially the same method used for the purification of dog heart 15-OH-PGDH. The fraction precipitated between 30% and 70% saturation of ammonium sulfate was used. In the Sephadex G-100 chromatography, the enzyme activity was eluted from the column at 1.54-2.12 of Ve/Vo. In the DE-52 chromatography, the enzyme was absorbed on the column equilibrated with 10 mM phosphate buffer (pH 7.4) and the major enzyme activity was eluted from the column with the 10 mM phosphate buffer containing 500 mM KCl.

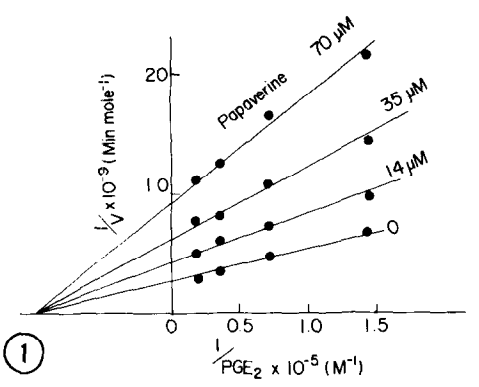
Assay of 15-OH-PGDH activity —— The 15-OH-PGDH activity was routinely assayed by the formation of 15-keto-PGE_2 as described by Änggård and Samuelsson (10). The standard assay mixture contained, in a total volume of 0.50 ml, 28 nmoles of PGE_2, 600 nmoles of NAD^+, 50 μmoles of potassium phosphate buffer (pH 7.4), 0.5 μmole of GSH, and the enzyme. Reaction was initiated by the addition of PGE_2 dissolved in 5 μl of ethanol, and incubation was performed at 37° for 20 min and terminated by dipping in an ice-water bath. For the swine lung and chicken heart enzymes, incubation was performed at 45°. The formation of 15-keto-PGE_2 was determined from the absorption at 500 nm after addition of 30 μl of 2N NaOH. The apparent molar extinction coefficient of 15-keto-PGE_2 was 31,000 under the conditions.

The activity of dog heart 15-OH-PGDH was measured spectrofluorometrically with a Hitachi fluorescence spectrophotometer (model, MPF-4) from the PGE_2-dependent formation of NADH, since the 15-OH-PGDH activity was low in this tissue. The assay mixture contained, in a total volume of 100 μl, 5.6 nmoles of PGE_2, 120 nmoles of NAD^+, 10 μmoles of Tris-HCl buffer (pH 8.7), 20 nmoles of dithiothreitol, 2 μg of bovine serum albumin and the enzyme. After incubation at 37° for 60 or 90 min, 2.5 ml of 0.1 M cyclohexylaminopropane sulfonate buffer (pH 11.5) containing 10% isopropanol (13) was added to stop the reaction. The control reaction mixture contained the same components except for the omission of PGE_2.

Assay of cAMP-PDE activity: All tissues were homogenized with 4 volumes of 0.17 M Tris-HCl (pH 8.0) containing 5 mM $MgSO_4$ at 0°. The homogenates were centrifuged at 100,000 g for 1 hour. The clear supernatant fluid was used as a source of cAMP-PDE. The cAMP-PDE activity was assayed by essentially the same method as described by Pichard and Cheung (14).

RESULTS AND DISCUSSION

Papaverine was found to inhibit NAD^+-linked 15-OH-PGDH partially purified from guinea pig lung. Figure 1 shows the double reciprocal plots of the PGE_2 concentration versus reaction



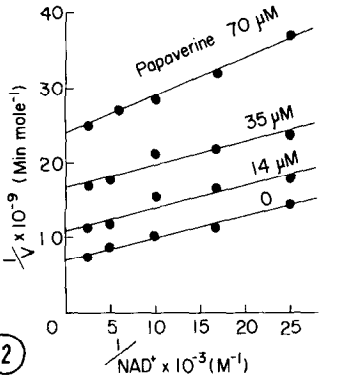


Fig. 1. Inhibition of guinea pig lung 15-OH-PGDH by papaverine with the varying concentrations of PGE_2 as substrate at a fixed concentration of NAD^+ (1.2 mM).

Fig. 2. Inhibition of guinea pig lung 15-OH-PGDH by papaverine with the varying concentrations of NAD^+ as cofactor at a fixed concentration of PGE_2 (56 μM).

velocity in the presence of various concentrations of papaverine. Papaverine exhibited a noncompetitive inhibition with regard to PGE_2 with the Ki value of 26 μM (The Km value for PGE_2 was 10 μM). The inhibition of papaverine was uncompetitive with regard to NAD^+ (Fig. 2).

The inhibition of papaverine is reversible. This conclusion is based on the following experiment: the enzyme was incubated at 37° with papaverine at a concentration of 100 μM for 45 min and then the enzyme activity was measured after dilution. The inhibition was not increased by the preincubation of the enzyme with papaverine.

The experiments were extended to partially purified 15-OH-PGDH preparations of other tissues, including heart and kidney, of other animal species in order to clarify whether the 15-OH-PGDH inhibition was a general property of papaverine, or whether the inhibitory effect of papaverine was restricted to those organs in which it exerts its pharmacodynamic effects. Papaverine inhibited

Table I. Inhibition by papaverine of NAD^+-linked 15-OH-PGDH and cAMP-PDE from different sources

Enzyme sources	I_{50}		
	15-OH-PGDH[a)]	low Km[b)] cAMP-PDE	high Km[c)] cAMP-PDE
Guinea pig lung	26 μM	2.0 μM	21 μM
Swine lung	120	N.D.	N.D.
Chicken heart	98	N.D.	N.D.
Dog heart	70	5.0	200
Rat kidney	400	2.7	75
Dog kidney	68	2.3	105

Values are expressed as the concentrations of papaverine required for 50% inhibition of the enzyme activities. The following concentrations of substrates were used: a) 56 μM PGE_2, b) 0.14 μM cAMP, and c) 500 μM cAMP. N.D.; not determined.

all the 15-OH-PGDH activities in swine lung, dog and chicken heart, and dog and rat kidney, besides guinea pig lung (Table I). The concentration for half-maximal inhibition were lowest in guinea pig lung and highest in rat kidney.

The inhibitory effect of papaverine on 15-OH-PGDH was compared with its effect on cAMP-PDE. The low Km and high Km cAMP-PDE activities were assayed in the 100,000 g supernatant fraction of the tissue homogenates. Papaverine strongly inhibited the low Km cAMP-PDE. Its concentrations required for half-maximal inhibition of the low Km cAMP-PDE were one order of magnitude lower than those of 15-OH-PGDH. On the other hand, the high Km cAMP-PDE were inhibited by the drug in similar concentrations to 15-OH-PGDH.

The present study showed that papaverine inhibits the NAD^+-linked 15-OH-PGDH. Since the 15-OH-PGDH has been shown to be specific for prostaglandins (10), this finding suggested that

prostaglandins may be involved in part in the action of papaverine. Until now, the relaxant effect of papaverine on the smooth muscle has been attributed to the inhibition of cAMP-PDE with resultant increase in the cAMP concentration in tissue. This finding that papaverine also inhibits NAD^+-linked 15-OH-PGDH lends additional support to the role of cAMP in the pharmacological action of papaverine. When papaverine inhibits the NAD^+-linked 15-OH-PGDH activity, PGE_1 and PGE_2 may accumulated in the tissue, which would elevate the cAMP level by stimulating its synthesis. On the other hand, papaverine is a potent inhibitor of cAMP-PDE. Therefore, papaverine appears to raise the cAMP level in tissue both by stimulating cAMP synthesis and inhibiting its degradation.

Since papaverine was found to inhibit NAD^+-linked 15-OH-PGDH, other cAMP-PDE inhibitors were tested for the inhibition activity toward the 15-OH-PGDH reaction. No significant inhibition of the guinea pig lung 15-OH-PGDH was produced by 1.4 mM theophylline or 0.4 mM dipyridamole.

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