REGULATION OF PROSTAGLANDIN METABOLISM: ACTIVATION OF 15-HYDROXYPROSTAGLANDIN DEHYDROGENASE BY CHLORPROMAZINE AND IMIPRAMINE RELATED DRUGS

Hsin-Hsiung Tai* and Charles S. Hollander**

* Department of Medicine, The Genesee Hospital and The University of Rochester School of Medicine and Dentistry Rochester, New York 14607
and
** The Endocrine Division, Department of Medicine New York University Medical Center New York, New York 10016

Received December 1,1975

SUMMARY: Chlorpromazine and imipramine related drugs were found to activate swine renal 15-hydroxprostaglandin dehydrogenase maximally about 2 fold. Activation by these drugs was uncompetitive with respect to both prostaglandin E_1 and NAD+. Hill plots of the rate of reaction against either substrates have a slope of approximately 1, which is not altered by the presence of the activator indicating no cooperativity between the substrate site and putative activator site. Ability of chlorpromazine and imipramine related drugs to activate this enzyme suggests that these drugs may facilitate the inactivation of prostaglandins in vivo.

INTRODUCTION

15-Hydroxyprostaglandin dehydrogenase (15-OH-PGDH), the first enzyme in the catabolic pathway of prostaglandins, catalyzes NAD^+ -dependent oxidation of 15-hydroxyl group of prostaglandins and has been considered a key enzyme in regulating the biological activity of prostaglandins (1). Previously we reported that thyroid hormone analogues were potent inhibitors of this enzyme from swine kidney (2). Subsequently, this observation was extended to the enzyme from chicken heart (3) and human placenta (4). Other pharmacological agents such as xylocaine (5), furosemide (6) and indomethacin (3, 7) were also found to inhibit this enzyme. These effectors may elevate intra-cellular levels of prostaglandins by inhibiting 15-OH PGDH. Recently, we found that psychotropic drugs related to chlorpromazine and imipramine are able to exert positive modulation by activating this enzyme. In the present communication we report our findings along with kinetic studies of the mechanism of this activation.

MATERIALS AND METHODS

Chlorpromazine hydrochloride and trifluorperazine dihydrochloride were obtained from Smith, Kline, and French Laboratories. Acetophenazine dimaleate and fluphenzine dihydrochloride were donated by Schering Corporation. Chlorprothiexene was given by Hoffman-La Roche Inc. Imipramine hydrochloride was obtained from CIBA Pharmaceutical Co. Desipramine hydrochloride was obtained from Lakeside Laboratories. Amithriptyline hydrochloride and protriptyline hydrochloride were given by Merck, Sharp, and Dohme Research Laboratories. We are indebted to these pharmaceutical companies for their generous supply of psychotropic drugs. Prostaglandin E_1 was a kind gift of Dr. John Pike of the Upjohn Company. NAD^+ was obtained from Sigma Chemical Company. [5,6-^{3}H] prostaglandin E_1 was purchased from New England Nuclear.

15-OH-PGDH was purified from swine kidney by ammomium sulfate precipitation, DEAE-cellulose chromatography, Sephadex G-100 gel filtration and hydroxylapatite c chromatography as previously described (2). In some cases the Sephadex G-100 fraction was further purified by blue dextran-Sepharose affinity chromatography instead of hydroxylapatite c chromatography (8). The Sephadex G-100 fraction, after concentration by ammonium sulfate precipitation and desalting by Sephadex G-25, was applied into a blue dextran-Sepharose column (1 x 20 cm) equilibrated with 5 mM potassium phosphate buffer, pH 7.5 containing 1 mM EDTA and 0.5 mM dithiotheitol. The column was washed with the same buffer until the effluent contained no proteins. 15-OH-PGDH was then eluted with 200 ml of a linear gradient of KCl from 0 to 1.0 M in the same buffer. The active fractions were pooled and stored at - 76° C. The final preparation was purified 400 fold with a specific activity of 653 mU per mg of protein at 37° C under following standard assay conditions. The standard assay mixture contained: EDTA, 1 mM; NAD^+, 0.5 mM; PGE_1, 28 µM and enzyme in a final volume of2ml of 0.1 M potassium phosphate buffer, pH 7.5 at 25° C. The initial rate of formation of NADH was obtained by measuring the linear increase in fluorescence intensity at 450 nm with excitation at 360 nm by a Farrand Spectro-fluorometer 801 attached with a recorder. The enzyme was alternatively assayed with [^{3}H] PGE_1 (50,000 cpm, 56 nmoles) as substrate to determine the formation of 15-oxo-PGE_1. Incubations were carried out under standard assay conditions for 10 min before termination with 50 µl of 12 M formic acid. The reaction mixture was then extracted with two 3 ml portions of ethylacetate. The combined extracts were evaporated under N_2 at 40°C. The residue was spotted on silica gel G plate (2 x 20 cm) and developed in the organic layer of the solvent system of ethylacetate-acetic acid-iso-octane-water (11:2:5:10). Both PGE_1 and 15-oxo-PGE_1 were localized by exposure to iodine vapor. Each zone was scraped off the plate and the radioactivity was determined by liquid scintillation counting. The conventional method of determing 15-oxo-PGE_1 by alkalinization (9) could not be used because of the formation of turbidity as a result of the poor solubility of psychotropic drugs under alkaline conditions.

RESULTS AND DISCUSSION

The concentration dependent activation of purified swine renal 15-OH-PGDH by two representative psychotropic drugs, chlorpromazine and imipramine as measured by the increased formation of NADH is shown in Fig. 1. Activation of the enzyme as measured by the increased formation of 15-oxo-PGE_1 followed the same patterns. The concentration required for activating 15-OH PGDH by

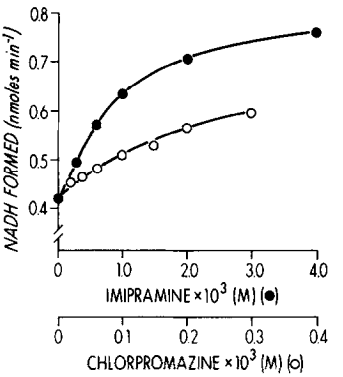

Fig. 1 Effect of chlorpromazine and imipramine on swine renal 15-OH PGDH activity.

The enzyme was assayed in the presence of indicated drugs under standard assay conditions. 11.4 µg of hydroxyapatite fraction was used per assay.

TABLE I

Effect of Various Psychotropic Drugs on Swine Renal

15-Hydroxyprostaglandin Dehydrogenase Activity

Concentration dependent activation of 15-OH PGDH by various drugs was assayed as described in Fig. 1. V is the maximal velocity at infinite activator concentration, while Vo is the velocity in the absence of activator. Ka is the concentration of activator giving half maximal activation.

Drug	V/Vo	Ka (mM)
Imipramine	2.07	1.25
Desipramine	1.83	1.67
Amitriptyline	2.08	1.82
Protryptyline	1.91	1.43
Chlorpromazine	1.63	0.15

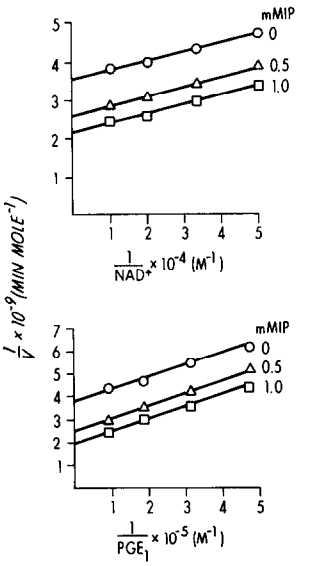

Fig. 2 Activation of swine renal 15-OH PGDH activity by imipramine (IP) with NAD^+ as the variable substrate at fixed concentration of PGE_1 (28 μM) (upper half) and with PGE_1 as the variable substrate at fixed concentration of NAD^+ (0.5 mM) (lower half). The concentrations of imipramine were varied as indicated. 2.5 μg of blue dextran-Sepharose fraction was used per assay.

chlorpromazine is one order of magnitude lower than that by imipramine although greater maximal activation was achieved by imipramine. Activation of the enzyme activity appears to follow hyperbolic kinetics (Hill coefficient n=1) for both types of drugs indicating the presence of a saturable site for the activators. Maximal stimulation by chlorpromazine and imipramine studied at near saturated concentrations of both substrate and coenzyme were about 163% and 207% respectively. The concentrations required for half maximal stimulation by chlorpromazine and imipramine are 0.15 mM and 1.25 mM respectively. Table I listed the degree of maximal stimulation and the concentration required for half maximal stimulation by a variety of psychotropic drugs. It is interesting to note that all four tricyclic antidepressant drugs activated 15-OH-PGDH to about the same degree, while chlorpromazine was the only antipsychotic tranquilizer tested which possessed activating properties. Indeed other

antipsychotic tranquilizers such as fluphenazine and acetophenazine exhibited a slight inhibitory effect at 0.1 mM.

The nature of this activation was then investigated by kinetic studies. With NAD^+ as the variable substrate, imipramine gave an uncompetitive activation pattern in the double reciprocal plot (upper half of Fig. 2). When PGE_1 was used as the variable substrate, imipramine again exhibited an uncompetitive activation pattern (lower half of Fig. 2). Similar activation patterns with respect to NAD^+ and PGE_1 were also observed with chlorpromazine. The uncompetitive activation with respect to either substrates suggested that imipramine interacts with the enzyme at a site distinct from both substrate sites. One attractive possibility for the mechanism of activation of this type of activator is that there may be a change in the nature of the active site induced by the presence of the activator located at another site. However, swine renal 15-OH-PGDH does not show a sigmoid response to PGE_1 even over a wide range of concentrations. This lack of a sigmoid response to PGE_1 is shown in the Hill plots of Fig. 3, in which the line without added imipramine gives a slope of 1.02. The effect of imipramine is illustrated by other lines, in which the addition of either 0.5 mM imipramine (slope, 0.95) or 2mM imipramine (slope 0.97) does not significantly alter the slope of a Hill plot against substrate. The other activator, chlorpromazine, was equally without any effect on the slopes of Hill plots against PGE_1 concentrations. Likewise, Hill plots with regard to coenzyme concentrations also showed slopes of approximately 1 both in the absence and presence of activators. It, therefore, appears that if one interprets the Hill number as indicative of cooperativity between sites, there is no indication of cooperativity between the substrate site and the putative activator site.

Although the mechanism of activation remains obscure at this point, it is interesting to note that xylocaine, a potent inhibitor of swine renal 15-OH-PGDH, also showed uncompetitive type of inhibition with respect to both substrate and coenzyme (5). Kinetic studies of mixed effect of

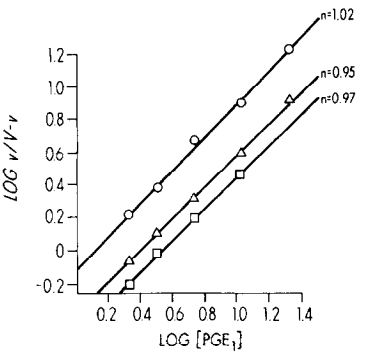

Fig. 3 Hill plots of swine renal 15-OH PGDH in the presence and absence of imipramine. Concentrations of PGE_1 are micromolar. Assay conditions were the same as those of Fig. 2. ○, control, no added imipramine; △, 0.5 mM imipramine; □, 2mM imipramine.

imipramine and xylocaine on swine renal 15-OH-PGDH indicated that the activator and the inhibitor probably interact with the enzyme at the same site (5). Whether the different type of effectors invoke a different conformational change in the enzyme such that the rate of product release was oppositely affected, or these effectors may exert some other mechanisms will require a homogenous enzyme preparation for further experimentation.

Psychotropic drugs have been widely used for the control of threatened abortion. Tothill et al (10) first hypothesized that these drugs might exert their action by preventing the liberation of prostaglandins and thereby blocking their action on the uterus. Lee (11) further supported the thesis with the finding that these drugs indeed inhibited prostaglandin synthesis in cell-free homogenates of guinea pig lung. Our observations that these psychotropic drugs also activate 15-OH-PGDH thereby faciltating the inactivation of biologically active prostaglandins appear to provide an additional possible explanation for their clinical utility in threatened abortion.

Acknowledgments: One of us (H. H. Tai) is indebted to Dr. William Y. Chey for his continued interest and support throughout this study. This work was supported by grants from USPHS (5 RO1-AM14314-4), The New York Heart Association and the Isaac Gordon Center of Gastroenterology of the Genesee Hospital, Rochester, New York.

References:

1. Samuelsson, B., Granstrom, E. Green, K., and Hambert, M. (1971) Ann. N. Y. Acad. Sci., 180, 138
2. Tai, H. H., Tai, C. L., and Hollander, C. S. (1974) Biochem. Biophys. Res. Commun., 57, 457
3. Lee, S. C., and Levine, L. (1975) J. Biol. Chem., 250, 548
4. Thaler-Dao, H., Saintot, M., Baudin, G., Descomps, B., and Crastes de Paulet, A. (1975) International Conference on Prostaglandins Abstract, p. 118
5. Tai, H. H. and Hollander, C. S. (1975) Advances in Prostaglandin and Thromboxane Research, Raven Press, New York, in Press
6. Paulsrud, J. R., Miller, O. N., and Schlegel, W. (1974) Fed. Proc. 33. 590
7. Hansen, H. (1974) Prostaglandins 8, 95
8. Thompson, S. T., Cass, K. H. and Stellwagen, E. (1975) Proc. Nat. Acad. Sci., U.S.A. 72, 609
9. Anggard, E., Larson, C., and Sameulsson, B. (1971) Acta Physiol. Scand., 81, 396
10. Tothill, A., Bamford, D., and Draper, J. (1971) Lancet ii (7720) 381
11. Lee. R. E. (1974) Prostaglandins 5, 63