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Arachidonic Acid Metabolites

Editor

Ruth Johnsson Hegyeli, M.D.

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Ruth Johnsson Hegyeli, M.D.
Assistant Director for International Programs
Office of the Director
National Heart, Lung, and Blood Institute
National Institutes of Health
Bethesda, Maryland

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Preface

Research on arachidonic acid metabolites is becoming increasingly important in studies on cardiovascular and pulmonary diseases. The role of these metabolites as vasodilators and/or vasoconstrictors appears to be significant in a number of other diseases as well. At the same time, improved methods are becoming available for the measurement of these metabolites as they are formed and released within the organism. This volume reviews the state of knowledge on arachidonic acid metabolites and atherosclerosis and identifies areas in which further research could be most rewarding.

The volume opens with a review of current research on the biosynthesis and function of a stable metabolite of prostacyclin; four major functions important to the regulation of the circulation are identified. The role of thromboxane in renal disease, as elucidated in animal studies, is then addressed. In another chapter, the therapeutic effect of prostacyclin on myocardial tissue is detailed through studies designed to ascertain the specific actions associated with this effect. A subsequent chapter explores the hypothesis that a deficiency of prostacyclin synthesis may play a role in the pathogenesis of atherosclerosis; the results of several pharmacological studies of prostacyclin analogs are of interest here.

The role of arachidonic acid metabolites in the interaction of granulocytes with the vascular wall is explored in order to better understand how chronic dialysis may accelerate atherosclerosis; the effects of certain pharmacological agents, including aspirin, on granulocyte/endothelial cell interaction are considered. Additional pharmacological reports include the kinetics of aspirin and the inhibition of thromboxane generation and the effects of loop diuretics on the prostaglandins. A final chapter on pharmacology reviews available information on the selective inhibition of platelet thromboxane production.

Two chapters on the clinical aspects of arachidonic acid close this volume. These include one on the effect of dietary polyunsaturated fats in the development of atherosclerosis, drawing on recent

observations in Greenland Eskimos, and another on the role of arachidonic acid metabolism in diabetes mellitus.

In sum, this volume brings together up-to-date information from important research efforts in both Italy and the United States on arachidonic acid metabolites. In doing so, the contributors point to priority areas for future research—both experimental and clinical. Although research in this area is relatively new, it is developing apace and has important implications for cardiovascular and many other related diseases. This book will be of special interest to both scientists and clinicians working in cardiology, pharmacology, and pulmonary diseases.

Claude Lenfant, M.D.
Rodolfo Paoletti, M.D.
Antonio M. Gotto, Jr., M.D.
Eugene Passamani, M.D.
Ruth Johnsson Hegyeli, M.D.

Contributors

L. Caparrotta
Department of Pharmacology
Padua University
Largo E. Meneghetti 2
35131 Padua, Italy

Chiara Cerletti
Istituto di Ricerche Farmacologiche "Mario Negri"
Via Eritrea 62
20157 Milan, Italy

G. Davì
Institute of Clinical Medicine
University of Palermo
90127 Palermo, Italy

Giovanni De Gaetano
Istituto di Ricerche Farmacologiche "Mario Negri"
Via Eritrea 62
20157 Milan, Italy

Elisabetta Dejana
Istituto di Ricerche Farmacologiche "Mario Negri"
Via Eritrea 62
20157 Milan, Italy

Aldo Del Maschio
Istituto di Ricerche Farmacologiche "Mario Negri"
Via Eritrea 62
20157 Milan, Italy

G. Fassina
Department of Pharmacology
Padua University
Largo E. Meneghetti 2
35131 Padua, Italy

Paola Filabozzi
Department of Pharmacology
Centro di Studio per la Fisiopatologia dello Shock C.N.R.
Catholic University School of Medicine
Via Pineta Sacchetti 644
00100 Rome, Italy

Garret A. FitzGerald
Division of Clinical Pharmacology
Vanderbilt University
Nashville, Tennessee 37232

Ferruccio Galletti
Universita di Napoli
II Facoltà di Medicina e Chirurgia
II Clinica Medica
Nuovo Policlinico
Via Sergio Pansini
80131 Naples, Italy

John G. Gerber
Division of Clinical Pharmacology C-237
Departments of Medicine and Pharmacology
University of Colorado School of Medicine
4200 East Ninth Avenue
Denver, Colorado 80262

Harry S. Jacob
Department of Medicine
University of Minnesota Medical School
University of Minnesota Hospital
Minneapolis, Minnesota 55455

Saulo Klahr
Departments of Medicine and Pharmacology
Washington University School of Medicine
660 South Euclid Avenue
St. Louis, Missouri 63110

Howard R. Knapp
Division of Clinical Pharmacology
Vanderbilt University
Nashville, Tennessee 37232

Roberto Latini
Istituto di Ricerche Farmacologiche "Mario Negri"
Via Eritrea 62
20157 Milan, Italy

John C. McGiff
Department of Pharmacology
New York Medical College
Valhalla, New York, 10595

Aubrey R. Morrison
Departments of Medicine and Pharmacology
Washington University School of Medicine
660 South Euclid Avenue
St. Louis, Missouri 63110

Alan S. Nies
Division of Clinical Pharmacology C-237
Departments of Medicine and Pharmacology
University of Colorado School of Medicine
4200 East Ninth Avenue
Denver, Colorado 80262

Rodolfo Paoletti
Institute of Pharmacology and Pharmacognosy
University of Milan
Via A. Del Sarto 21
20129 Milan, Italy

Paola Patrignani
Department of Pharmacology
Centro di Studio per la Fisiopatologia dello Shock C.N.R.
Catholic University School of Medicine
Via Pineta Sacchetti 664
00100 Rome, Italy

Carlo Patrono
Department of Pharmacology
Centro di Studio per la Fisiopatologia dello Shock C.N.R.
Catholic University School of Medicine
Via Pineta Sacchetti 644
00100 Rome, Italy

Joseph P. Pieroni
Department of Pharmacology
New York Medical College
Valhalla, New York 10595

Mabel Purkerson
Departments of Medicine and Pharmacology
Washington University School of Medicine
660 South Euclid Avenue
St. Louis, Missouri 63110

E. Ragazzi
Department of Pharmacology
Padua University
Largo E. Meneghetti 2
35131 Padua, Italy

Robert N. Saunders
Department of Platelet Research
Sandoz Research Institute
East Hanover, New Jersey 07936

A. Strano
Institute of Clinical Medicine
University of Palermo
90127 Palermo, Italy

Elena Tremoli
Institute of Pharmacology and Pharmacognosy
University of Milan
Via A. del Sarto 21
20129 Milan, Italy

Patrick Y.-K. Wong
Department of Pharmacology
New York Medical College
Valhalla, New York 10595

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This latest symposium on arachidonic acid metabolites was co-chaired by Dr. Claude Lenfant, Director, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland and by Professor Rodolfo Paoletti, Director, Institute of Pharmacology and Pharmacognosy, University of Milan, Italy.

Contents

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6-Keto-Prostaglandin E_1: Biosynthesis and Physiological Function

Patrick Y-K. Wong, Joseph P. Pieroni, and John C. McGiff

Department of Pharmacology, New York Medical College, Valhalla, New York 10595

Prostacyclin (PGI_2), discovered in blood vessels by Moncada et al. in 1976, is the most potent antiaggregatory substance so far described (1,2). It has been assumed to be the principal product of enzymatic transformation of the cyclic endoperoxide prostaglandins, PGG_2 and PGH_2 in all vascular elements (3). Further, most of the prostaglandin-mediated circulatory effects are considered to be mediated by PGI_2 (4); other prostaglandins identified in vascular tissues have been suggested to be either artifacts or relatively unimportant. However, several recent studies by different groups of investigators preclude the unqualified acceptance of prostacyclin as the only significant naturally occurring vascular prostaglandin. Consider the following: (a) In some blood vessels, there is evidence that prostacyclin is not the principal product of arachidonic acid metabolism (5). (b) PGE_2, a classical prostaglandin also synthesized within the vascular wall, may be the principal modulator of the vascular action of vasoactive polypeptides and adrenergic nervous activity (6). (c) Prostacyclin may not be a circulatory hormone due to its intrinsic instability and vulnerability to rapid inactivation by 15-hydroxy-prostaglandin dehydrogenase (15-OH-PGDH), an enzyme abundantly present in blood vessels and other tissues (7). (d) In some tissues or cell types, such as the liver and platelets which lack 15-OH-PGDH activity (8,9), prostacyclin can be transformed

to the stable metabolite 6-keto-PGE$_1$ by the enzyme 9-OH-PGDH (10). In this chapter we review the current status of 6-keto-PGE$_1$ and its biological function.

CHEMISTRY AND BIOSYNTHESIS OF 6-KETO-PGE$_1$ IN THE LIVER

In 1978, Axen and Smith reported the first chemical synthesis of 6-keto-PGE$_1$ (11) and, subsequently, showed 6-keto-PGE$_1$ to be a potent inhibitor of platelet aggregation (12). There are several reports of sustained biological activity of PGI$_2$ that are difficult to explain in view of its inherent instability. As an explanation for such findings, we suggest that in certain tissues 6-keto-PGE$_1$ may be generated from prostacyclin via the 9-OH-PGDH pathway. First demonstrated in the liver (13), this enzyme will catalyze the oxidation of the 9-OH group of 6-keto-PGF$_{1\alpha}$ to a ketone, a function similar to the enzymatic activity that converts PGF$_{2\alpha}$ directly to PGE$_2$. As the liver is a major site of PGI$_2$ clearance, the generation of a stable, active metabolite of prostacyclin within the liver may be of considerable importance for cardiovascular homeostasis. However, the renal cortex also has the capacity to synthesize 6-keto-PGE$_1$ (14), and we suggest that, under some conditions, the kidney may contribute to circulating levels of 6-keto-PGE$_1$ as has been suggested for prostacyclin (15). Before discussing the role of 6-keto-PGE$_1$ as a putative mediator of renin secretion, those studies that have indicated its formation from PGI$_2$ in liver and platelets are considered.

We infused radiolabeled PGI$_2$ into the isolated liver of the rabbit (13). Of the total radioactivity recovered in the liver perfusate, 7% was found to be tritiated water after distillation of the aqueous phase of the perfusate. Recovery of tritiated water indicated the loss of tritium at the 9-position as [9-^{3}H]-PGI$_2$ was infused into the liver; the formation of tritiated water suggested that PGI$_2$ had undergone oxidation at C9 and was converted to 6-keto-PGE$_1$ under these conditions. Any 6-keto-PGE$_1$ formed would be unlabeled and,

therefore, undetectable by radiometric methods. The formation of this nonradioactive prostaglandin was assessed by scraping the 6-keto-PGE$_1$ zone from the thin-layer chromatographic (TLC) plate, eluting the silica gel, suspending the residue in saline, and testing for biological activity. The material in the 6-keto-PGE$_1$ zone was found to have biological activity identical to that of authentic 6-keto-PGE$_1$, namely, contraction of rat stomach strip and bovine coronary artery and inhibition of adenosine diphosphate (ADP)-induced platelet aggregation. Further, alkali treatment abolished the platelet antiaggregatory activity of the 6-keto-PGE$_1$ standard as well as that of the material recovered from the 6-keto-PGE$_1$ zone on TLC plates. Thus, 6-keto-PGE$_1$ differs from both PGI$_2$ and 6-keto-PGF$_{1\alpha}$ in being unstable at alkaline pH. The bovine coronary and rabbit mesenteric arteries are the only tissues shown to differ qualitatively in their response to 6-keto-PGE$_1$ when compared to PGI$_2$. They are contracted by 6-keto-PGE$_1$ and relaxed by PGI$_2$ (13,16,17). Jackson et al. (18) have recently applied gas chromatography–mass spectrometry (GC-MS) analysis in determining plasma levels of 6-keto-PGE$_1$ in human subjects and found concentrations less than 30 pg/ml. Taylor et al. (19) injected 11-β[^{3}H] PGI$_2$ into the dog. Within 40 sec, 6-keto-PGE$_1$ was the major metabolite, representing 18% of radiolabeled material in plasma, declining to 4% by 5 min and not detectable 20 min after injection. Valuable insights may be gained from studies aimed at determining which tissues contribute to plasma levels of 6-keto-PGE$_1$.

BIOTRANSFORMATION OF PROSTACYCLIN IN HUMAN PLATELETS

Several groups have presented evidence for enzymatic conversion of PGI$_2$ to an active metabolite by platelets. Wong et al. (9) isolated 9-OH-PGDH activity primarily from the cytoplasmic fraction of human platelets and purified the enzyme by DEAE-cellulose followed by Sephadex G-200. Gel electrophoresis and isoelectric focusing resulted in a single band of enzyme, having a molecular weight of 60,000, a pH optimum of 8.5 to 9.0, an isoelectric point

of 5.0, and a requirement for NAD^+. Purified platelet 9-OH-PGDH metabolized the methyl ester of [11-^{3}H]-PGI_2 to a product identified by its mobility on TLC plates as [11-^{3}H]-6-keto-PGE_1 methyl ester. The methyl ester of PGI_2 was used in this study because it is at least 10 times more stable than PGI_2, thereby favoring reaction of purified platelet enzyme with PGI_2 methyl ester rather than with the hydrolysis product, 6-keto-$PGF_{1\alpha}$ methyl ester. Thus, significant amounts of unreacted PGI_2 methyl ester and the reaction product, 6-keto-PGE_1 methyl ester, were recovered. PGI_2 methyl ester was labeled in the 11-position rather than the 9-position in order to detect 6-keto-PGE_1 by radiochromatogram scanning. The 6-keto-PGE_1 methyl ester zone on the TLC plate yielded material that inhibited platelet aggregation induced by ADP, whereas the radioactive peak associated with 6-keto-$PGF_{1\alpha}$ methyl ester did not yield such material. As noted previously for 6-keto-PGE_1, alkali treatment abolished the platelet antiaggregatory activity of the material obtained from the 6-keto-PGE_1 methyl ester zone.

Pace-Asciak (20) reported 9-OH-PGDH activity capable of converting 15-keto-13,14-dihydro-$PGF_{2\alpha}$ to 15-keto-13,14-dihydro-PGE_2 in homogenates of rat kidney. Subsequently, activity was found in rabbit kidney (21) and rat liver (22). In these organs, both $PGF_{2\alpha}$ and its 15-keto-13,14-dihydro metabolite were oxidized. In contrast, purified 9-OH-PGDH from human platelets did not convert 15-keto-13,14-dihydro-$PGF_{2\alpha}$ to the corresponding metabolite of the E series (9). Differences in preferred substrate may indicate the existence of species- and tissue-specific forms of the enzyme. The capacity of platelets to generate 6-keto-PGE_1 has been suggested by other studies (23–26). Although the study by Wong et al. (9) concerned the activity of purified 9-OH-PGDH of platelets, PGI_2 was shown to gain access to 9-OH-PGDH of the intact platelet because, in the same study, washed human platelets also converted [11-^{3}H]-PGI_2 methyl ester to [11-^{3}H]-6-keto-PGE_1 methyl ester. Unlike PGI_2, 6-keto-$PGF_{1\alpha}$ was not metabolized by washed platelets although the purified platelet enzyme did transform it to 6-keto-PGE_1, a finding which may relate to the failure of infused PGI_2 to be converted to a biologically active compound (18). As the purified

platelet enzyme metabolized 6-keto-PGF$_{1\alpha}$, forming 6-keto-PGE$_1$, it would appear to be difficult to reconcile with the ineffectiveness of 6-keto-PGF$_{1\alpha}$ in causing cardiovascular changes when large amounts are infused either into the venous or arterial sides of the circulation. An explanation resides in the possible inability of platelets to bind 6-keto-PGF$_{1\alpha}$ (27). In support of a critical role of platelet receptors to prostaglandin-platelet interactions, Schafer et al. (27) have shown that the affinity of PGI$_2$ for the platelet receptor was 1,000 times greater than that of 6-keto-PGF$_{1\alpha}$. It is probable then, that uptake by platelets is an obligatory step prior to metabolism by the 9-OH-PGDH. An additional factor that may contribute to the inability of 6-keto-PGF$_{1\alpha}$ to be metabolized by platelets relates to the presence of several isomeric forms of 6-keto-PGF$_{1\alpha}$ (28). Thus, the 6-keto-PGF$_{1\alpha}$ formed in a given tissue may not be in the same isomeric form as the synthetically prepared compound. The possibility that 6-keto-PGE$_1$ may be formed from PGI$_2$ through an unknown intermediate should also be considered.

IS 6-KETO-PGE$_1$ GENERATED INTRAVASCULARLY?

Evidence has been accumulating that PGI$_2$ may be transformed to an active product rather than inactivated by hydrolysis or enzymic degradation. Gimeno and co-workers (16) exploited the differential responses of the bovine coronary artery strip to 6-keto-PGE$_1$ (contraction) and PGI$_2$ (relaxation) in an investigation of the biotransformation of PGI$_2$ and 6-keto-PGF$_{1\alpha}$ by human plasma. When PGI$_2$ was incubated for 1- to 2.5-hr periods, material was generated in the incubate which contracted the coronary strip. After shorter periods of incubation, less than 30 min, only PGI$_2$-like activity, inducing relaxation, was found in the plasma incubate. As incubated platelet-poor and platelet-rich plasma showed similar responses, Gimeno et al. attributed the transformation of PGI$_2$ to a plasma factor. An additional finding of these authors merits comment. When 6-keto-PGF$_{1\alpha}$ was initially tested on the coronary strip, no activity was detected, yet tone increased following incubation of 6-keto-PGF$_{1\alpha}$ in plasma for 2 hr.

Hoult et al. (29) subsequently demonstrated prolongation of platelet antiaggregatory activity and enhanced spasmogenic effects after incubating PGI_2 in human plasma. The altered responses were not seen in a platelet-poor plasma incubate but could be reproduced by incubation with the resuspended platelet pellet, suggesting that biotransformation of PGI_2 was due to a platelet factor. It should be recalled that the enzymatic activity required to effect the generation of this material has been found in platelets by Wong et al. (9). The product of platelet transformation of PGI_2 has been tentatively identified by Hoult et al. (29) as 6-keto-PGE_1, based on chromatographic mobility and spasmogenic effects. Recent evidence suggests formation of 6-keto-PGE_1 *in vivo*. Stoff et al. (30) have described decreased platelet aggregability associated with elevated cyclic adenosine monophosphate (cAMP) levels in platelet-rich plasma in patients with Bartter's syndrome. The defective aggregation and the elevation of cAMP were sensitive to indomethacin treatment and were dependent on a stable plasma factor, the stability of which was the major criterion distinguishing the factor from PGI_2. The mobility of the stable factor on high performance liquid chromatography was identical to that of 6-keto-PGE_1.

Assuming that 6-keto-PGE_1 is generated intravascularly through the action of platelet 9-OH-PGDH activity, the question arises as to which is the immediate precursor. From the results of Schafer et al. (27), 6-keto-$PGF_{1\alpha}$ does not seem to be the endogenous substrate. The findings in this important study suggest that the failure of infused 6-keto-$PGF_{1\alpha}$ to evoke significant cardiovascular effects can be explained by the absence of ar uptake-binding mechanism for 6-keto-$PGF_{1\alpha}$ in the platelet membrane. Such high-affinity binding sites were demonstrated in platelet membranes for PGI_2, indicating that PGI_2, released from the vascular wall or infused, might ultimately find access to the platelet 9-OH-PGDH, a cytosolic enzyme. Our guess, then, is that PGI_2 may be the immediate precursor of 6-keto-PGE_1 since incubation of [11-^{3}H]-PGI_2 methyl ester with platelets resulted in the formation of [11-^{3}H]-6-keto-PGE_1 methyl ester.