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European Symposium Volume 7

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Edited by:

J. E. DUMONT, J. NUNEZ and R.M. DENTON

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Scientific Committee:

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J. Nunez
G. Schultz

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FOREWORD

During the last few years, substantial progresses have been achieved in the elucidation of systems of cell regulation in superior organisms. In the field of hormone and neurotransmitter action at least three major breakthroughs have greatly stimulated our interest and understanding : first, these problems can now be studied at the molecular level; second, several hormones are now revealed to act as neurotransmitters; third, all these agents act in different cells on the same fundamental regulation systems. It seems clear now that a limited number of regulation models can account for the main characteristics of such different extracellular signals as hormones, neurotransmitters, ions, etc. All these models imply a primary interaction of the signal with a specific protein, the receptor. Such receptors may belong to the plasma membrane, but also to other subcellular structures. Several types of receptors may correspond to one signal; each type of cell is submitted to several controls; the cell itself may modulate its response to extracellular signals. In any case, the primary interaction of the signal with its receptor leads to a cascade of intracellular events taking place at the level of the plasma membrane, of specific intracellular enzymes, of protein synthesis machinery, etc. The known physiological responses to the signals are the more of less distal consequences of these cascades.

Those ideas led a group of researchers to organize each year since 1976 a four day International Symposium in a small village on the Alsatian side of the Vosges, first at the Bischenberg Centre, last year at Saint Odile. They believed that it would be of great interest to compare results and concepts derived from studies in widely different areas but bearing all on the mechanisms of cell regulation. Moreover, the need to organize such a meeting at a European level was obvious, as a simple inventory demonstrated the existence of many groups of international reputation which had no regular direct contacts, such as the Laurentian or Gordon Conferences in America. For these groups we wished to promote the regular cross fertilization of ideas and techniques. This organization was made possible by the help of the "Institut National de la Recherche Médicale" and of the "Délégation Générale à la Recherche Scientifique et Technique" (France).

The meetings have been successfull as they allowed, in a relaxed and informal atmosphere, to review and discuss recent advances in various fields of cell regulation, which are presented either as scattered and partial communications in general congresses or at length in separate specialized colloquia. The interdisciplinary character of the symposium obliges everybody to remain comprehensible for a general audience. Thus, it has become a very useful forum for European researchers in the field, who attend regularly not only to talk but also to listen and learn. It is the hope of the organizers that they will, in time, constitute a nucleus for European collaboration in the field. The symposium has therefore been organized each year.

The success of a meeting should not necessarily imply the publication of its proceedings. In this case the periodicity of the meetings allows to review regularly recent advances in the various fields of cell regulation by extracellularr signals. All authors have been asked to present a rather brief synthetic view of their subject and their research. The length and depth of these reviews should place them between reviews in Molecular and Cellular Endocrinology and those of Physiological Reviews. This should benefit researchers in the same field, but also non specialists and students. This book should give them brief and authoritative introductions and syntheses of the state of the art in various fields without having to scan the very dispersed specialized articles The very rapid publication allows the reviews to be up to date; the predominantly European participation will ensure a European flavor and a fair consideration of European litterature which, for various reasons, is sometimes conveniently forgotten. The conferences on fundamental biological problems (eg. membranes, DNA organization, etc.) which have always constituted a significant part of the Bischenberg meeting have not in general been printed in a book which is dedicated to cell regulation. The editors have discussed the possibility of editing the discussion of each presentation. However, full publication of the discussion was thaught to be both financially and materially difficult and might stiffen the informal character of this discussion. Summaries of the discussion were prepared for Volume 3 but these are open to the criticism that they may reflect more the opinion of the commentator than a true summary. They have therefore been omitted from later volumes. Finally, the problem of the lecturer who attends

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the meeting, all expenses being paid, and fails to deliver the promised manuscript had to be faced. In these cases, it has been decided to publicize this omission in the introduction or table of contents. This year we did not receive the contribution of Dr S. Hammarström (Stockholm).

In summary we would like this book to provide to those who are unable to attend the meeting a good reflection of its scientific information and, as to the series, when anybody in need of a short, synthetic and up to date review on cell regulation by hormones or neurotransmitters will turn first to Hormones and Cell Regulation, the editors will think that the endeavour was worthwhile. This year we wish to thank Dr J. McCormack, Dr R. Brownsey, Dr A. Halestrap and Mrs Wilmes who carried out with much enthusiasm and proficiency the bulk of the work on the organization of the meeting.

CONTENTS

ARACHIDONIC ACID CASCADE

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ACTIONS OF PROSTACYCLIN AND THROMBOXANES: PRODUCTS OF THE ARACHIDONIC ACID CASCADE

BRENDAN J.R. WHITTLE,
Department of Prostaglandin Research, Wellcome Research Laboratories Langley Court, Beckenham, Kent BR3 3BS, U.K.

INTRODUCTION

The unsaturated 20-carbon lipids known as prostaglandins have many potent and varied actions on cell function. Because of these actions, members of the prostaglandin family have been implicated in regulation or modulation of cellular activity and may also act as specific local hormones. These products are synthetized from polyunsaturated fatty acids containing three, four or five double bonds, which are present in the phospholipids of mammalian cell membranes. The predominant prostaglandin precursor in most animal and human cells is the essential fatty acid eicosatetraenoic acid, more commonly known as arachidonic acid, from which is derived the prostaglandins (PG's) containing two double bonds[1,2] such as PGE_2, $PGF_{2\alpha}$, PGD_2 prostacyclin (PGI_2), as well as thromboxane A_2, as is shown in the simplified metabolic pathway (Fig. 1). Dihomo-γ-linolenic acid gives rise to prostaglandins which have only one double bond such as PGE_1[1,2], but these products are found in very low concentrations in mammals, and thus their physiological importance is obscure.

Arachidonic acid can be released from membrane phospholipids by lipases, notably phospholipase A_2, which can be activated by many different stimuli. Thus hormonal, chemical and even slight mechanical perturbation of the cell membrane is sufficient to activate these enzymes and once released, the arachidonic acid is available for rapid metabolism into various oxygenated products by distinct enzymatic processes[3]. One metabolic pathway involves lipoxygenase enzymes (Fig. 1) which can give rise to non-cyclized hydroperoxy and subsequently hydroxy acids including 12-hydroxyeicosatetraenoic acid (12-HETE)[4]. A 5-lipoxygenase enzyme can also give rise to a series of conjugated trienes, the leukotrienes which have potent chemotactic and bronchospastic actions[5]. The lipid-peptide members of this series, LTC_4 and LTD_4 can account for the biological properties of the SRS-A (slow reacting substance of anaphylaxis) and the biosynthesis and chemical identification of these products are dealt with elsewhere.

The other main route of arachidonic acid metabolism involves the enzyme-complex termed cyclo-oxygenase (previously described as prostaglandin synthetase) which forms an unstable cyclic endoperoxide with a 15-hydroperoxy substituent, PGG_2[6]. The non-steroid anti-inflammatory drugs such as aspirin and indomethacin inhibit subsequent prostaglandin generation by inhibiting this enzyme[7]. The endoperoxide

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PGG_2 is further transformed into another unstable endoperoxide, having a 15-hydroxy substituent PGH_2. The endoperoxide can be broken down either enzymatically or by non-enzymatic decomposition (the half-life in aqueous solution is about 5 min at 37°C) to the stable now classical prostaglandins PGE_2 and $PGF_{2\alpha}$ as well as to PGD_2, a 17-carbon hydroxy acid (HHT) and malondialdehyde. In addition, the endoperoxides are also metabolised by two distinct enzymes into either prostacyclin (PGI_2) or thromboxane A_2 (TXA_2). Both these products are chemically unstable at physiological temperatures and pH; the half-life of prostacyclin at pH 7.4 is 3 mins at 37°C whereas that for TXA_2 is 30 secs. The respective chemical degradation products 6-oxo-$PGF_{1\alpha}$ and TXB_2 (Fig. 1) have little biological activity, in contrast to their unstable immediate precursors[8].

Isolation and identification of the unstable but highly reactive cyclo-oxygenation products of arachidonic acid, namely the endoperoxides, prostacyclin and TXA_2 has greatly assisted the interpretation of the potential importance of prostaglandins as biological regulators of cellular activity. Indeed, it may well be that these products, rather than the more classical prostanoids PGE_2 and $PGF_{2\alpha}$, which have been extensively investigated in the last decade, are of greater physiological and pathological importance[9]. In the present paper, therefore, the biological interactions between prostacyclin and TXA_2 in a variety of systems will be discussed. It is of biological significance that TXA_2 and prostacyclin have directly opposing actions in several processes such as on platelet function, vascular smooth muscle, pulmonary function and gastro-intestinal integrity. Thus prostanoid mediated control of cellular activity may reflect an interactive modulating influence of both substances, with an imbalance resulting in dysfunction in the cell or tissue.

BIOSYNTHESIS OF THROMBOXANE A_2 AND PROSTACYCLIN

Thromboxane A_2

The aggregation of human platelets by arachidonic acid and the prostaglandin endoperoxides, as well as other endogenous aggregating agents such as collagen, is accompanied by the release of a potent labile vasoconstrictor and pro-aggregating substance identified as thromboxane A_2 (TXA_2)[10]. The enzyme which converts the endoperoxides into TXA_2, thromboxane synthetase, is localized in the high-speed particulate fraction of human and horse platelets[11], and it has been solubilized and separated from the cyclo-oxygenase enzyme which has allowed detailed studies on its activity[12].

Besides platelets, other cells have been demonstrated to synthetize TXA_2, including rabbit and human polymorphonuclear leukocytes, human lung fibroblasts and macrophages from mouse, rat and guinea-pig. Whole tissues have also been shown to generate TXA_2 including, guinea pig lungs, rabbit and cat spleen, rabbit iris and

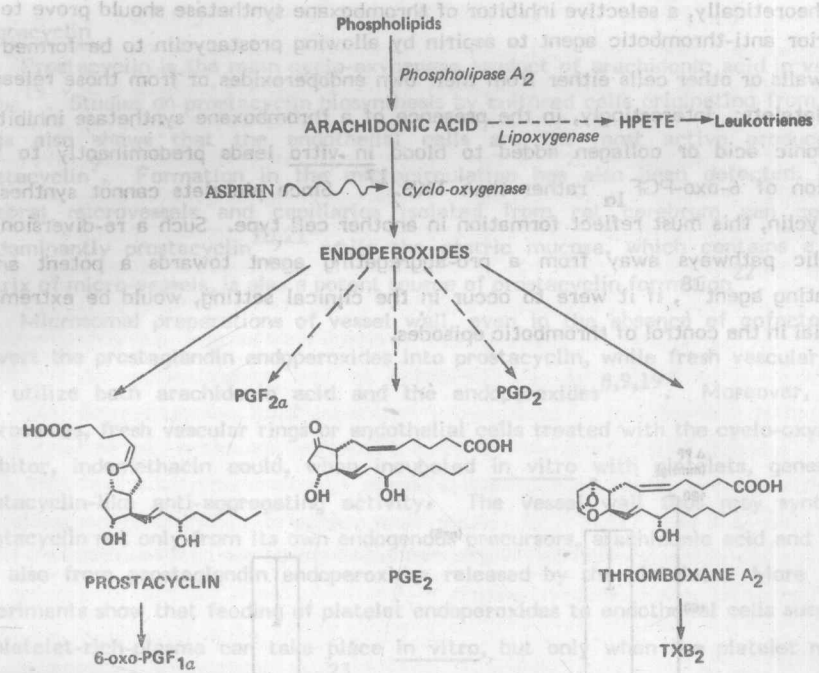

Fig. 1. Metabolism of arachidonic acid by the cyclo-oxygenase and lipoxygenase pathways.

conjunctiva, human umbilical artery, rabbit and rat kidney and rabbit pulmonary artery[8], although the exact location or cellular type possessing thromboxane-synthetase in these tissues is not known. Indeed, it is likely that some degree of the production of TXA_2 by these tissues is due to the presence of entrapped platelets or migratory cells in the tissue microcirculation.

Several groups of compounds have been reported to inhibit the TXA_2 formation[11,13]. The finding that imidazole inhibited TXA_2 production[14] has led to the synthesis of many substituted derivatives of greater potency and selectivity than the parent molecule. Such derivatives include 1-butylimidazole and 1-benzylimidazole. This latter compound inhibits the formation of thromboxanes[15] in human and dog platelets in vitro and likewise can inhibit the formation of the potent vasoconstrictor TXA_2 from arachidonic acid by dog platelets in blood (Fig. 2) when infused into an incubation coil in situ[16].

Theoretically, a selective inhibitor of thromboxane synthetase should prove to be a superior anti-thrombotic agent to aspirin by allowing prostacyclin to be formed by vessel walls or other cells either from their own endoperoxides or from those released from platelets. Interestingly, in the presence of a thromboxane synthetase inhibitor, arachidonic acid or collagen added to blood in vitro leads predominantly to the formation of 6-oxo-$PGF_{1\alpha}$ rather than TXB_2[17]. Since platelets cannot synthesize prostacyclin, this must reflect formation in another cell type. Such a re-diversion of metabolic pathways away from a pro-aggregating agent towards a potent anti-aggregating agent[18], if it were to occur in the clinical setting, would be extremely beneficial in the control of thrombotic episodes.

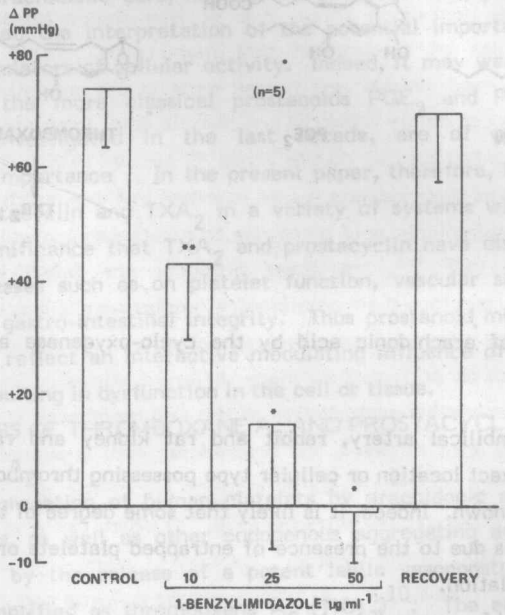

Fig. 2. Vasoconstriction in the canine gastric circulation by TXA_2 generated from arachidonic acid (AA) in blood, and its inhibition by 1-benzylimidazole (BZI). 'Control' represents response to AA (100 μg) injected intra-arterially into a delay coil so as to incubate with blood for 30 sec before reaching the stomach. BZI (10-50 μg ml^{-1} final concentration in blood) was infused intra-arterially so as to incubate with blood 30 sec prior to reaching injection site for AA. Results, shown as change in gastric perfusion pressure (Δ PP) are mean ± s.e. mean of 5 experiments, * $P < 0.05$, ** $P < 0.01$. Data is taken from Whittle, Kauffman and Moncada, (1981)[16].

Prostacyclin

Prostacyclin is the main cyclo-oxygenase product of arachidonic acid in vascular tissue[19]. Studies on prostacyclin biosynthesis by cultured cells originating from vessel walls also shows that the endothelial cells are the most active producers of prostacyclin[9]. Formation in the microcirculation has also been detected. Bovine cerebral microvessels and capillaries isolated from rat cerebrum can generate predominantly prostacyclin[20,21] while the gastric mucosa, which contains a dense matrix of micro-vessels, is also a potent source of prostacyclin formation[22].

Microsomal preparations of vessel wall, even in the absence of cofactors can convert the prostaglandin endoperoxides into prostacyclin, while fresh vascular tissue can utilize both arachidonic acid and the endoperoxides[8,9,19]. Moreover, vessel microsomes, fresh vascular rings or endothelial cells treated with the cyclo-oxygenase inhibitor, indomethacin could, when incubated *in vitro* with platelets, generate a prostacyclin-like anti-aggregating activity. The vessel wall thus may synthesize prostacyclin not only from its own endogenous precursors, arachidonic acid and PGH_2, but also from prostaglandin endoperoxides released by the platelets. More recent experiments show that feeding of platelet endoperoxides to endothelial cells suspended in platelet-rich-plasma can take place *in vitro*, but only when the platelet number approaches the normal blood levels[23]. However, the concept of endoperoxides released from platelets being utilized by endothelial cells has not yet been fully evaluated *in vivo*, and it may well be that such a biochemical co-operation occurs under pathophysiological conditions. Thus, the adherence of the platelet to the vessel wall, one of the first responses of the vascular tissue to injury, would provide the close proximity that would be needed for such co-operation between platelet and endothelial cells. It is also possible that other formed elements of blood such as white cells, which produce endoperoxides and TXA_2 could interact with the vessel wall to promote formation of prostacyclin especially under conditions of local vascular damage. Moreover, leukocytes themselves appear to generate prostacyclin in whole blood, an observation made with the use of a thromboxane synthetase inhibitor[17].

15-Hydroperoxy arachidonic acid (15-HPAA), a lipid peroxide, is a potent inhibitor of prostacyclin generation by vessel wall microsomes or by fresh vascular tissue[24]. Likewise, other fatty acid peroxides and their methyl esters can potently inhibit prostacyclin formation *in vitro*. Interestingly, high concentrations of lipid peroxides have been demonstrated in advanced atherosclerotic lesions. Lipid peroxidation induced by free radical formation is known to occur in vitamin E deficiency, the ageing process and perhaps also in the hyperlipidaemia which accompanies atherosclerosis. Thus accumulation of lipid peroxides in, for example, atheromatous plaques could predispose to thrombus formation by inhibiting generation of prostacyclin by the vessel wall[25].

Arachidonic acid can be metabolised into lipid peroxides by action of lipoxygenases, generating 5-, 12-, or 15-hydroperoxy eicosatraenoic acid (HPETE's). These metabolites, like other lipid peroxides can inhibit the prostacyclin synthetase in vitro and could therefore be involved in the biochemical regulation of prostacyclin formation. A modulator role for the lipoxygenase product 12-HPETE on arachidonate metabolism in platelets has thus been been proposed by Siegel and co-workers[26]. In homogenates of gastro-intestinal tissue, prostacyclin formation is significantly elevated by the lipoxygenase inhibitors, NDGA and BW755C. Although this latter compound is a dual cyclo-oxygenase-lipoxygenase inhibitor in most tissues and cells[27], BW755C inhibits cyclo-oxygenase in gastro-intestinal tissue only at high concentrations[28]. In the absence of cyclo-oxygenase inhibition at low concentrations of BW755C, a substantial enhancement of prostacyclin formation from endogenous substrate in rat gastric mucosal homogenates was observed (Fig. 3). This action may be related to the inhibition of endogenous lipoxygenase products, thus removing their inhibitory actions on prostacyclin production[29]. A further consequence of inhibition of the arachidonate lipoxygenase pathway could be diversion of substrate to the cyclo-oxygenase pathway, thus increasing prostaglandin formation.

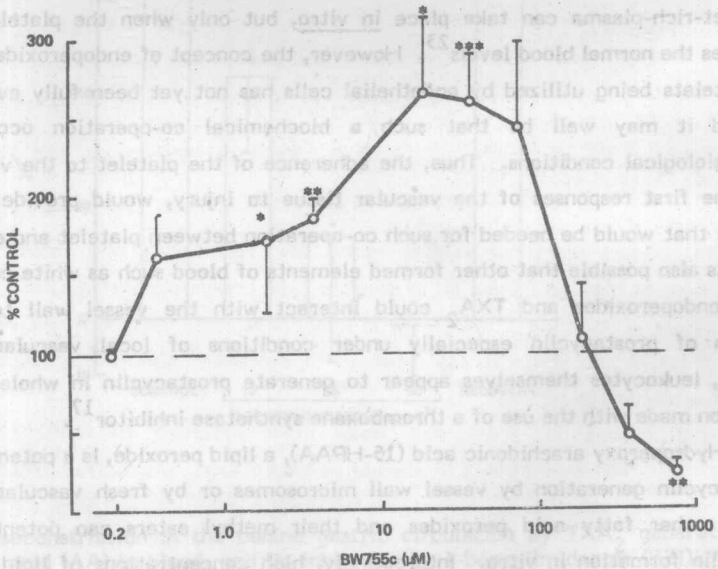

Fig. 3. Stimulation and inhibition of prostacyclin formation from endogenous substrate in homogenates of rat gastric mucosa by BW755C. Results, expressed as % of the control value are following 10 min pre-incubation (at 22°C) with BW755C, and are mean ± s.e. mean of 5-15 experiments for each value. The level of statistically significant difference from control is shown as * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Data is taken from Boughton-Smith & Whittle, (1982)[29].

ACTIONS OF THROMBOXANE A_2 AND PROSTACYCLIN ON PLATELETS

Thromboxane A_2

The endoperoxides PGG_2 and PGH_2 induce aggregation and the release of the platelet constituents when added to platelet suspensions. However, Hamberg, Samuelsson and colleagues were able to demonstrate that during platelet aggregation induced by arachidonic acid or the endoperoxides, a further product, TXA_2 is also generated[10]. TXA_2 is a more potent inducer of aggregation than the endoperoxides themselves, and it was proposed that TXA_2 is the cyclo-oxygenase product from arachidonic acid which mediates platelet aggregation and release reaction stimulated under patho-physiological circumstances by such endogenous agents as collagen. The question remains as to whether the endoperoxides have intrinsic pro-aggregatory activity or whether they act only after conversion to TXA_2. Thus, when their further conversion to TXA_2 is blocked by use of a thromboxane synthetase inhibitors, PGG_2 and PGH_2 may exert a direct activity on platelets, perhaps on the TXA_2 receptors. Interestingly, chemically stable epoxy methano endoperoxide analogues (which cannot be converted into TXA_2), possess intrinsic pro-aggregatory and vascular actions[30] and are thought to act as direct thromboxane mimics despite that apparent lack of structural similarity (Fig. 4). Under normal conditions when platelets are activated and the endogenous arachidonic acid cascade is triggered by PLA_2 activation, the prostaglandin endoperoxides thus generated may well exert their action following rapid conversion to the more potent TXA_2. The interaction of these products of arachidonic acid metabolism via the cyclo-oxygenase pathway with the so-called third pathway of platelet aggregation (believed to be cyclo-oxygenase and ADP independent) is not yet clear.

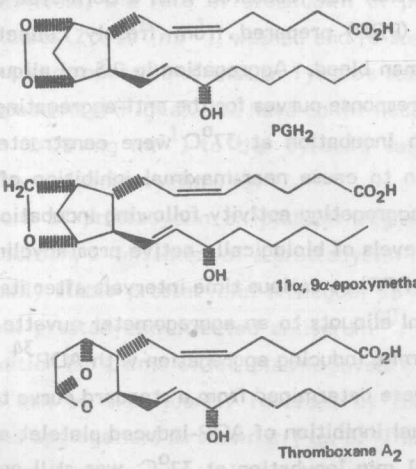

Fig. 4. Structure of the endoperoxide PGH_2, a 11, 9, epoxymethano PGH_2 analogue (U-46619) and thromboxane A_2.

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