# ADVANCES IN PHARMACOLOGY AND THERAPEUTICS

Proceedings of the 7th International Congress of Pharmacology

*General Editors: J. R. BOISSIER, P. LECHAT & J. FICHELLE*

## Volume 4

# PROSTAGLANDINS-IMMUNOPHARMACOLOGY

Editor: B. B. VARGAFTIG

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*Paris*

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# Introduction

The scientific contributions at the 7th International Congress of Pharmacology were of considerable merit. Apart from the sessions organised in advance, more than 2,200 papers were presented, either verbally or in the form of posters, and the abundance of the latter in the congress hall is a good indication that this particular medium of communication is becoming increasingly attractive to research workers, and offers scope for discussions which combine an elaborate, thorough approach with a certain informality.

It would have been preferable to have published the entire congress proceedings within the framework of the reports. That was, however, physically impossible, and the organisers had to adopt a realistic solution by publishing only the main lectures, symposia and methodological seminars. The amount of material presented necessitated the printing of ten volumes, each volume containing congress topics regrouped according to their relevant content and subject areas. This system of division may give rise to criticism on account of its artificiality, and we readily admit that certain texts could have been placed in more than one volume. We are asking the reader to excuse this arbitrariness, which is due to the editors' personal points of view.

I draw attention to the fact that most of the symposia finish with a commentary which the chairmen had the option of including, presenting their personal opinions on one or several points. We think that such an addition will facilitate reflection, discussion, indeed even controversy.

The launching of the scientific programme for this congress began in September 1975 on returning from the last meeting in Helsinki. Long and delicate discussions took place in the Scientific Programme Committee and with the International Advisory Board. Should it be a pioneer, 'avant-garde' congress? Or one laid out like a balance-sheet? Should we restrict the congress to the traditional bounds of pharmacology, or extend the range of papers to cover the finest discipline? The choice was difficult, and the result has been a blend of the two, which each participant will have appreciated in terms of his training, his tastes, and his own research.

A certain number of options, however, were taken deliberately: wide scope was given to toxicology, from different points of view, and to clinical pharmacology, a subject much discussed yet so badly practised; the founding of two symposia devoted

to chemotherapy of parasitic diseases which are still plagues and scourges in certain parts of the world; a modest but firm overture in the field of immunopharmacology, which up until now was something of a poor relation reserved only for clinical physicians; the extension of methodological seminars, in view of the fact that new techniques are indispensable to the development of a discipline.

We have been aware since the beginning that, out of over 4,000 participants who made the journey to Paris, not one could assimilate such a huge body of knowledge. Our wish is that the reading of these reports will allow all of them to become aware of the fantastic evolution of pharmacology in the course of these latter years. If one considers pharmacology as the study of the interactions between a "substance" and a living organism, then there is no other interpretation. Nevertheless, one must admit that there exists a period for describing and analysing a pharmacological effect, and that it is only afterwards that the working mechanism can be specified; a mechanism which will permit these "substances" to be used for the dismantling and breaking down of physiological mechanisms, a process which justifies Claude BERNARD'S term, "chemical scalpel".

The reader will be able to profit equally from more down-to-earth contributions, more applied to therapeutics, and less "noble", perhaps, for the research worker. He will realise then that his work, his research and his creative genius are first and foremost in the service of Man, and will remember this statement from Louis PASTEUR:

> "Let us not share the opinion of these narrow minds who scorn everything in science which does not have an immediate application, but let us not neglect the practical consequences of discovery."

I would like to renew my thanks to my colleagues in the Scientific Programme Committee and also to the members of the International Advisory Board, whose advice has been invaluable. I owe a particular thought to J J BURNS, now the past-president of IUPHAR, who granted me a support which is never discussed, and a staunch, sincere friendship. The Chairmen have effected an admirable achievement in the organisation of their proceedings, and in making a difficult choice from the most qualified speakers. The latter equally deserve our gratitude for having presented papers of such high quality, and for having submitted their manuscripts in good time.

The publisher, Robert MAXWELL, has, as always, put his kindness and efficiency at our service in order to carry out the publication of these reports. But none of it would have been possible without the work and competence of Miss IVIMY, whom I would like to thank personally.

My thanks again to the editors of the volumes who, in the middle of the holiday period, did not hesitate to work on the manuscripts in order to keep to the completion date.

Finally, a big thank you to all my collaborators, research workers, technicians and secretaries who have put their whole hearts into the service of pharmacology. They have contributed to the realisation of our hopes for this 7th International Congress, the great festival of Pharmacology. Make an appointment for the next one, in 1981, in Tokyo.

Jacques R BOISSIER

Chairman

Scientific Programme Committee

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# Contents

# Prostaglandins, Thromboxanes and Prostacyclin

# Prostaglandins

**Ulf S. von Euler**

Karolinska Institutet, S-104 01 Stockholm, Sweden

I wish to express my thanks to the sponsors and organizers of this Symposium on Prostaglandins for the invitation to give an introductory talk. The title given to me, "Prostaglandins: discovery, chemistry, assay, possible biological role" covers a wide field, and I hope it will be accepted if I restrict myself mainly to the first and the last part where I can speak with more confidence than in the other sections.

As usual in analogous cases there have been many steps leading up to the discovery of prostaglandins. Biological actions of human seminal fluid on smooth muscle were noted by Kurzrok and Miller in 1930 (1) who used isolated human uterus strips. Shortly afterwards Goldblatt (2) and myself independently observed such actions and also noted a lowering effect on the blood pressure. The chemical nature of the active principle was unknown to all of us at this time. The basic chemical characterization came in 1935 when we were able to show that the active principle was a lipid soluble acid, and it was then given the name "Prostaglandin" (3). A chemically related activity was found in the vesicular gland of the monkey Macacus rhesus and was called "vesiglandin" in order to differentiate it from the human and ovine product which had a stronger effect on intestinal and uterine muscle relative to the blood pressure lowering effect. The chemical properties observed at this time distinguished these bioactive substances from others with similar pharmacological effects, such as acetylcholine, histamine, substance P and others, and it became clear that prostaglandins represented a novel group of hormone-like compounds.

As to the names, prostaglandin and vesiglandin, for these apparently closely related substances, it must be born in mind that the term prostate was often used undiscriminately at this time for the gland proper as well as for the vesicular gland. From our early experiments it was clear, however, that the vesicular gland and not the prostate glands of the sheep was a major source of the active compounds.

The cycling of the active principles between ether or chloroform and an aqueous phase at acid or alkaline reaction became a particularly useful means of purifying the active principles, although the oily character of the active acidic residue was an obstacle for further purification. By treatment with baryta some water insoluble inactive soapy material could be removed, leaving a dry water-

soluble barium salt which was suitable as standard and showed high stability.

Further characterization at this time (before 1940) included the finding that solubility of prostaglandin in a nonpolar solvent like petroleum ether was low, and also some observations suggesting that the active principle was an unsaturated compound. Since the alkali salts were readily soluble in water and dialysable, it was also assumed that the compound was relatively low molecular.

At this state the chemical investigations were continued by Bergström, leading to the isolation in 1957 of the first prostaglandin and the chemical characterization of this group of substances in 1962 (4).

From the early observations on the high concentrations of prostaglandins (PG) in seminal fluid and in the seminal vesicles or the homologous organs, the vesicular glands of some species, it appeared that these organs were the site of origin for the formation of the active compounds. This was shown in experiments by Eliasson (5) who found that on incubation of minced vesicular glands from sheep the amount of extractable prostaglandins strongly increased. Attempts to use arachidonic acid as precursor were not successful at this time, but some years later biosynthesis along the same lines was independently achieved by the research groups headed by Bergström and van Dorp in 1964.

After the clarification of the chemical composition of the active compounds, various members of the PG family could be produced by biosynthesis, in many cases using the biosynthetic enzymes from sheep vesicular gland. Furthermore, partial synthesis could be achieved from the prostaglandin derivative found in large amounts in the gorgonian Plexaura homomalla, and by complete synthesis, making prostaglandins available for study. Metabolizing enzymes were described, and the formation and disposition of PG in the organism was investigated. Improved assay methods led to the important conclusion that PG was formed or could be formed almost anywhere in the organism by means of a synthetase or cyclic oxygenase acting on precursors, occurring in and liberated from lipid fractions of the cells.

Continued work led to the discovery of two new kinds of derivatives, the thromboxanes (6) and the prostacyclin (7) which appear to be of great physiological significance.

The rapid development especially in the chemical sector of the PG:s has naturally raised the question of their physiological functions.

Some of the early observations on the high biological activity of the PG:s on various parts of the vascular system, and on smooth muscle organs like the intestine and the uterus, suggested that PG plays a physiological role in these areas. To what extent PG:s contribute physiologically to the activity pattern of these organs is still debatable, however. At any rate the effects elicited by administration of PG:s are quite marked, such as vasodilatation, fall in blood pressure, and contraction of the gut and the uterus. The effects of PG on the kidney circulation is likely to be of special physiological significance. It has also been noted that some prostaglandins exert a vasoconstrictor action in certain vascular areas.

Of the numerous biological actions which have been reported in the literature I shall only dwell on a few, which seem to me particularly interesting.

The discovery by Vane (8) that aspirin and some other drugs with analgetic and antiphlogistic effects act by inhibiting PG synthesis, has perhaps more than any-

thing else helped to clarify the physiological or pathophysiological actions of the prostaglandins. This finding strongly suggests that many forms of inflammatory pain which are relieved by aspirin in one way or other depend upon PG action. This again suggests that the formation of PG is associated with pain sensations in situation of tissue damage and by its release enhances the pain signals.

In high-doses PGE, causes pain on intradermal or intramuscular injection. It seems, however, that the most important action of PGE is to cause hyperalgesia. PGE, is particularly active in this respect.

When PGE is added to bradykinin or histamine in subdermal infusion strong pain occurs (9). Itching is observed when PGE is combined with histamine. Hydroperoxides of arachidonic, linoleic and linolenic acids increase the intensity of pain. Apparently PGE sensitizes the pain fibres to mechanical or chemical stimuli by a cumulative and long-lasting action. PGE may also cause erythema in low doses and edema, possibly by increasing the effects of histamine or bradykinin.

In certain other instances a physiological function may be ascribed to PG, namely as a regulator of gastric juice secretion and as a modulator of adrenergic neurotransmission. Thus it appears that inhibition of PG synthesis tends to produce gastric hypersecretion sometimes with gastric ulcer formation as final result. Administration of PGE on the other hand strongly inhibits gastric secretion as originally discovered by Robert (10). If cell damage to the gastric mucosa-chemical, thermal, mechanical - causes release of PGE this may effectively counteract an enhanced secretion of gastric juice, induced by the same stimuli, and could thus be of physiological significance and beneficial to the organism.

To a certain extent prostaglandins of the E-type also seem to interfere with sympathetic neurotransmission under physiological conditions. Thus PGE compounds strongly inhibit nerve stimulation responses in various areas as originally found by Hedqvist (11). Moreover, inhibition of PG synthesis tends to increase the nerve - induced response in some organs, suggesting that prostaglandins exert a physiological inhibition.

## Prostaglandins and Reproduction

The finding of large amounts of prostaglandins in the seminal vesicles or in the homologue organ, the vesicular gland of some animals including man, suggests that it has some as yet unknown function in reproduction. Several possibilities might be considered. Firstly: PG may act on the secreting gland itself. Since PG inhibits secretion of gastric juice it is at least conceivable that it may inhibit the secretion of the seminal vesicle, which is the only secretion product, except the bile, which is accumulated and stored as such and subject to intermittend expulsion. In view of the irregular intervals of evacuation a homeostatic mechanism curtailing its formation might well be conceivable.

A second possibility is that accumulation of PG influences afferent impulses from the genital area which may induce sexual activity. Relatively little is known about the role of nerve impulses from the accessory genital glands and their central connections. The same is true for factors, physiological and others, which enhance or suppress the input of such stimuli to the brain, such as metabolites, drugs etc.

Of other possible functions various actions on the genital tract in the female should be considered. Thus a local action on the uterus has been shown to occur with prostaglandin preparations. The first experiments in vivo of this kind were

made some 30 years ago by Karlsson and 10 years later by Eliasson and Posse (12). Since then the contracting effect on the uterus has been studied extensively for the purpose of inducing abortion and supporting labor. It is even possible that prostaglandins play a role in normal parturition as suggested by Karim.

The production of prostaglandins in the seminal vesicles in man is also reflected in the excretion of the major metabolites in urine. From the amounts excreted in male and female urine the synthesis of PGE and PGF has been estimated in the two sexes. Against values of approximately 50-350 μg PGE in 24 hours in males, stand 20-40 μg in females. For PGF the difference is smaller but still appreciable.

Prostaglandins and Platelet Aggregation

A few years ago it was observed (6) that the endoperoxides $PGG_2$ and $PGH_2$ could be transformed to a non-prostaglandin compound which was named thromboxane $A_2$ or $TXA_2$. The corresponding microsomal synthetase system has also been described (13). Both the endoperoxides and $TXA_2$ contract rabbit aorta strips and cause platelet aggregation in vitro.

Another very important step was taken when Vane's group in 1976 (7) discovered that blood vessel microsomes contain an enzyme capable of transforming PG endoperoxides to an unstable compound which prevents platelet aggregation and also acts vasodilating in some areas. The potential usefulness of the new compound, prostacyclin or $PGI_2$, was immediately recognized and a large number of studies have been reported. The discoverers suggested in their original paper that generation of the new compound by vessel walls could be the biochemical mechanism underlying their unique ability to resist platelet adhesion. It appears that there exists a kind of balance between pro- and anti-aggregatory forces in the vascular endothelium and that prostaglandins may play a fundamental role in this process. Thus the enzyme forming prostacyclin can utilize endoperoxides liberated from platelets and thereby counteract platelet aggregation. The effects on vascular width might form a similar balanced system, with a regulating effect on blood pressure. Since prostacyclin is an unstable product a successful utilization of the potentially beneficial effects of the new compound would require that a stable compound of similar biological activity should be found. Progress along these lines have already been reported. Thus Fried and Barton in Chicago have synthesized a "new" prostaglandin which is a stable analog of prostacyclin, being a methyl ester of 13, 14-dehydroprostacyclin. Whereas natural prostacyclin has a half-life of only 5 minutes the new compound retains its full effect for 20 hours or more.

As for the vasodilator effect Kadowitz and his group in New Orleans have shown that the new synthetic compound markedly increases renal and pulmonary blood flow in experimental animals.

The formation of thromboxane $A_2$ by the platelets is inhibited by aspirin, which might suggest that this drug could be helpful in preventing clotting and thereby heart attacks. Clearly aspirin treatment would be likely to influence the formation of prostacyclin as well. At any rate a nation-wide Government-sponsored study is under way in U. S. A. with the aim to find out whether aspirin will reduce clotting.

Adrenergic neurotransmission. As shown by Hedqvist (1970) $PGE_1$ and $PGE_2$ inhibit the mechanical response to nerve stimulation in the isolated perfused cat spleen and also reduce the output of labelled noradrenaline previously added to the preparation. After administration of a PG synthetase inhibitor the response to nerve stimulation is increased. The inhibitory effect of PGE on the mecha-

nical response of the isolated vas deferens of the guinea-pig is particularly striking, in that $PGE_1$ is concentrations of a few ng per ml bath fluid may annul the mechanical response. This effect appears especially at low stimulation rates. The direct effect of noradrenaline on the isolated guinea pig vas deferens is increased by $PGE_1$, indicating that inhibition after nerve stimulation is a prejunctional effect. Other experiments have shown that the guinea-pig vas deferens contains and releases PGE. In agreement with this finding, addition of a synthetase inhibitor increases the mechanical response of the organ to postganglionic nerve stimulation (transmurally).

Similar inhibitory actions of PGE on the adrenergic neurotransmission have been demonstrated in the isolated rabbit heart.

A blocking effect of $PGE_1$ has also been demonstrated on the action potentials of cerebellar Purkinje cells, inhibited by noradrenaline (14).

From this brief and necessarily incomplete review it appears that prostaglandins take part in a number of physiological processes and exert a regulatory function and serve as modulator for a number of mechanisms. Of special interest appears the stimulating effect on adenylate cyclase whereby PG can exert actions on numerous metabolic and other processes. The modulatory effect on nerve transmission likewise seems to open new vistas not only for the peripheral autonomous system but also for the central nervous system. Effects on secretory functions and on platelets exemplify the vast scope of actions. Although the large quantities of PG in the seminal fluid of man and some animals have not been adequately explained, they point to important action in the reproduction area. The actions on the uterus have attracted much interest and led to extensive studies on their applicability for fertility control. The effects on vascular smooth muscle, especially in the kidney, and in the bronchial tree may be of great physiological significance. Finally, the discovery of the mode of action of non-steroid anti-inflammatory agents has revealed a fundamental role of PG in the genesis of pain, fever, and inflammation, thereby throwing light on phenomena which stand in the centre of patho-physiological symptomatology.

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# Prostaglandin Endoperoxides and Thromboxanes: Biochemistry and Biological Role

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Mechanistic studies on the biosynthesis of prostaglandins using isotopic oxygen indicated that an endoperoxide structure was involved as intermediate (1). Several years later two arachidonic acid derived endoperoxides, $PGG_2$ and $PGH_2$, were isolated (for a review, see ref. 2). These derivatives were very unstable ($t_{1/2}$ = 5 min in aqueous medium). Of particular interest was the finding that they showed biological effects (contraction of vascular and respiratory smooth muscle, platelet aggregation) which could not be explained by their conversion to the known prostaglandins. This indicated to us that they had either inherent biological effects or that they were converted to unknown derivatives.

Further work on the role of the endoperoxides in platelet aggregation showed that an aggregating factor formed by human platelets after addition of arachidonic acid could not be due to the endoperoxides $PGG_2$ and $PGH_2$. This led to the discovery of thromboxane $A_2$ ($TXA_2$), which is a highly unstable ($t_{1/2}$ = 30 sec) bicyclic derivative of $PGH_2$ (3-5). It is hydrolyzed to the stable thromboxane $B_2$ ($TXB_2$). $TXA_2$ is a very potent aggregating agent and causes contraction of vascular and respiratory smooth muscle. It is considerably more potent than the endoperoxides.

Recently, in a search for thromboxane forming enzymes using the endoperoxide $PGH_2$ as substrate, it was found that arteries transform this compound into an antiaggregating derivative (PGX) (6). Chemical studies have shown that it is an enol ether (7). PGX is now called prostacyclin ($PGI_2$). The transformations mentioned above are shown in Fig. 1.

The enzyme (thromboxane synthetase) catalyzing the conversion of $PGH_2$ to $TXA_2$ has been solubilized and purified from platelets and lung tissue. It catalyzes also the formation of malondialdehyde and HHT from $PGH_2$. Several inhibitors of the enzyme have been described. The most potent inhibitor described so far, an azoderivative, inhibits $PGH_2$ induced release of ADP and aggregation. This indicates that thromboxane synthetase inhibitors should be of considerable interest for therapeutic control of platelet functions (8).