PROSTAGLANDINS

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PROSTAGLANDINS*

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PREFACE

It was a great pleasure for us to welcome old and new friends to this conference. Three years ago we held a Symposium on Prostaglandins† at the Worcester Foundation, and it was a sad time, for Dr. Gregory Pincus had just died. He spoke often about potential luteolytic and luteotropic factors, and his vision has since been justified, for one of the major developments in the field of reproductive physiology since 1967 has been the demonstration of the remarkable effects of prostaglandins on steroidogenesis, luteolysis, abortion and in termination of pregnancy.

At this recent meeting, owing to the intensity of interest and relevance of prostaglandins to population control, there were three sessions on reproduction, one of which was added during the conference. Unfortunately, some highly significant areas of research could not be included in the program, and to those working in these areas we extend our sincere apologies.

We wish to thank the more than 500 registrants from 28 countries for their attendance, and we are grateful to the Academy staff and our chairmen for all their help and enthusiasm. It is a particular pleasure to thank our organization secretary, Dorothy Schnurr, for helping us from the first letters about the Conference to the typing and proofing of the last pages of the Proceedings.

The photo offset process has been used, since the prime purpose of this Conference was to elicit information and ideas for rapid dissemination by the Academy throughout the world, to all those with immediate interests in the prostaglandins, and also to those who have yet to turn their attention to this remarkable field.

We also wish to express our appreciation and indebtedness to the following organizations for their financial support:

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Jane Shaw and Peter Ramwell
Conference Chairmen

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INTRODUCTORY REMARKS

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Dr. Ramwell and Dr. Shaw, as organizers of this Symposium, sponsored by The New York Academy of Sciences, have kindly asked me to deliver an introductory address. I have accepted this honor with great pleasure, and I wish also to express my cordial thanks for the invitation to attend this Symposium. Many of those present today attended the Nobel Symposium in Stockholm on Prostaglandins four years ago. Since then we have witnessed a steady increase in interest in the field of prostaglandin studies, not the least of which has been in the clinical area. This is also reflected in the growing literature on prostaglandins so carefully collected and generously distributed by Dr. Pike and Dr. Weeks to the benefit of research workers in the field.

It is now about 40 years since Kurzrok and Lieb, in this country, made their original observations on the action of human seminal plasma on uterine strips (Kurzrok & Lieb, 1930). The type of effects observed by them, and later by Goldblatt and me (Goldblatt, 1933, 1935; von Euler, 1934), were at a time when interest was expanding rapidly in the field of hormones and biogenic amines. The classification of the active principle as a fatty acid enabled us to differentiate it from other similarly acting autopharmacologically active compounds. The problem of finding a suitable source of material was solved, fortunately, when we found that sheep vesicular glands contained considerable quantities of the pharmacological activity.

I shall not dwell on the early work in which some hundred kilograms of vesicular glands from young Icelandic sheep were collected and finally provided us with a moderate quantity of a barium salt which served as a provisional standard and was easier to handle than the oily compound from the earlier preparations. I then approached Sune Bergström, my research colleague in the Chemistry Department, who then steadily continued the purification work which ended, as we know, with elucidation of the chemical structure and was followed by studies on biosynthesis by van Dorp and Bergström and their collaborators.

Even during the early period it was possible, with a reasonable degree of confidence, to ascribe a number of biological actions to this group of substances provisionally called, "prostaglandins", because of their high potency and solubility properties. Their occurrence in large amounts in accessory male genital gland secretion suggested that they

may have something to do with genital function. Such actions might be either on the smooth muscle of the accessory male genital organs (the seminal vesicles, prostate and vas deferens) or on the female reproductive organs. One possibility seemed to be a function concerning regulation of the emptying of the male glands. Particularly in the light of some recent observations in several laboratories, including our own, this possibility still appears to merit consideration. It is true that the tissue concentrations of prostaglandins vary within wide limits in different animals, but so does the sensitivity of tissues to these agents. The seminal vesicle secretion in the guinea pig contains only small amounts of active prostaglandins, but the sensitivity of the vas deferens in this animal to PGE_1 and PGE_2 is extremely high, actions having been observed at less than 10^{-10}M concentrations. Furthermore, the contractile effect of noradrenaline on the isolated vas deferens of the guinea pig and other animals is greatly augmented by these prostaglandins. It is therefore conceivable that the action of the released transmitter *in vivo* is enhanced or potentiated in a similar way, which may facilitate expulsion of the gland contents. Under certain conditions the prostaglandins can also inhibit the contraction of vas deferens elicited by electrical stimulation, as will be reported by Dr. Hedqvist during this Symposium. Both the facilitatory and inhibitory effect of members of the PGE series on this organ may represent different facets of a regulating mechanism for the adequate release of male genital products whereby weak stimuli are inhibited and stronger stimuli reinforced. There is some attraction in the concept of a secretion product controlling its own expulsion.

The growing evidence that other members of the same family occur in a variety of organs raises the question what function these may have. The striking contractile effects elicited by human seminal plasma on isolated strips of human pregnant uterus were initially observed. From a study of the contractile pattern of the uterine muscle Eliasson has suggested a role for prostaglandins in facilitating sperm transport in the female reproductive organs. This may even involve the muscular movements of the oviducts.

Considering the varying actions of different members of the prostaglandin family one is reminded of the steroids which serve a multitude of functions, often widely different. Another fact which has gradually been established is the ubiquity of the prostaglandins which are by no means restricted to the sexual organs, even if no other tissues show similar high concentrations as is found in certain male genital glands.

To judge at present what may be the most important physiological function would be largely conjecture. It is tempting, on the other hand, to pay special attention to two kinds of actions, one of which has been known for a couple of years, whereas the second has only been discovered recently. I am referring to the antilipolytic effect of PGE_1 discovered by Steinberg and co-workers in 1963, and to the inhibitory action of adrenergic nerve transmission

discovered by Hedqvist. Both of these effects can be elicited by very low concentrations and may consequently occur in all organs where PGE_1 or PGE_2 are endogenous or are available through the circulation. The inhibitory effect of PGE_1 or PGE_2 on adrenergic neurotransmission is interesting also from the point of view that these are the first naturally occurring factors which act as inhibitors of transmission in very low concentrations. As will be further reported, the action can, under certain conditions, be conducive to complete transmission block *in vitro*.

As to the mechanism of the antilipolytic action of PGE_1 the studies on lipolysis have indicated that it interferes with the formation of cyclic AMP. This raises the question as to whether the action of PGE_1 on adrenergic neurotransmission is also in some way associated with changes in intracellular levels of this cyclic mononucleotide. The connection between PGE_1 and noradrenaline in the transmission process is an intriguing one and would indicate that further studies along this line may be fruitful.

Another effect which seems to be of special interest, both from a theoretical point of view and for possible practical use, is the antinidatory action of $PGF_{2\alpha}$. Further studies on the occurrence and release of prostaglandins in the ovary might add to the interesting hypothesis proposed by Pharriss.

Clearly, only a few aspects of the physiology of the prostaglandins have been touched upon in this brief introductory survey, with an emphasis on physiological action. Many more important findings, particularly the possible role of the prostaglandins in the central nervous system, in which our Conference Chairmen and their group, and Horton and co-workers in England, have made so many outstanding contributions, would merit a thorough discussion. This will no doubt come in the Symposium, and I must ask your indulgence for my rather arbitrary choice of examples. These have, however, seemed to me to make further studies desirable in the future, certainly among several other lines of prostaglandin research.

I have not, on this occasion, entered into the presently favored field of clinical or therapeutical application of various members of the prostaglandin group. Such applications seem to hold many promises and will, no doubt, form an important part of the proceedings of this Symposium.

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THE BIOLOGICAL SIGNIFICANCE OF THE PROSTAGLANDINS

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The biological significance of prostaglandins at the time of this meeting lies primarily in their ubiquity, high potency and remarkable diversity of pharmacological effects. The promise of these compounds lies in three areas, namely cell regulation, pathology and therapeutics.

Distribution

The ubiquity of the prostaglandins in the animal kingdom at least, is currently being established, and of particular value is the demonstration of the 15 epi PGA_2 compounds in coral by Weinheimer and Spraggins (1969); a systematic survey in the plant, as well as the animal kingdom will be useful. Within the individual animal organism the prostaglandins have been demonstrated in most tissues and cells. As indicated earlier (Shaw & Ramwell, 1969), their absence from a particular cell type may prove valuable in obtaining a model for study of the mechanism of prostaglandin action (see Shaw et al, this conference).

Biosynthesis

It is now clear that biosynthesis occurs so rapidly that the values reported for tissue prostaglandin content may not reflect the endogenous level. This is particularly the case in view of four points: (i) bioassays may only reflect the content of the smooth muscle stimulating prostaglandins (i.e., PGE and PGF compounds†), (ii) there are three groups of intracellular metabolizing enzymes present, namely the 15 dehydrogenase, reductases and the β oxidases, (iii) tissue synthesis may be stimulated by a wide variety of conditions such as ischemia, hypotonic solutions, etc. (Ramwell & Shaw, 1970), (iv) the direction of synthesis, i.e., the relative proportion of PGE and PGF compounds formed in tissues from their common precursor, appears to be readily modified. Whether or not qualitative changes in prostaglandin synthesis occur in single cell types on stimulation remains to be determined. Studies of the prostaglandin composition and especially prostaglandin turnover in single cell types are required. Correlation of this information with cyclic AMP concentrations is an attractive goal. Other

†the rat uterus from ovariectomized rats is also sensitive to both PGA_1 and PGA_2 at <5 ng/ml.

studies are needed on the binding and possible storage sites of prostaglandins. Little has been published in this area, but it is clear that prostaglandins bind to plasma proteins and cell membranes in a manner analogous to that of free fatty acids(Shio et al,1971). Also needed is information on the prostaglandin synthetase enzyme complex,and in particular,on the hormone sensitive step which presumably is an acid hydrolase,perhaps a phospholipase A,which releases the polyenoic precursors(see Änggård,1969).

Potency and Diversity of Effects

The highly active nature of the primary prostaglandins on various types of smooth muscle,target organs of the trophic hormones,on certain exocrine functions and on the formed elements of the blood is being established. None of the conventional drug antagonists block the action of prostaglandins,and it is believed that the diversity of action is due to prostaglandins effecting a key regulatory system such as adenyl or guanyl cyclases through which the action of humoral agents may be expressed. Unfortunately,little is known of the effect of prostaglandins on growth or morphology; some changes in feather growth have been observed (Kischer,1969).

Cell Regulation

Exogenous prostaglandins usually result in either increased or decreased intra-cellular concentrations of cyclic AMP. Only a few cell types containing hormonally sensitive adenyl cyclase systems are unresponsive to prostaglandins. The evidence available indicates the need for an intact or nearly intact cell membrane for detecting the inhibitory effects of prostaglandins on cyclic AMP accumulation,which encourages speculation that the site of action in the plasma membrane may be located between the receptor and catalytic sites of the adenyl cyclase system. However, other enzymes such as guanyl cyclase,may be involved,and moreover,there is still little information as to the action of prostaglandins on phosphodiesterase. No account is being taken in the above studies of the effects of humoral stimulation on the endogenous content of prostaglandins. Prostaglandins have also been shown to stimulate ^{32}P incorporation into ATP by isolated mitochondria(Polis et al,1969), and there is presently speculation as to the likely effect of prostaglandins on protein synthesis since there are a number of reports indicating that exogenous cyclic AMP may modify RNA and protein synthesis. In view of the therapeutic potential of prostaglandins,studies should be instituted as soon as possible as to the effects of prostaglandins in modifying cyclic AMP control of RNA (and possibly DNA) (see Pastan & Perlman,1970;Knopp et al,1970).

Pathology

Detection of the release of prostaglandins from inflamed tissue in anaphylactic shock,certain types of tumors and cell damage is significant for the prostaglandins themselves cause whealing,flaring and are leucotactic. These effects,together with the known action of prostaglandins on red cells and platelets indicate that the microcirculation

may be a significant target for prostaglandins released under pathological circumstances, and suggest that some of the symptomatology of shock may be due in part to prostaglandin release; recent results indicate that prostaglandins are released in trauma and shock. Moreover, the direct and indirect effects of prostaglandins on lipid and carbohydrate metabolism raise the question as to the implication of prostaglandins in performance deterioration observed in different types of stress.

Therapeutics

The obvious areas of therapeutics for the prostaglandins per se are abortion, nasal congestion, stomach ulcers, asthma, hypertension, etc. Undoubtedly prostaglandin analogues will have a role, but the protean effects of prostaglandins are so great that the development of specific analogues will be a formidable undertaking and should be an interesting challenge for the students of structure-activity relationships.

However, the real potential is more likely to be in the development of prostaglandin antagonists; probable areas include the treatment of inflammation, premature labor and possibly dysmenorrhea. Yet another approach is to develop agents to inhibit prostaglandin synthesis and metabolism. It possibly goes without saying that every attempt should be made to identify diseases associated with under and overproduction of prostaglandins. A significant step forward is being made by van Dorp and his colleagues with respect to essential fatty acid deficiency.

Conclusions

The development of inexpensive procedures for the supply of prostaglandins by Corey and others should make it possible for the whole of the biomedical community to explore the properties of these compounds. Studies in cell biology and in physico-chemical models will now be possible and should rapidly lead to elucidation of the biological significance of the prostaglandins. Determination of the therapeutic implications of these substances will need prudence and time.

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PROGRAM NOTES ON STRUCTURES AND NOMENCLATURE

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The prostaglandins are cyclic, oxygenated, C_{20} fatty acids based on the prostanoic acid skeleton. In nature they are produced from the corresponding polyunsaturated fatty acids by a microsomal synthetase system. The structures of prostanoic acid, the six primary prostaglandins and their precursors are shown in Fig.1. The other natural prostaglandins (see Fig.2) are related to the E-type of prostaglandins, having suffered loss of the 11-hydroxyl as water, and in some cases bearing an additional hydroxyl group at C-19.

The prostaglandins are grouped according to the type of chemical functionality present, and by the degree of unsaturation. Thus prostaglandins are divided into the F, E, A and B-type by the nature of the five-membered ring functions (Fig.3) and into the mono-, bis- or tris-unsaturated classes, according to the number of carbon-carbon double bonds in the parent E-type prostaglandin. This class designation appears as a subscript in the names of the prostaglandins.

In addition to these features, all prostaglandin structures present the possibility of stereoisomers. However, only one isomer of each type has been isolated from mammalian sources to date. As an illustration, the stereochemical detail of the natural E-type prostaglandin is shown in Fig.4. The heavy and dashed lines are used to represent bonds projecting above and below the plane of reference, respectively, which is taken to be the average plane of the five carbon atoms in the ring. The two side chains project from opposite sides. The oxygen functions are on the same side as the carboxyl side chain.

The prostaglandins of any one class can be chemically inter-related. For example, the reduction of the carbonyl group of E prostaglandins produces two F prostaglandins, differing in configuration at the nine position (Fig.5).

The β-hydroxyketone function of E prostaglandins is not stable to acidic or basic condition. In fact, except in the pH range 5-8, dehydration occurs giving A-type prostaglandins (Andersen, 1969a). The latter rearrange to B prostaglandins under basic conditions (Fig.6).

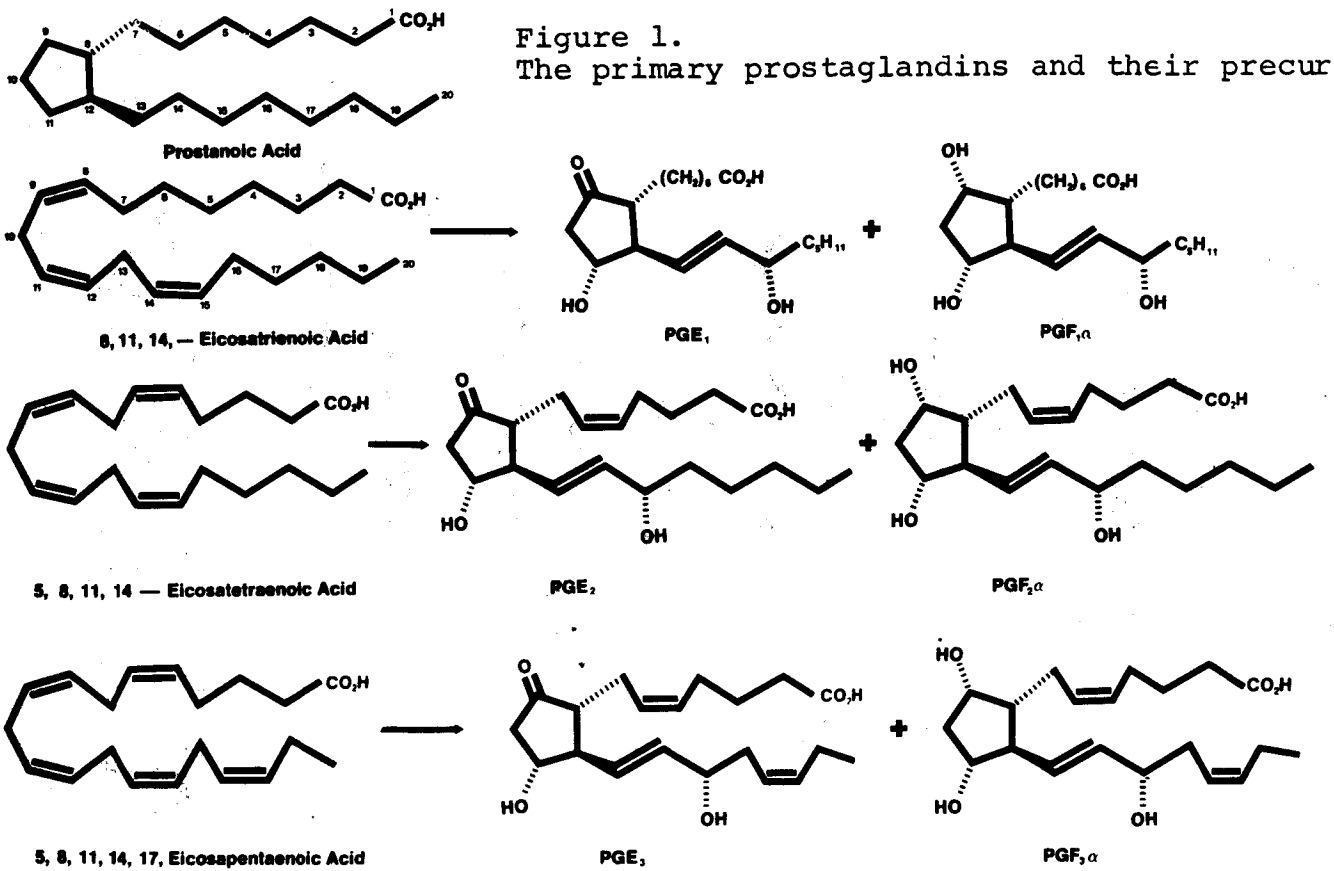
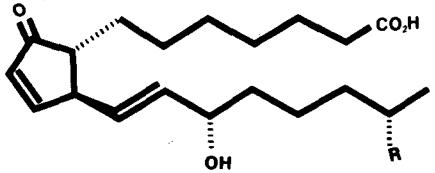
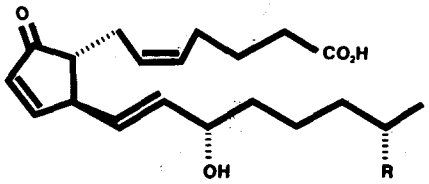


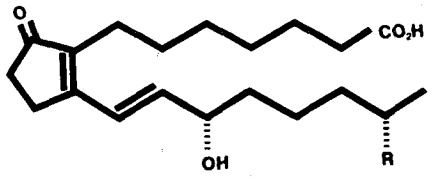
Figure 1.
The primary prostaglandins and their precursors.



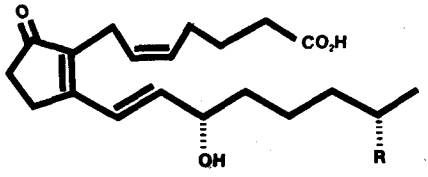
R = H PGA₁
R = OH 19-hydroxy-PGA₁



R = H PGA₂
R = OH 19-hydroxy-PGA₂



R = H PGB₁
R = OH 19-hydroxy-PGB₁



R = H PGB₂
R = OH 19-hydroxy-PGB₂

Figure 2. The naturally-occurring dehydrated prostaglandins.

NOMENCLATURE

A. Fatty Acids

Fatty acids are numbered with the carboxylic acid carbon as number one. The three unsaturated acid precursors of the prostaglandins are numbered in Fig.1. The trienoic acid is known as dihomo-γ-linoleic acid; the tetraenoic acid is