J. Pharm. Pharmac., 1976, 28, 389–392

# Prostaglandins—mediators, modulators or metabolites?

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The ubiquitous occurrence in animal tissues of the enzyme complex responsible for the biosynthesis of prostaglandins from unsaturated fatty acids, coupled with a remarkable variety of pharmacological activities which these compounds possess, has for long prompted investigators to seek a biological role for these substances. The observation that prostaglandin biosynthesis can be virtually abolished by drugs such as aspirin or indomethacin in non-lethal doses might suggest that whatever the role of prostaglandins, their formation cannot be essential for the continued existence of the organism.

Initially prostaglandins had been discovered in human semen and later in sheep vesicular glands—which suggested that they were peculiar to the male reproductive tract—hence the name 'prostaglandin'. Suggestions that this substance might in some way be involved in the processes of erection, ejaculation or emptying of the vesicles were subject to the objection that these mechanisms worked perfectly well in most species whose semen contains either no, or very little, prostaglandin. The role (if any) of these seminal prostaglandins remains a mystery. A further possibility was that prostaglandins in the ejaculate might in some way aid fertilization by an action on the female reproductive tract. Certainly they exert remarkable effects on reproductive smooth muscle—particularly that of the oviduct; moreover, they can be absorbed from the vagina in amounts sufficient to affect oviduct smooth muscle. But the crucial experiments in women would be difficult to perform.

Following the discovery of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) in bovine lung by Bergström, Dressler & others in 1962, the distribution of these compounds beyond the confines of the male reproductive tract, soon became apparent. At Miles-Ames Resesearch Laboratories we began to examine the actions of prostaglandins in other systems. Thus, in 1963, we discovered their actions on respiratory smooth muscle (Main, 1964) and on the cns (Horton, 1964).

The Swedes had previously reported (Bergström & others, 1962) that in the sheep, of all organs examined, the brain alone was devoid of prostaglandin-like activity. Our observations on the central actions of the prostaglandins, however, prompted a re-examination of this matter and Samuelsson reported in 1964 the isolation of $PGF_{2\alpha}$ from bovine brain. Much interest was aroused in the role of prostaglandins in the central nervous system. It was clear that prostaglandins of both E and F series were active on various pathways in the brain and spinal cord and on single neurons in the brain stem (see Horton, 1969, for references). But these observations, however interesting, might simply reflect the pharmacological activity of prostaglandins and have no bearing upon any role (physiological or pathological) in the central nervous system. We were able to identify both prostaglandins as natural constituents of the brain of the cat and chick, the two species in which these prostaglandins were so active pharmacologically (Horton & Main, 1967). Attempts to map the distribution of prostaglandins in different regions of the dog brain were complicated by the presence of at least four prostaglandins. Nevertheless, it was evident that all areas of the brain and spinal cord examined appeared to contain much the same concentration of all four prostaglandins (Holmes & Horton, 1968). Subcellular fractionation of brain homogenates failed to substantiate the findings of others that these substances are confined to nerve ending fractions—by far the largest component being in the cytoplasmic fraction (Hopkin, Horton & Whittaker, 1968).

Attempts to demonstrate the release of prostaglandins from the brain either by examination of ventricular fluid or superfusates failed to show convincing evidence that amounts could be increased by drugs or electrical stimulation, with the one exception that 5-hydroxytryptamine caused increased output of prostaglandin E-like material (Holmes, 1970).

### *Modulators at adrenergic nerve endings*

It was at this point that in collaboration with Davies and Withrington, I was able to demonstrate the presence of prostaglandin $E_2$ ($PGE_2$)-like material in splenic venous blood of the dog during, and after, splenic nerve stimulation (Davies, Horton & Withrington, 1968). Thus it appeared that neuronal activity, in the peripheral nervous system at least, could be associated with increased prostaglandin output. But whatever the role of prostaglandins

might be in the brain, it was quite clear that in the periphery they were not acting as mediators (at least of adrenergic neurons in the splenic nerve). One possibility was that the prostaglandins so released might modify the action of the transmitter at the post-synaptic site. Indeed, we had already shown years earlier that prostaglandin $E_1$ ($PGE_1$) can greatly reduce pressor responses to adrenaline and noradrenaline (Holmes, Horton & Main, 1963). No significant difference in the capsular or vascular responses of the dog spleen to noradrenaline, adrenaline, angiotensin or splenic nerve stimulation could be detected during an infusion of $PGE_1$ at 2·5 $\mu$g $min^{-1}$. A similarly negative result with $PGE_2$ was found against adrenaline and noradrenaline (Davies & Withrington, 1968).

Shortly after these observations were published, various workers in Stockholm began to report that in a variety of systems, $PGE_1$ and $PGE_2$ given intra-arterially can reduce noradrenaline output from nerve endings, the latter being measured directly by fluorimetry or by counting of the labelled transmitter with which the nerve terminals had been previously loaded (see Hedqvist, 1973; Horton 1973, for references). Moreover, treatment with a biosynthesis inhibitor such as eicosatetraynoic acid or indomethacin potentiated the responses to nerve stimulation. Hedqvist postulated that $PGE_2$ released in response to nerve stimulation acts upon adrenergic nerve terminals to reduce release of transmitter in response to continued neuronal activity. A crucial piece of evidence necessary to substantiate this hypothesis is the identification and quantitation of the prostaglandin released in response to nerve stimulation. Much of the evidence for this modulator mechanism is based upon work on the isolated Tyrode-perfused cat spleen, but there is, in fact, very little compelling evidence that that organ will release prostaglandins under anything approaching physiological conditions in response to splenic nerve stimulation. In my laboratory, Bedwani & Millar (1975) therefore set out to compare the output of prostaglandins from the cat and the dog spleen and to identify the isolated material conclusively by mass spectrometry. After much careful experimentation they concluded that whereas the dog spleen releases microgram quantities of $PGE_2$ and $PGF_{\alpha 2}$ (identified by mass spectrometry) in response to splenic nerve stimulation, the cat spleen under similar conditions releases undetectable or barely detectable (low nanogram) amounts. Thus in the dog, splenic nerve stimulation causes copious release of prostaglandins, but these have little or no effect, whereas in the cat, despite the effect of $PGE_2$ on transmitter release, little or no $PGE_2$ is released on nerve stimulation.

A recent paper by Dubocovitch & Langer (1975) throws further doubt on the general validity of this hypothesis. They confirmed the inhibitory effect of $PGE_2$ on transmitter overflow. They were able to detect the release from the cat spleen of a PGE-like substance in response to nerve stimulation, but only after 3 h of saline perfusion. Abolition of the output of this substance with indomethacin did not affect either responses to nerve stimulation or noradrenaline overflow. In contrast, blockade of the prejunctional $\alpha$-adrenoceptors with phenoxybenzamine or phentolamine increased transmitter overflow by 6·5- and 8·3-fold respectively.

Perhaps it is appropriate to recall the words of Davies and Withrington, written in 1968: 'It is possible that the effects of intra-arterially infused prostaglandin have no bearing on the function of prostaglandin $E_2$ which is released from the spleen on nerve stimulation. The functions of the prostaglandin may be concerned with intracellular events at the post-synaptic membrane which cannot be mimicked by injecting prostaglandin'.

*Mediator between uterus and ovary*

At a symposium on 'Drugs of Animal Origin' held at the Mario Negri Institute, Milan in 1966, Professor Jung of Freiberg reported that when two strips of uterine muscle are suspended separately in one organ bath, stretching of one muscle is followed by contraction of the other (Jung, 1967). When N. L. Poyser graduated from the School of Pharmacy in 1968, he set out to follow up this and other observations which suggested that physical stimuli applied to the uterus led to the release of pharmacologically-active substances. One such substance was luteolysin, a substance produced by the guinea-pig uterus and believed to be involved in controlling the life span of the corpus luteum. It was known that the formation of luteolysin could be stimulated by the insertion of foreign bodies into the uterine lumen. Poyser repeated these experiments *in vitro* examining the bathing fluid for substances which contract smooth muscle. He detected a difference in biological activity of fluid from 'distended' and 'non-distended' uteri. The substance differed from all known pharmacologically-active substances except the prostaglandins and the evidence from parallel assays suggested that it could be $PGF_{2\alpha}$. Its identity was proved conclusively by gas chromatography-mass spectrometry (Poyser, Horton & others, 1971). About the same time Pharriss, a reproductive

physiologist working with the Upjohn Company, made the empirical but important observation that $PGF_{2\alpha}$ is luteolytic in the pseudopregnant rat (Pharriss & Wyngarden, 1969). This luteolytic effect was quickly confirmed in other species including the guinea-pig (Blatchley & Donovan, 1969).

Since we had shown that one stimulus to luteolysin production, namely a foreign body in the uterus, could stimulate $PGF_{2\alpha}$ production, the finding of its luteolytic activity prompted us (and others) to test the hypothesis that the uterine luteolytic hormone itself might be $PGF_{2\alpha}$. Oestrogen treatment on day 5 or 6 of the normal oestrous cycle was also known to cause premature luteolysin release. In collaboration with Drs Blatchley and Donovan at the Institute of Psychiatry we examined utero-ovarian venous blood from guinea-pigs on day 7 for $PGF_{2\alpha}$ content. We found that oestrogen-treated guinea-pigs had higher levels of $PGF_{2\alpha}$ than vehicle-injected controls. With the same collaborators we then examined $PGF_{2\alpha}$ output from the uterus on different days of the normal cycle. Until about day 11 little could be detected. There was then an increase reaching a maximum on day 15. This pattern of production fitted well with that expected if it were the natural luteolytic hormone. Poyser also showed that the capacity of uterine homogenates to produce $PGF_{2\alpha}$ increased as the cycle progressed paralleling the *in vivo* output.

We then tried to interfere with prostaglandin production and action by synthetase inhibition and active immunization respectively. Insertion of paraffin wax pellets in the uterine lumen shortened the cycle slightly, but when the pellets had been impregnated with indomethacin to provide a slow release form of this drug, the cycle was greatly lengthened. Similarly, active immunization of guinea-pigs using a $PGF_{2\alpha}$-bovine serum albumin conjugate caused a lengthening of the cycle comparable to that produced by hysterectomy, whereas the control animals had normal cycles. These various lines of evidence suggest that $PGF_{2\alpha}$ is implicated in the control of the oestrous cycle and since it possesses all the properties described for luteolysin it seems reasonable to conclude that the two are identical (see Horton & Poyser, 1976 for references).

One further line of evidence supported this view. In the pregnant guinea-pig the conceptus secretes an anti-luteolytic factor which prevents degeneration of the corpus luteum which would have occurred by day 15 of the normal cycle. Miss Maule Walker and Dr Poyser have, therefore, compared the output of $PGF_{2\alpha}$ from the uterus on day 15 in the non-pregnant and pregnant guinea-pig. In the latter $PGF_{2\alpha}$ output is low (comparable to that normally seen early in the cycle) whereas in the former it was high. Evidently the antiluteolytic factor depresses $PGF_{2\alpha}$ production, which again supports the view that $PGF_{2\alpha}$ has a role as a luteolytic hormone in the guinea-pig.

The local nature of the effect of the uterine hormone has been well documented the hormone produced by one horn having an effect (in the guinea-pig) only on the ovary of the same side. The route by which the prostaglandin formed in the uterus reaches the corpora lutea appears to be vascular. The pathway in the guinea-pig has not yet been elucidated although in the sheep a countercurrent mechanism between the utero-ovarian vein and the ovarian artery has been postulated (see Horton & Poyser, 1976, for references.)

It thus seems fairly well established from this and other work that in guinea-pigs, sheep and a few other species, $PGF_{2\alpha}$ has a physiological role as a *mediator* between the uterus and the corpus luteum thus exerting a control of oestrous cycle length.

*Prostaglandins as metabolites*

Finally, I will consider briefly the possibility that the prostaglandins, despite their remarkable potency, are metabolites with no special function. The release of prostaglandins occurs in response to a wide range of physical and chemical stimuli, accompanying contraction of smooth muscle, glandular secretion, neuronal activity, phagocytosis and distension or stretch. Gilmore, Vane & Wyllie (1969) pointed out that 'structural deformation' of cell membranes appears to be the only feature common to all these situations. Some conformational change associated perhaps with the passage of ions or of carrier molecules might involve chemical changes in the phospholipids—resulting in the cleavage of unsaturated fatty acids which are then converted to prostaglandins as mere by-products of cellular activity. Escape of such pharmacologically-active substances into the circulation would not cause unwanted effects because of the efficient mechanisms in the lungs for their removal and inactivation.

There is evidence that prostaglandins released in some situations do, indeed, represent metabolites of unstable and more active precursors. The recent studies of Nugteren & Hazelhof (1973) and of Hamberg & Samuelsson (1973, 1976) have established that intermediates in the conversion of arachidonic acid to the primary prostaglandins are two, unstable but highly active endoperoxides, which have been named prostaglandin $G_2$ and prosta-

glandin $H_2$. Moreover, $PGG_2$ is a precursor of a new series of compounds, the thromboxanes.

It has been known for some years that attempts to measure levels of prostaglandins in samples of mixed venous blood were liable to be erroneous owing to the release of much larger quantities of prostaglandins from the aggregating platelets at the time the blood was collected. For a while it was believed that $PGE_2$ so released was itself involved in the processes of platelet aggregation—for it was formed and released in response to aggregating agents like thrombin and when added to platelets it could potentiate the aggregating action of adenosine diphosphate. Moreover, blockade of prostaglandin formation with aspirin inhibited platelet aggregation.

It is now established, however, that it is the unstable endoperoxides and thromboxane $A_2$ which are responsible for the aggregation of platelets. These endoperoxides have a half life of a few minutes and are degraded on extraction mostly to $PGE_2$ unless very special precautions have been taken to prevent this conversion and to detect the endoperoxide. Furthermore, most of the endoperoxide is converted in the platelet to the even more unstable product thromboxane $A_2$ which also aggregates platelets. This has a half life of 30 s and degrades to thromboxane $B_2$, a compound lacking in biological activity.

It is conceivable that a similar relationship holds at other sites where the release of $PGE_2$ has been reported. Thus, for example, the $PGE_2$ detected leaving an organ on adrenergic nerve stimulation may represent degraded endoperoxide which has performed some function possibly at or near its site of release, perhaps within the post-synaptic membrane. The discovery of these highly unstable yet potent endogenous compounds poses many problems for those trying to elucidate the biological significance of this group of compounds.

*Acknowledgements*

I should like to acknowledge the valuable contributions of those who worked in collaboration with me at the School of Pharmacy: I. H. M. Main, C. J. Thompson, S. W. Holmes, J. R. Bedwani, Eve Willman, P. B. Marley, Judy Hopkin, Margaret Stewart, P. M. Wright, J. L. H. Laity, Heather Emslie (née Davis), R. L. Jones and N. L. Poyser.

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