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PROSTAGLANDINS, PROSTACYCLIN, AND THROMBOXANES

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John C. McGiff

Department of Pharmacology, New York Medical College, Basic Science Building, Valhalla, New York 10595

INTRODUCTION

Like gold, the future of arachidonic acid metabolites appears boundless. The first prostaglandin metabolites of arachidonic acid, discovered almost twenty years ago, were the stable species, PGE_2 and $PGF_{2\alpha}$ (1) (Figure 1). They arise from unstable intermediates, PGG_2 and PGH_2, the prostaglandin cyclic endoperoxides (2). PGG_2 and PGH_2 also undergo enzymic transformation to unstable products, thromboxane A_2 (TxA_2) and prostacyclin (PGI_2), which have dominated this area of research since their discovery several years ago (3, 4). When at last it appeared that the final entry had been made with the discovery of prostacyclin in 1976, another major pathway of arachidonic acid metabolism was reported in 1979 to give rise to a group of compounds, the leukotrienes, which rival prostaglandins in their complexity and diversity (5).

In 1966, the first symposium dedicated to prostaglandins was held in Stockholm under the auspices of the founding fathers of the field: von Euler and Bergström (6). Thirty-five papers were read in a single session, lasting two days. Few of the seventy-six participants could have predicted the prodigious multiplication of their efforts that occurred over the next decade. von Euler, codiscoverer of prostaglandins, however, recognized their potential. In his welcoming address to the symposium von Euler stated ". . . we have indeed in prostaglandin a unique hormone. . . . its scope of action is still wider, however, and it may well be that the prostaglandins represent a group of compounds which are involved in a variety of actions ranging

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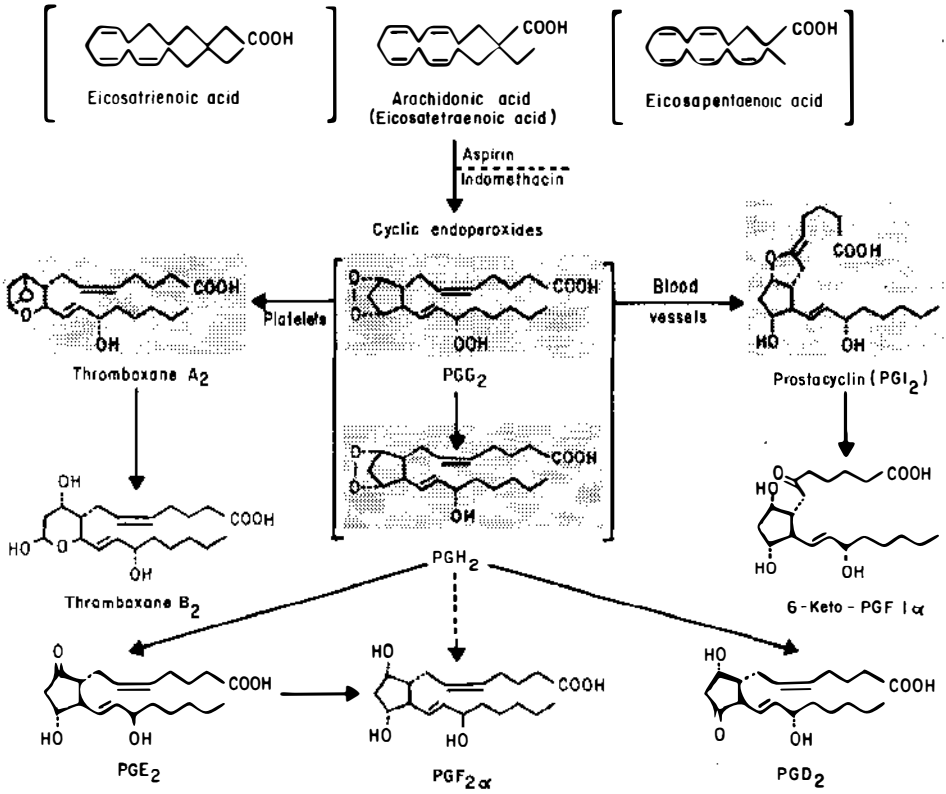

Figure 1 Metabolism of arachidonic acid to form prostaglandins and thromboxanes. The more unstable products are indicated by shading. Eicosatrienoic and eicosapentaenoic acids, bracketed at the top of the figure, give rise to products (not shown) having one and three double bonds, respectively.

from effects on the central nervous system to intricate metabolic actions, thus justifying their very special chemical configuration" (7), a prediction fulfilled within less than a decade. The magnitude of the impact of arachidonic acid metabolites on all fields of biology is evident on reviewing the proceedings of the fourth international prostaglandin conference held in Washington DC in 1979 (8). Three volumes, running to 2000 pages, drew upon 900 authors. The first paper of the conference announced the discovery of leukotrienes (5), which will predictably result in our viewing the biology of health and disease in a new way, just as the prostaglandins did when they captured the attention of the scientific world less than 20 years ago. The leukotrienes generated by a lipoxygenase pathway are not considered in this review; rather the biology of those products formed by the

cyclooxygenase pathway of arachidonic acid metabolism, the prostaglandins and thromboxanes, is considered.

Several of the papers presented at the first symposium described unique properties of prostaglandins that heralded the most important of the recent discoveries in this field. The full significance of these properties could not be appreciated until a decade later when the labile members of the arachidonic acid cascade were identified: PGG_2 and PGH_2 in all tissues (9), TxA_2 in platelets (3), and PGI_2 in the vasculature (4). Platelet aggregation and subsequent adhesion to the blood vessel wall as affected by PGI_2–TxA_2 interactions have occupied center stage since the discovery of prostacyclin in 1976 (4), and rightly so since this area holds great promise, already partially realized, for designing novel measures for the prevention and treatment of vascular diseases (10). This important area was prefigured in the 1966 symposium by the historical paper of Kloeze (11), "Influence of Prostaglandins on Platelet Adhesiveness and Platelet Aggregation," a study that has led to new insights into atherosclerosis and thromboembolism in terms of a dynamic interaction between the endothelium and platelet, one in large part determined by the outcome of thromboxane-prostacyclin interactions. The relationship of prostaglandins to hypertension, which has received a great deal of attention over the past decade, was addressed by two investigators, Muirhead (12) and Lee (13). They were the first to appreciate the importance of prostaglandins and other vasoactive lipids to the regulation of renal function and to the control of blood pressure. Their studies revealed that the renal medulla was a rich source of highly potent vasoactive lipids and set in motion a number of significant studies which identified the importance of prostaglandin-related mechanisms to the regulation of renal blood flow and extracellular fluid volume, as well as to blood pressure control (14).

Two papers presented at the symposium dealt with the capacity of prostaglandins to influence the responses to hormonal stimulation of rat epididymal pads and rabbit renal collecting tubules. These papers opened a Pandora's box for they indicated that the ability of prostaglandins of the E series to dampen the effects of vasopressin on permeability of collecting ducts (15) and to counteract the lipolytic activity of catecholamines, glucagon, ACTH, and TSH (16) was related to prevention of hormonal induced elevation of cyclic AMP levels within these tissues. TxA_2 and prostacyclin have opposing effects on cyclic AMP levels within platelets, effects suggested to be critically related to aggregation of platelets (17). Increased levels of cyclic AMP are associated with the ability of prostacyclin and antiplatelet drugs to inhibit platelet clumping (10). These effects of prostaglandins and thromboxanes on basic cellular mechanisms involving a second messenger help explain their capacity to modulate the actions of diverse

hormonal stimuli, such as catecholamines, angiotensins, and kinins (18). An effect of prostaglandins on a final common pathway, therefore, is implicit in their role as modulators. The concept of prostaglandins as modulators has been extended to the renin-angiotensin (19), the kallikrein-kinin (20), and the autonomic nervous systems (21). It has led to a better understanding of the complex network of interacting hormonal and nervous factors that determines the integration of bodily function as in the respiratory, circulatory, and digestive systems. The pathophysiology of diseases can also be better understood in terms of abnormalities of prostaglandin metabolism, for example, deficiencies as found in hypertension (22), or excessive production as in some forms of arthritis (23).

BIOSYNTHESIS OF PROSTAGLANDINS

With the exception of seminal fluid, prostaglandins are not stored; in response to diverse stimuli prostaglandins enter the extracellular space upon synthesis which is reflected in elevated levels in plasma, urine, and other biological fluids. After removal of the stimulus, prostaglandin levels rapidly subside as a result of metabolism, diffusion, and removal in blood, lymph, and urine. Prostaglandins are derived from twenty carbon polyunsaturated fatty acids of which arachidonic acid is the most abundant; they are usually found in low concentrations as free acids. Release of the precursor from storage in bound form, chiefly phospholipids, is brought about by a group of enzymes, acylhydrolases, such as phospholipase A_2 and triglyceride lipase (24). Hormonal and other stimuli including antigen challenge, thrombin, and collagen which induce prostaglandin synthesis cause release of arachidonic acid from stored forms by stimulating acylhydrolases. For hormones such as kinins and angiotensins which promote prostaglandin synthesis, the intensity and the range of activity of the peptide hormone are affected as a result of the released prostaglandins (14).

The phospholipases of cell membranes, particularly phospholipase A_2, have been assumed to play an important role in prostaglandin biosynthesis because tissue phospholipids are the richest source of arachidonic acid. However, arachidonic acid has been shown to be released by the actions of enzymes other than phospholipase A_2. In the ovary where there is a high concentration of cholesterol arachidonate, cholesterol esterase activity can be stimulated by LH, leading to prostaglandin synthesis (25). Further, one of the mechanisms releasing arachidonic acid from thrombin-stimulated platelets may involve the sequential operation of several enzymes. A phosphatidylinositol-specific phospholipase C forms a diglyceride, followed by hydrolysis of the ester bond linking arachidonic acid with the C-2 hydroxyl

group of the diglyceride which is catalyzed by a diglyceride lipase (26). An alternative mechanism is also based on initial stimulation of phospholipase C with subsequent phosphorylation of the diglyceride, resulting in the formation of phosphatidic acid. The latter stimulates phopholipase A_2 of platelets (27). Similar mechanisms for the release of arachidonic acid may operate in the kidney and vascular wall (28).

After arachidonic acid is released from tissue stores, its conversion into prostacyclin, prostaglandins, and thromboxanes occurs in two steps. The first step is catalyzed by cyclooxygenase which is found in nearly all mammalian cells. This results in oxygenation and cyclization of the fatty acid, forming the unstable intermediates, the cyclic endoperoxides (2). The second step involves enzymes which are tissue specific, and results in the formation of products characteristic of a tissue such as thromboxane in platelets and prostacyclin in blood vessels. The prostaglandin synthase complex of bovine vesicular glands has been solubilized and resolved into two components: the cyclooxygenase, also designated prostaglandin endoperoxide synthase since its reaction products are the cyclic endoperoxides, and the prostaglandin endoperoxide PGE isomerase (29). The responsible activities have been partially purified and, respectively, convert arachidonic acid to PGG_2 and PGH_2, and PGH_2 to PGE_2. The final derivatives that are formed vary with the type of tissue, the physiological state of the animal, and the presence of injury or disease. The importance of the influence of adjacent cells on prostaglandin production by a particular cell type has been studied by Tomasi et al (30). Tomasi et al (30) showed that the rate of formation of PGI_2 by the sinusoidal cells of the liver, which include endothelial elements, can be influenced by contiguous parenchymal cells, the hepatocytes, through an effect on cyclic nucleotide levels in the sinusoidal cells.

Glucocorticoids reduce the capacity of tissues to produce prostaglandins in response to hormonal stimulation (31). As the steroids' effect was overcome by adding arachidonic acid, they were thought to act by inhibiting phospholipase activity. Indeed, the antiphospholipase activity of steroids is highly correlated with their anti-inflammatory potency (32). As several hours were required after administration of glucocorticoids before this effect was evident, inhibition of phospholipase activity, like other steroidal effects, appeared to be the result of changes in protein synthesis.

Nonsteroidal anti-inflammatory drugs, such as aspirin and indomethacin, inhibit cyclooxygenase (33) and will, therefore, prevent formation of the prostaglandin endoperoxides resulting in decreased formation of the final products, the prostaglandins and thromboxanes. Because the capacity of the vascular wall to generate prostacyclin is less affected by aspirin-like drugs

than that of the platelet to form TxA_2 (10), low doses of aspirin are used to inhibit thromboxane generation by platelets in order to prevent thrombosis and embolism in patients at risk. In part, this selective effect of aspirin derives from the inability of platelets to synthesize cyclooxygenase which is inhibited for the life of the platelet, as aspirin is irreversibly acetylated to the enzyme complex (34). In contrast, the vascular wall can regenerate cyclooxygenase (35). The dose of aspirin is critical—probably 200 mg every third day (36) is sufficient to suppress platelet formation of TxA_2 by more than 90%—as the therapeutic objective will be defeated if prostacyclin formation by blood vessels is diminished. However, there are other means available to modify metabolism of arachidonic acid in platelets which will decrease their aggregability. For example, selective inhibition of thromboxane synthesis by an agent such as imidazole (37) has the advantage of sparing prostacyclin synthesis. Perhaps the most intriguing approach, one that does not require taking a drug, is based on diet and, therefore, could lead to prophylactic measures for large populations. Thus, a recent study has suggested that it might be possible to modify the development of vascular disease through diet (38). Two groups were studied: the Eskimos of Greenland, who demonstrate delayed atherosclerosis and low incidence of myocardial infarction, and Danes, who demonstrate the susceptibility of western man to vascular disease. An important difference between Eskimos and Danes, one perhaps accounting for the difference in susceptibility to vascular disease, is the principal polyunsaturated fatty acid found in the lipid fractions of blood, eicosapentaenoic acid in the Eskimo and arachidonic acid in the Dane. Eicosapentaenoic acid differs from arachidonic acid (eicosatetraenoic acid) in the degree of unsaturation, five versus four double bonds (Figure 1). Thus, eicosapentaenoic acid gives rise to products having three double bonds as designated by the subscript in TxA_3 and PGI_3. TxA_3, unlike TxA_2 which arises from arachidonic acid, has been reported to be unable to cause platelet aggregation (39). On the other hand, PGI_3 possesses antiaggregatory activity. Needleman et al (40) have shown that eicosapentaenoic acid is readily incorporated into platelet phospholipids and released simultaneously with arachidonic acid by acylhydrolases. However, eicosapentaenoic acid is a poor substrate for the cyclooxygenase but competes very effectively with arachidonic acid for metabolism by this enzyme, resulting in reduced formation of TxA_2 by platelets and thereby suppressing aggregation. Aggregation of platelets and their subsequent adhesion to the vascular wall may be the initiating lesion in atherosclerosis and is directly related to arterial thrombus formation. These studies offer the remarkable prospect of reducing thrombosis and atherosclerosis through enrichment of the diet with either eicosapentaenoic acid or, its precursor fatty acid, linolenic acid.

IDENTIFICATION OF PROSTAGLANDINS AND THROMBOXANES

The rapid development of prostaglandin and thromboxane research owes a great debt to two methods: gas-liquid chromatography–mass spectroscopy (GC-MS), definitive, precise and expensive; and bioassay, not nearly as specific, more sensitive, and much less expensive. Each method is demanding; when used properly together, they can lead to amazing discoveries. It is not unexpected that bioassay methods should play such an important role in all aspects of prostaglandin and thromboxane research. Prostaglandins were originally discovered in seminal fluid as a result of their ability to affect the state of contraction of smooth muscle (7). Unexpected responses of smooth muscle to biological fluids have historically provided important clues to the presence of previously unrecognized biologically active substances; kinins, substance P, and SRS-A were discovered in this manner (41).

The studies of Bergström between 1956 and 1960, which culminated in the isolation of PGE_1 and $PGF_{1\alpha}$ in pure crystalline form from sheep prostatic glands made use of the rabbit duodenum to trace the biological activity of prostaglandins through each stage of the isolation procedure. "With this biological assay an improved isolation procedure was worked out" (1). Retention of biological activity at each step indicated preservation of the original material, undegraded in the extract. After isolation from the prostate glands, the prostaglandins were resolved by gas-liquid chromatography and identified by mass spectroscopy. GC-MS has become the standard procedure for separation and structural determination of stable arachidonic acid metabolites. Although these procedures were adequate for identification of the stable metabolites of arachidonic acid, which do not degrade rapidly at neutral pH, the labile metabolites evaded identification. TxA_2, for example, has a half-life of 30 sec in solution at neutral pH. The introduction to this field in 1967 by Ferreira & Vane (42) of an adaptation of bioassay methods, the superfusion system, permitted direct assay before degradation of unstable biologically active material in the effluent of organs, in the circulating blood, and in the perfusates of tissues (Figure 2). This method proved to be decisive for the detection and subsequent characterization of labile and biologically active arachidonic acid metabolites, such as TxA_2 and prostacyclin.

Thromboxane

For the identification of TxA_2, the superfusion bioassay technique was the reference assay that provided biological criteria for characterization and subsequent identification of thromboxane. The first description of TxA_2 was

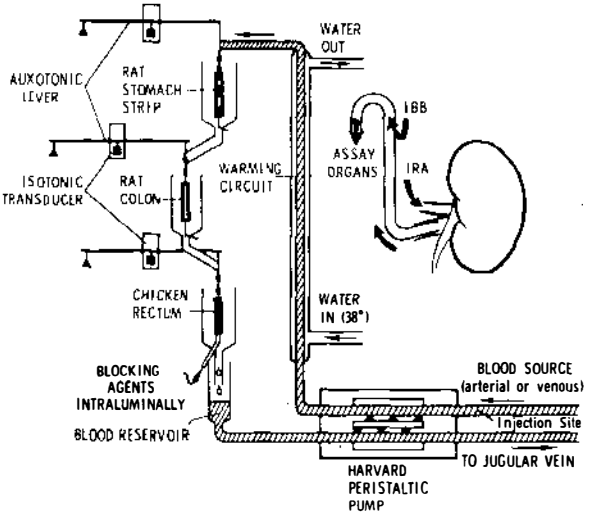

Figure 2 Schematic diagram of blood-bathed organ system (one bank). Blood is withdrawn at the rate of 10 to 15 ml/min by a pump. After traversing a constant-temperature circuit, the blood cascades over three assay organs arranged in series. Changes in the length of the assay organs are transduced and recorded on a multichannel recorder. The blood is collected in a reservoir and returned at a constant rate to the animal. Selective blockade of assay organs is possible by direct intraluminal application of a blocking agent. A major use of this method is shown by the schematized kidney on the right. Thus, a substance may be given into the renal artery (IRA) and its effects on assay organs compared to its direct effects on administration into the extracorporeal circuit IBB (into the bathing blood) indicated by "injection site" in the diagram of the assay system. Indirect effects of hormones may thereby be determined, e. g. release of substances from the kidney by angiotensin II. [Printed with permission of the American Heart Association, Inc. From reference (49).]

based on superfusion bioassay by Piper & Vane in 1969 (43), six years before the chemical structure was announced. They detected the release from the passively sensitized guinea pig lung, when challenged with antigen, of a labile material that contracted rabbit aorta and affected the other assay tissues proportionately much less than stable prostaglandins, musculotropic effects that distinguished it from the known prostaglandins. It was designated rabbit aorta contracting substance (RCS) and, as its release was inhibited by indomethacin, RCS was considered to arise from the cyclooxygenase pathway of arachidonic acid metabolism. Arachidonic acid had been known to be converted in platelets to one or more novel metabolites that stimulated platelet aggregation, an effect which could not be accounted for by any of the prostaglandins then available. The labile cyclic endoperoxides first identified in platelets in 1974 were compared to RCS. They were judged wanting because their half-lives were longer and they had less rabbit aorta contracting activity than RCS (10). Within a year the major product

of arachidonic acid metabolism in platelets was shown to be a novel, unstable compound. It was designated thromboxane A_2 because it was first identified in thrombocytes (platelets) and had a six-membered oxane ring in place of the cyclopentane ring of prostaglandins (Figure 1). It was later confirmed that TxA_2 was also produced by the immunologically challenged guinea pig lung (10) and accounted for most of the activity identified as RCS on bioassay. TxA_2 not only promoted platelet aggregation but constricted blood vessels (10), effects that could be prevented or overridden by PGI_2. The release of TxA_2 probably signifies one form of tissue response to injury. The biochemical studies that resulted in the chemical identification of TxA_2 were conducted by Hamberg, Svensson & Samuelsson (3) of the Karolinska Institute, whose members not only included the founding fathers of the field but also Samuelsson and his associates who, together with Vane and Moncada of the Wellcome Laboratories, have guided and shaped the destiny of this area within the last decade.

Prostacyclin

Prostacyclin was discovered through the inspired use of two bioassay methods, the superfusion system and the platelet aggregometer, by Moncada et al (4). It was originally found to arise from transformation of prostaglandin endoperoxides by a microsomal enzyme of blood vessels and was subsequently shown to be formed by vascular tissues of all species (10). The release from vascular tissues of an unstable substance, a potent inhibitor of platelet aggregation and differing from the known prostaglandins in its effects on assay tissues, suggested the appearance of a novel material. As release could be inhibited by low doses of indomethacin, this substance was considered to be a labile arachidonic acid metabolite arising from the cyclooxygenase pathway. In these studies the aggregometer was used to measure the inhibitory effects of this material on platelet clumping induced by aggregating agents, such as ADP and arachidonic acid. Aggregometry provided the decisive biological criterion, inhibition of platelet clumping, for defining the most important characteristic of the then unknown arachidonic acid metabolite, PGX, as PGI_2 was designated before its chemical structure was identified (44). When coupled to the superfusion bioassay system, the biological characterization of prostacyclin by aggregometry was highly discriminating and distinguished it from all other arachidonic acid metabolites. These studies preceded the announcement of the chemical structure of prostacyclin by one year (45).

The Superfused Blood-Bathed Tissue Assay

The remarkable utility of the superfusion technique is best demonstrated when applied to the detection of prostaglandins and other autacoids in the blood (Figure 2). Studies based on superfusion bioassay have uncovered

interactions of vasoactive polypeptides with prostaglandins. They have greatly contributed to understanding the renin-angiotensin and kallikrein-kinin systems. Prostaglandin-dependent mechanisms are involved in controlling release of the enzymes, renin (46) and kallikrein (47). In addition, prostaglandins in turn released by angiotensins and kinins determine the final effects of these hormonal systems by either amplifying, attenuating, or reversing the effects of the peptide hormones (48).

The first study to identify prostaglandin-peptide interactions made use of stimulation of the renin-angiotensin system by constricting a renal artery of the anesthetized dog (49). Blood was diverted from selected sites within the circulation to superfuse serially arranged assay tissues which continuously monitored changes in the blood levels of prostaglandins and angiotensins; the blood was returned to the venous side of the circulation (Figure 2). This technique permits determining the time course of release of prostaglandins from an organ, an estimate of the amount released in response to a stimulus, and the fate of the prostaglandins after their release into the circulation, e.g. whether they are metabolized on passage across the lungs. Within 2–4 min after constricting the renal artery, prostaglandin-like substances appeared in the venous effluent of the ischemic kidney as determined

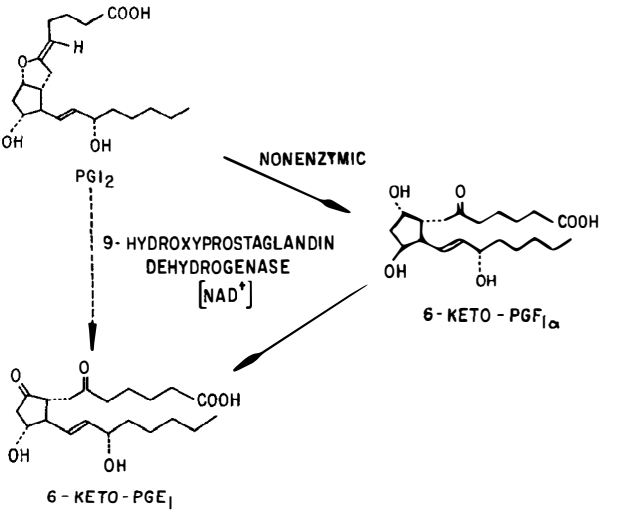

Figure 3 Chemical structures of PGI_2, 6-keto-$PGF_{1\alpha}$, and 6-keto-PGE_1. Spontaneous hydrolysis of PGI_2 forms 6-keto-$PGF_{1\alpha}$, which differs from 6-keto-PGE_1 in the substituent at C-9. The enzyme 9-hydroxyprostaglandin dehydrogenase is probably responsible for transforming the hydroxyl group to a ketone. Alternatively, 6-keto-PGE_1, may be formed from PGI_2 through an unknown intermediary pathway. [Printed with permission of the American Heart Association, Inc. From ref. (90).]

by contraction of the assay tissues. They were not detected by the assay tissues which monitored aortic blood, denoting destruction of the prostaglandins released from the ischemic kidney on passage across the lungs. However, angiotensin-like activity appeared in the aortic blood several minutes after induction of renal ischemia. This was followed within several minutes by the release of prostaglandins into the venous blood of the contralateral kidney. It was concluded that renal arterial constriction caused the rapid release of prostaglandins from the ischemic kidney and that angiotensin II induced the contralateral kidney to produce prostaglandins.

Blood samples were obtained when the assay tissues responded maximally to the released prostaglandins. Assay in vivo of the acidic lipids, extracted and purified in the blood samples, confirmed the estimates of changes in prostaglandin levels in the circulation determined by the superfusion blood-bathed organ system. Further, infusion of angiotensin II in amounts estimated to be present in aortic blood provoked release of prostaglandin-like substances from the kidney (50). With no other method or combination of methods could an integrated picture of the circulatory response to a stimulus have been obtained as simply. This includes radioimmunoassay (RIA) since at the time this study was conducted in 1969, antisera of sufficient specificity to measure prostaglandins in plasma were not available.

Assay of Prostaglandins and Their Metabolites

Levels of PGE_2 and $PGF_{2\alpha}$ in arterial blood are low, normally less than 20 pg/ml, because of the efficiency of pulmonary degradation which removes in excess of 90% of these prostaglandins from the venous blood as it passes through the lungs (42). However, it is possible to measure in plasma the 13,14-dihydro-15-keto metabolites of PGE_2 and $PGF_{2\alpha}$, which have relatively long biological half-lives. Changes in the plasma levels of these metabolites may indicate alterations in prostaglandin metabolism and have been used for this purpose to ascertain changes in prostaglandin formation, such as induced by administration of captopril, the antihypertensive drug which inhibits angiotensin converting enzyme (51).

Venous levels of prostaglandins reflect generation within the vascular territory drained by the vein. They have provided important information on changes in prostaglandin formation by the kidney (52) and gravid uterus (53), which vary with experimental conditions and hormonal background. In resting conscious dogs, renal venous concentrations of PGE_2 and $PGF_{2\alpha}$ are low, usually less than 50 pg/ml (52). They increase by more than tenfold in response to stimulation of renal prostaglandin synthesis by either norepinephrine, angiotensin, or bradykinin (48). Another index of renal prosta-

glandin production is the measurement of urinary levels of prostaglandins which appears to be a reliable index of changes in intrarenal production (54). Changes in total body synthesis of prostaglandins can be estimated by measurement of major urinary metabolites, such as the C-16 metabolite, the tetranor dicarboxylic acid (55). Measurements of tissue levels of prostaglandins are of questionable value because they reflect generation during preparation of the tissue, not in situ levels.

RIA

The importance of validating RIA with another method is evident from reviewing the history of PGA_2. PGA_2, unlike PGE_2 and $PGF_{2\alpha}$, was not destroyed by the lungs and therefore could act as a circulating hormone (56). Because a PGA compound dilated blood vessels, lowered blood pressure, and promoted salt and water excretion (57), PGA_2 was considered to be the renal antihypertensive substance that counteracted those forces that elevated blood pressure. A substance, immunoreactive with antibodies to PGA, was described in the plasma of normal man (58). Based on these studies, it was concluded that concentrations of PGA_2 in the plasma were in excess of 1 ng/ml. However, several studies based on mass spectrometry, which is capable of detecting concentrations of PGA_2 as low as 5 pg/ml of plasma, failed to disclose the presence of PGA_2 in plasma (55). The material in plasma which cross reacted with PGA antibodies, therefore, could not have been PGA_2. PGA_2 presumably arose from nonenzymic dehydration of PGE_2 during storage and sample preparation. These studies indicate that claims of specificity for measurements of prostaglandins by RIA should not be accepted unless verified by other methods, preferably GC-MS. Identification of any substance must be considered tentative until confirmed by chemical methods. Unknown products of arachidonic acid metabolism, or one or more metabolites of the parent compound as well as unrelated material, may cross react with antibody to the prostaglandin being measured. Thus, the plasma concentrations of the metabolites of the major prostaglandins are estimated to be more than fiftyfold those of the parent compound (55).

The lessons to be learned from the experience with PGA_2 cited above have already been forgotten by those who, in their haste to measure circulating levels of prostacyclin, relied on RIA of the stable degradation product, 6-keto-$PGF_{1\alpha}$, of prostacyclin. PGI_2, if released unmetabolized from tissues, may function as a circulating hormone as it escapes pulmonary degradation (10). However, measurements of prostacyclin based on RIA of the stable hydrolysis product, 6-keto-$PGF_{1\alpha}$ may be misleading because of (*a*) possible differences in binding of PGI_2 and 6-keto-$PGF_{1\acute{a}}$ by plasma proteins; (*b*) cross reactivity of antisera to 6-keto-$PGF_{1\acute{a}}$ with prostaglandins and their metabolites, including a newly described active metabolite of prostacy-

clin, 6-keto-PGE_1 (59); (*c*) platelet metabolism and sequestration of PGI_2 and 6-keto-$PGF_{1\alpha}$, both before and during preparation of the sample (60). The operation of one or more of these factors probably accounts for the reported large discrepancies between RIA and bioassay of prostacyclin-related material in the blood (61). Further, prostacyclin can be metabolized rapidly to 6,15-diketo-$PGF_{1\alpha}$ (62) in blood vessels and to 6,15-diketo-13,14-dihydro-$PGF_{1\alpha}$ in the lungs and kidney (63). Measurement of one of the metabolites of prostacyclin should provide a more reliable index of changes in prostacyclin production than measurement of 6-keto-$PGF_{1\alpha}$, which arises nonenzymically. Once again, studies based on superfusion bioassay which measures biologically active material have provided useful information not now available with RIA. This is evident in two recent studies which demonstrated release of prostacyclin-like material from the kidney in response to infusion of either angiotensin II (64) or bradykinin (65) into the renal artery. The clinical studies of Masotti et al (66), also based on the blood superfused bioassay method, have provided important observations on some determinants of circulating levels of prostacyclin-like material. These studies support the concept that prostacyclin is a circulating hormone that can be released by diverse stimuli from the lungs and systemic vasculature into the blood where it affects platelet aggregability. The combined experience with PGA_2 and PGI_2 forces the general conclusion that bioassay has proved to be a more reliable index than RIA of changes in blood levels of biologically active metabolites of arachidonic acid, particularly in the initial phases of the development of an assay.

PROSTAGLANDINS AND FUNCTION

The present stage of development of physiological studies on arachidonic acid metabolites resembles the field of peptide hormones in 1953 when the synthesis of oxytocin was announced, an achievement that resulted in remarkable advances in peptide physiology over the ensuing decades. In the preface to the proceedings of a symposium on peptide hormones held in 1955, Gaddum indicated the potential importance to physiology of naturally occurring potent polypeptides (41), a statement relevant to the present search for physiological roles for prostaglandins:

> The fact that most of these substances are active in very low concentrations suggests that they have physiological functions, and many of those who work in this field must have indulged in wild private speculations based on this fact, but in most cases the evidence has been vague and the speculations have not been published. There can, however, be little doubt that some at least of these substances play fundamental physiological roles.

Most of the important actions of prostaglandins are a consequence of their local effects achieved at very low concentrations, whereby they act as tissue

or local hormones. The potency of prostaglandins is evident from studies which indicate effects of PGE_1 at concentrations less than 10 pg/ml (10^{-11} M), a level that approaches one molecule per cell. At these concentrations PGE_1 has been reported to affect the shape of red blood cells (67) and stimulate formation of cyclic AMP (68).

Prostaglandins are perhaps most important to defensive mechanisms that protect organ function and the integrity of the organism. The contribution of prostaglandin-dependent mechanisms to homeostasis is evident in the cytoprotective function of prostaglandins in the gastrointestinal tract (69), the adaptation of the fetal and maternal circulations during gestation (70), and the support of the renal circulation (52) during severe physical stress and disease or when affected by drug-induced changes, such as depletion of extracellular fluid volume by diuretic therapy. There is a great deal of evidence obtained from studies of diverse tissues and various hormonal systems which indicates the importance of prostaglandins to maintaining organ function when the *milieu interieur* is altered. Prostaglandin-dependent mechanisms are, then, evoked locally in an attempt to reestablish normal function through regulation of blood flow and metabolism.

Prostaglandins, when they act as agents of defense, are released by a variety of stimuli that alter the basal or resting state of the animal. For example, a surge of local prostaglandins in response to tissue hypoxia contributes to reactive hyperemia in skeletal muscle (71). The importance of prostaglandin-dependent mechanisms in facilitating the transition from the basal state to a condition of stress can be viewed at several levels in terms of their participation in the circulatory adjustments that are evoked. Some of the most important roles of prostaglandins are in those intrinsic mechanisms regulating the microcirculation, particularly adjustment of local blood flow to changing metabolic requirements of the tissue (71), as well as to local autoregulatory mechanisms that stabilize blood flow to a tissue. Prostaglandin-dependent mechanisms not only contribute to the response of the microcirculation to the requirements for increased nutrient delivery by increasing blood flow and vascular permeability, but also participate in local metabolic processes, such as glucose uptake (72). As in many of the circulatory actions of prostaglandins, their effects on tissue metabolism and vascular permeability are related to the kallikrein-kinin system. The importance of prostaglandin-kinin interactions to the regulation of the microcirculation was first indicated by Messina et al who demonstrated that indomethacin blunted the dilator action of bradykinin on the microvasculature of skeletal muscle (73). Some of the most important interactions involving prostaglandins and vasoactive polypeptides which operate at the level of the microcirculation occur in the kidney and contribute to the regulation of renal blood flow and its zonal distribution, as well as glomerular filtration rate and salt and water reabsorption.

THE KIDNEY

The general conclusion that prostaglandins act as agents mediating mechanisms of defense and are released by a variety of stimuli which alter the resting state of the animal can be deduced from studies on the renal circulatory effects of prostaglandin interactions with the renin-angiotensin system (52). The relationships of prostaglandins with the renin-angiotensin system are complex and involve the regulation of renin release (46), as well as modulation of the actions of angiotensins (50). Interaction of these systems was first reported in 1970; infusion of angiotensin II increased the concentration of PGE_2 in renal venous blood, associated with blunting of the renal vasoconstrictor and antidiuretic actions of angiotensin II (50). When angiotensin II did not release prostaglandins from the kidney, or when release was inhibited by treatment with indomethacin (74), the renal vasoconstrictor and antidiuretic effects of the peptide were augmented. It was concluded that release of PGE_2 by exogenous angiotensin II modulated the effects of the peptide hormone on renal function. Prostaglandin synthesis by the kidney has been shown to be increased to maintain renal blood flow in the face of stimulation of the renin-angiotensin system by diverse stimuli (52, 75) and was reflected by large increases in the concentration of PGE_2 in renal venous blood (52). In the dog the renal venous concentration of PGE_2 over a hundredfold range was highly correlated with the level of plasma renin activity. The prostaglandin-dependent component of the renal circulation in animals with high plasma renin activity induced by either surgical stress or hemorrhage was exposed by inhibiting prostaglandin synthesis (52). Intravenous administration of indomethacin to the stressed dog caused renal blood flow to decrease as renal production of PGE_2 declined, despite an attendant increase in renal perfusion pressure. In contrast, in the resting dog the activity of the renin-angiotensin system was low and indomethacin did not affect renal blood flow. Stimulation of the renin-angiotensin system by renal ischemia also activated a prostaglandin mechanism which protected renal function. The importance of this mechanism to the rabbit made hypertensive by constriction of one renal artery became evident after administration of indomethacin (76). Inhibition of renal prostaglandin synthesis precipitated malignant hypertension associated with rapid deterioration of renal function in those rabbits in which sustained renal ischemia had developed after clipping the renal artery. Thus activation of the renin-angiotensin system by acute stress or by renal ischemia evokes a prostaglandin-dependent mechanism which supports the renal circulation. Withdrawal of this support results in decreased renal blood flow and elevation of blood pressure. Drug-induced activation of the renin-angiotensin system also elicits this protective mechanism (47). The same mechanism contributes to the maintenance of renal function in patients with renal disease (77) and is

evoked in normal individuals when subjected to the stress of sodium deprivation (78).

The prostaglandin mechanism activated by stimulation of the renin-angiotensin system not only affects total renal blood flow but also influences zonal distribution of blood flow within the kidney, herein providing an example of the generally accepted role of prostaglandins as local hormones (79). Zonal distribution of renal blood flow, e.g. whether more blood is diverted to the outer cortex or to the inner cortex and medulla, is considered an important determinant of salt and water excretion. Itskovitz et al (79) have studied the effects of renal prostaglandin synthesis on the distribution of blood flow in the isolated canine kidney. This experimental preparation is ideally suited to study prostaglandin mechanisms affecting hemodynamics because blood flow in the isolated kidney is highly dependent on renal prostaglandin biosynthetic capacity. As blood levels of PGE_2 increased, reflecting renomedullary production, blood flow increased proportionately more to the inner cortex and medulla than to the outer cortex. Inhibition of prostaglandin synthesis with indomethacin reversed these renal hemodynamic changes. Thus, blood flow to the inner cortex and medulla declined much more than to the outer cortex and was correlated with falling PGE_2 concentrations in the blood. The renin-angiotensin system has also been shown to participate, perhaps, through angiotensin I, in the regulation of blood flow to the same zone, the inner cortex and medulla. Itskovitz et al (80) have obtained evidence that supports a separate role for angiotensin I, that is, one not requiring conversion of angiotensin I to angiotensin II. They have suggested that a balanced mechanism, involving both angiotensin I and PGE_2, participates in the regulation of the medullary circulation, PGE_2 increasing and angiotensin I decreasing renomedullary blood flow. The nephropathy of analgesic abuse has been suggested to be due to medullary ischemia, secondary to reduced synthesis of one or more vasodilator prostaglandins of medullary origin (81), perhaps contributed to by the unopposed constrictor action of angiotensins on renal medullary blood vessels.

Renin Release

The focus of prostaglandin interactions with the renin-angiotensin system shifted in 1974 when it was reported that prostaglandins also participated in the regulation of renin release (46). Administration of arachidonic acid increased renin release. As this effect was prevented by indomethacin, it indicated that transformation of arachidonic acid to a prostaglandin was required. Subsequently, several prostaglandins have been shown to increase renin release. There is evidence that supports the participation of a prostaglandin-dependent component in each of the several signals that affect renin release: tubular, neural, and vascular (82). For example, stimulation of

renin release by a tubular mechanism has been suggested to adjust glomerular filtration rate to the reabsorptive capacity of the individual nephron. This mechanism, referred to as tubuloglomerular feedback, may involve a prostaglandin which induces renin release in response to a signal arising in the renal tubules, such as delivery or reabsorption of sodium chloride at the macula densa (83). It is uncertain that all of the known signals that can release renin must operate through a prostaglandin mechanism. There are studies which do not support this view (84) and which suggest that a prostaglandin mechanism serves only to amplify some of these signals. Nonetheless, the possibility exists that a prostaglandin mechanism may be the final common pathway for the multiple signals capable of releasing renin. This mechanism may operate through effects on cyclic nucleotide levels within the cells of the juxtaglomerular apparatus where renin is formed and stored. Cyclic AMP has been shown to increase renin release (85) and in many tissues the levels of cyclic AMP are affected by prostaglandins (68).

The important question: "Which renal prostaglandin subserves the prostaglandin-dependent mechanism regulating renin release?" remains unanswered. The evidence to date supports prostacyclin, or a closely related compound, as the mediator (84). Renin is synthesized and stored in the vascular pole of the kidney, a site where prostacyclin is the presumed principal arachidonic acid metabolite, as the renal arterial tree has been shown to generate primarily prostacyclin (86). Prostacyclin, when infused into the kidney or when added to the medium containing renal slices, will release renin (84). Further, prostacyclin may be the responsible arachidonic acid metabolite participating in tubuloglomerular feedback. Thus, when this function was blunted by inhibition of prostaglandin synthesis, intraarterial infusion of PGI_2 restored glomerular feedback responses (83). However, there are recent studies which offer an alternative view, that an active metabolite of prostacyclin, 6-keto-PGE_1, is responsible for many of the effects previously attributed to prostacyclin, including release of renin (59). There are several reports of prolonged biological activity of PGI_2, which are difficult to explain in view of its inherent instability. In man, inhalation of PGI_2 resulted in prolonged resistance of platelets to the pro-aggregatory action of ADP (87). In studies using the hamster cheek pouch, ADP-induced thrombi formation had not returned to normal until 30 min after the infusion of PGI_2 had been stopped (88). Finally, after the addition of PGI_2 to renal slices, release of renin was stimulated for more than 30 min (89). These observations are consistent either with a sustained response to PGI_2 that persists much longer than the levels of PGI_2 in biological fluids or with transformation of PGI_2 to a more stable substance with prolonged biological activity.

Wong et al (59), in a study on prostacyclin metabolism in the rabbit liver, first raised the issue concerning enzymic transformation of either prostacyclin or its inactive degradation product, 6-keto-$PGF_{1\alpha}$, to an active and stable metabolite, 6-keto-PGE_1 (Figure 3). Material indistinguishable from 6-keto-PGE_1 by biological and chromatographic criteria was recovered from the liver perfusate. The enzyme responsible for this transformation, 9-hydroxyprostaglandin dehydrogenase, is present in the liver and has been identified in blood vessels and kidney (90) and most recently in platelets (60). Indeed, in view of the recent finding by Wong et al that 9-hydroxyprostaglandin dehydrogenase activity is high in human platelets (60), the question can be raised: Are the effects of prostacyclin on platelet aggregation dependent on its conversion to the biologically active metabolite, 6-keto-PGE_1? Stimulation of renin release previously attributed to PGI_2 may have resulted from its conversion to 6-keto-PGE_1 (Figures 3 and 4). Thus, the renin-releasing potency of 6-keto-PGE_1 is equivalent to or greater than prostacyclin (48). Like prostacyclin, 6-keto-PGE_1 is a potent inhibitor of platelet aggregation (90), reduces blood pressure, and decreases renal (91) and pulmonary vascular resistances (92). It also escapes pulmonary inactivation (91) and therefore may act as a circulating prostaglandin, a role

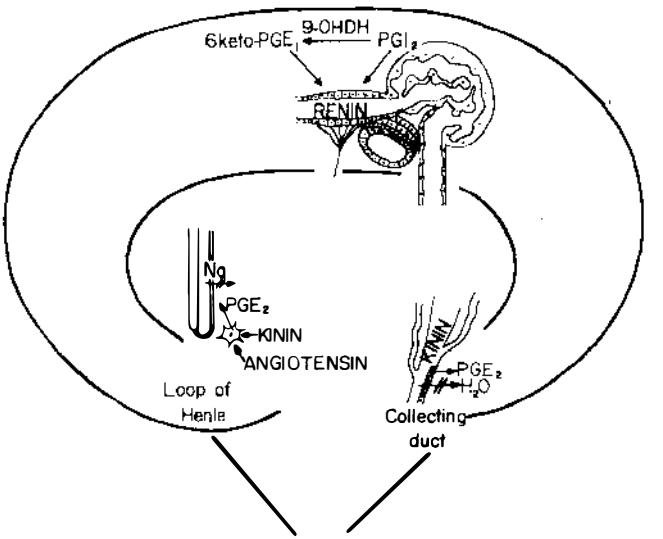

Figure 4 Interactions of the kallikrein-kinin and renin-angiotensin systems with prostaglandins within the kidney. Three major sites have been identified: On the left, within the interstitium of the medulla and adjacent loop of Henle; in the center the juxta-glomerular apparatus including the macula densa, the glomerulus, and afferent and efferent arterioles; on the right, kinin-prostaglandin interactions in the distal nephron and collecting ducts.

which has also been proposed for PGI_2. The highest activity of 9-hydroxy-prostaglandin dehydrogenase in the kidney was found in the cortex and the lowest in the papilla, corresponding to the zonal distribution of renin and prostacyclin.

Regional and Structural Variation in Renal Prostaglandin Synthesis

The prostacyclin-synthesizing capacity of the kidney is concentrated most heavily in cortical blood vessels, a site that favors interactions with renin. All renal arteries thus far examined, including the main renal artery, lobar, lobular, and interlobular arteries with attached afferent arterioles have similar capacities to convert arachidonic acid to prostaglandins, chiefly prostacyclin (86). Isolated glomeruli can also synthesize prostaglandins (93), whereas isolated convoluted tubules have a limited capacity to metabolize arachidonic acid (86). Generation of prostacyclin and 6-keto-PGE_1 by the blood vessels of the renal cortex may be related to the control of cortical vascular resistance, as well as to the regulation of renin release. In addition, either prostacyclin or 6-keto-PGE_1 after release from the kidney may act as a circulating hormone. Within the renal cortex an inhibitor of prostaglandin synthesis has been postulated, as slices or homogenates of the renal cortex have a limited capacity to metabolize arachidonic acid in contrast to isolated cortical blood vessels (86). The presence of an inhibitor of prostaglandin synthesis in the renal cortex could serve as a target for hormones and drugs that affect vascular resistance by influencing levels of vasodilator prostaglandins, such as PGI_2 or PGE_2 within the renal vasculature. For example, the renal vasodilator response to hydralazine, the antihypertensive agent, has been suggested to depend on drug-induced local stimulation of prostaglandin synthesis (94) because of increased efflux of prostaglandins into renal venous blood during vasodilatation (94) and abrogation of the hydralazine response with indomethacin (95).

A number of structures in all zones of the kidney have the ability to synthesize prostaglandins. The interstitial cells of the renal medulla which also synthesize the antihypertensive neutral lipid of Muirhead were first shown to generate prostaglandins (96). For most cell types or tissues, when prostaglandin synthesis is stimulated, a characteristic profile of arachidonic acid metabolites is released which determines the effects of changes in cyclooxygenase activity for that tissue (97). Thus, the pivotal intermediates, PGG_2 and PGH_2, arising from the initial transformation of arachidonic acid, are enzymically transformed to end-products in a manner characteristic for a tissue. That is, tissues are differentially endowed with enzymes that metabolize the prostaglandin endoperoxides to various tissue-specific end-products. The possibility exists that disease states and unphysiological con-

ditions will influence the activity of those enzymes that regulate the breakdown of prostaglandin endoperoxides, favoring the generation of products not usually associated with a tissue. On the other hand, disease or physical stress may cause expression of quiescent pathways of arachidonic acid metabolism in cells that respond only to injurious stimuli as, for example, the production of TxA_2 by the kidney when urine flow was obstructed by ureteral ligation (98). As TxA_2, PGI_2, PGE_2, and $PGF_{2\alpha}$ differ greatly in their biological properties, the principal product of prostaglandin endoperoxide metabolism is of great importance to tissue function. For example, PGE_2 inhibits the release of the adrenergic neurotransmitter (21), attenuates the vasoconstrictor action of angiotensin II, and affects salt and water transport (99), properties not shared with PGI_2. The major end-products of prostaglandin endoperoxides may be restricted to an anatomical compartment; for example, within the lung, the predominant prostaglandin of the respiratory tree is PGE_2, whereas pulmonary blood vessels form primarily PGI_2 (100, 101). In like manner, zones and structures within the kidney vary quantitatively and qualitatively in their capacity to synthesize prostaglandins, which is finally translated into alterations of renal function. Segregation of PGI_2 to the renal vasculature and PGE_2 to the urinary compartment has particular significance for renal function because of the different properties of PGI_2 and PGE_2.

Renal Prostaglandin-Kinin Interactions

In addition to the interstitial cells of the medulla and the renal blood vessels, the cells lining the collecting ducts have a large capacity to generate prostaglandins (102). Synthesis of PGE_2 by these cells and those of the distal nephron or adjacent areas, such as the interstitial cells, is related to the control of salt and water excretion, perhaps through interactions with the kallikrein-kinin system (103) (Figure 4). The conditions are favorable for kinin-prostaglandin interactions in the distal nephron and collecting ducts. Kallikrein has been shown to enter the tubular fluid in the distal convoluted tubules (104), where it liberates kinins from the precursor kininogen. Kinins so generated can affect prostaglandin synthesis by the cells that line the distal nephron and collecting ducts. PGE_2 released by kinins in the distal nephron inhibits ADH (15), contributes to the regulation of medullary blood flow (79), affects salt transport (99), and amplifies the effects of kinins on renal function (105). Studies based on infusion of bradykinin into the renal artery were the first to indicate that kinins could stimulate prostaglandin synthesis (20) and that the released prostaglandins amplified the vasodilator and diuretic actions of bradykinin and may be essential to some of the effects of peptide hormones on blood vessels and renal function (105). However, the contributions of prostaglandins to the renal effects of kinins

are disputed (106). This may derive in part from the inherent limitations in reconstructing the local effects of a hormone from the response evoked by infusion of the hormone into the arterial supply of an organ. For example, renal arterial infusion of bradykinin cannot reproduce the effects produced by stimulation of the renal kallikrein-kinin system as the latter is primarily localized to the distal nephron and the contiguous vasculature. This difficulty has been partially solved by using drugs that stimulate (mineralocorticoids) and inhibit (aprotinin) the kallikrein-kinin system (103), leading to changes in the tissue levels of endogenous kinins.

An intimate relationship between the kallikrein-kinin and prostaglandin systems was suggested from a study conducted in conscious rats which was based on measurements of urinary excretion of PGE_2 and kallikrein, reflecting the intrarenal activity of the prostaglandin and kallikrein-kinin systems, respectively (103). Either augmentation or depression of urinary kallikrein excretion evoked a corresponding change in excretion of PGE_2. Mineralocorticoids were given to increase the activity of the renal kallikrein-kinin system; the kallikrein inhibitor, aprotinin (trasylol), was used to reverse the stimulatory effects of mineralocorticoids on the kallikrein-kinin system. Either desoxycorticosterone acetate (DOCA) or aldosterone, administered over a period of ten to fourteen days in the conscious rat, produced an initial transient antinatriuresis. A diuresis appeared on the second day of steroid treatment and persisted for the remainder of treatment, associated with a threefold increase in excretion of kallikrein and PGE_2. Injection of aprotinin, despite continued treatment with the mineralocorticoid, caused a rapid decline in urinary kallikrein activity and a secondary reduction in excretion of PGE_2, sodium, and water. After three to four days, sodium and water excretion returned to the levels observed before administration of aprotinin despite sustained reduction in excretion of PGE_2 and kallikrein. The importance of renal prostaglandins to the excretion of water was suggested by a surge of prostaglandins into the urine during diuresis induced by the mineralocorticoid. Moreover, the fall in PGE_2 excretion, produced by aprotinin in rats receiving a mineralocorticoid, was associated with decreased water excretion lasting several days.

This study indicates functional coupling of the kallikrein-kinin system with prostaglandins. Further, in the basal state the activity of the renal prostaglandin system is regulated primarily through the kallikrein-kinin system. However, when the animal is stressed, the renin-angiotensin system supersedes the kallikrein-kinin system in determining the level of activity of renal prostaglandins (52). The interrelationships of angiotensins, kinins, and prostaglandins which are best delineated in the kidney (Figure 4) should be considered a paradigm for their interactions within other organs such as the brain, an area of great potential for future studies. These

interactions of prostaglandins and kinins occur primarily at the tissue or local level and are therefore registered within the organ where they originate. The most important findings on prostaglandin-kinin interactions, apart from those occurring intrarenally, are based on studies in vascular tissues.

VASCULAR SYNTHESIS OF PROSTAGLANDINS

Synthesis of prostaglandins by blood vessels was first reported in 1975 (107). Arachidonic acid was shown to be converted to prostaglandins by an isolated preparation of bovine mesenteric blood vessels. The prostaglandin biosynthetic capacity of mesenteric arteries and veins was similar when expressed in terms of unit wet weight of tissue. Bradykinin was found to augment release of prostaglandins from these blood vessels in keeping with previously described effects of the peptide on prostaglandin synthesis. However, there was evidence that bradykinin also affected the activity of prostaglandin-metabolizing enzymes. This possibility arose from the finding that bradykinin, which dilates arteries and constricts veins, caused an increased release of PGE_2 from arteries and $PGF_{2\alpha}$ from veins. The released PGE_2 may reinforce the vasodilator action of kinins as has been described in the kidney (105), uterus (53), and skeletal muscle (73). However, the venoconstrictor action of bradykinin may depend on the capacity of the vein to respond to it by increased synthesis of $PGF_{2\alpha}$, a known venoconstrictor (108). In keeping with this interpretation, contraction of the bovine mesenteric vein evoked by bradykinin was abolished by indomethacin (109). Bradykinin-induced generation of $PGF_{2\alpha}$ in mesenteric veins cannot be due simply to increased delivery of arachidonic acid to the prostaglandin-synthesizing machinery, as that should not alter the ratio of PGE_2 to $PGF_{2\alpha}$ formed. This ratio was 2:1 in untreated veins and arteries, whereas, after stimulation by bradykinin, it was 0.3:1 and 5.7:1, respectively; that is, the peptide caused selective release of PGE_2 from arteries and $PGF_{2\alpha}$ from veins. Bradykinin was postulated to stimulate PGE-9-ketoreductase, which catalyzes the stereospecific reduction of the 9-keto group of PGE_2 to form $PGF_{2\alpha}$. This enzymic activity, present in high concentrations in the cytosol of bovine mesenteric veins, was stimulated by bradykinin (109). Although the enzyme is also found in the cytosol of bovine mesenteric arteries, it could not be stimulated by bradykinin presumably because of destruction of the peptide by one or more kininases. The predominant kininase in the artery must have been kininase II which is identical with angiotensin-converting enzyme (110). Thus, after addition of an inhibitor of angiotensin-converting enzyme to the high speed supernate, bradykinin stimulated arterial PGE-9-ketoreductase (109). An important conclusion of

this study is that kininase II of vascular tissue will restrict the effects of kinins. Further, as potent angiotensin-converting enzyme inhibitors are available, it should be possible to enlarge the sphere of interaction of kinins with prostaglandins. For example, bradykinin has been reported to release PGE_2 from the isolated rabbit kidney (111), whereas arachidonic acid released prostacyclin, suggesting that kinins do not affect PGI_2 synthesis. However, bradykinin may be destroyed by kininase II of blood vessels before it can reach the site of prostacyclin synthesis in the renal vasculature. A study by Mullane et al (65) supports this explanation. They showed that bradykinin-dependent release of prostacyclin-like material into renal venous blood was enhanced by the presence of a converting enzyme inhibitor.

Bradykinin, which has limited access to the cytosol, may stimulate PGE-9-ketoreductase through an intermediate step, one involving cyclic GMP. Cyclic GMP, but not cyclic AMP, mimicked the effect of bradykinin on PGE-9-ketoreductase activity (112). Clyman et al (113) have shown that bradykinin increased the levels of cyclic GMP in human umbilical arteries without affecting those of cyclic AMP. The venoconstrictor action of the kinin has also been found to be associated with increased levels of cyclic GMP (114). Thus, bradykinin-induced constriction of bovine mesenteric veins may be mediated through accumulation of cyclic GMP, the latter increasing the activity of PGE-9-ketoreductase and thereby promoting formation of $PGF_{2\alpha}$. These findings recall two earlier studies: that increased levels of cyclic GMP can be associated with constriction of blood vessels (115) and that the biological activities of prostaglandins of the F series are related to the guanylate cyclase system as those of the E series are related to the adenylate cyclase system (116).

The ability of blood vessels to generate prostacyclin seems to be essential to prevention of platelet aggregation and deposition on endothelial surfaces, events which, as noted, can lead to alteration of the vascular wall and predispose to thromboembolism and perhaps initiate atherosclerosis (10). In some vascular beds prostacyclin probably also subserves vasodilator mechanisms and contributes to vascular tone (117). Terragno et al (70) have shown that fetal blood vessels have the greatest capacity to generate prostacyclin, a property that may be related to the very low peripheral vascular resistance of the fetal circulation. Prostacyclin is also a major product of the utero-placental complex (118) and may contribute to the ameliorating effects of pregnancy in both human and experimental hypertension (119). However, it is incorrect to conclude that prostacyclin is the only important vascular prostaglandin. The hormonal background, as well as the age of the animal and the presence of disease, may affect the prostacyclin biosynthetic capacity of blood vessels. Further, there are blood vessels, such as the umbilical artery and vein in which the major products of prostaglandin

synthesis are PGE_2 and $PGF_{2\alpha}$ (119). As the initial studies on vascular metabolism of arachidonic acid were carried out on large or medium size blood vessels, there was little information on the principal prostaglandin generated by microvessels. A recent study has indicated that the principal product of the microcirculation is PGE_2 (120), a finding which takes on great significance in view of the report of Messina & Kaley (121) that PGE_2, but not PGI_2, inhibited angiotensin II-induced constriction of arterioles. Thus, within the circulation there appears to be longitudinal variation in the principal prostaglandins generated, PGI_2 in the larger vessels, including veins, and PGE_2 in the microcirculation. PGE_2 appears to be the most important modulator of pressor systems and operates within the vascular wall to attenuate constriction induced by pressor hormones and noradrenergic nerve stimulation (18). For example, sympathetic nerve stimulation leads to prostaglandin release (21). The released PGE_2 moderates the effects of sympathetic nerve stimulation not only by counteracting vasoconstriction, but also by inhibiting the release of norepinephrine from nerve endings (21). PGI_2 may also modulate vascular responses to adrenergic stimuli in which case it acts primarily postsynaptically (122). Finally, a prostaglandin mechanism may also function as an integral part of the enigmatic mechanicochemical transducer of the vascular wall. Because of the varied effect of the products of arachidonic acid metabolism, an intramural system mediating either vasoconstriction or vasodilation is a prime candidate to act as the chemical transducer, responding to deformation or distention of the vascular wall by effecting changes in wall tension (123).

CONCLUSIONS

Prostaglandins are synthesized on demand by most cells and primarily act locally as tissue hormones. Prostaglandin synthesis begins with release of arachidonic acid or other 20 carbon polyunsaturated fatty acids from storage in bound form, chiefly by stimulation of phospholipases. Among the most important of the diverse stimuli that increase prostaglandin synthesis are the vasoactive peptides, angiotensins, and kinins. The intensity and the range of activity of peptide hormones are affected by prostaglandins.

Tissues are differentially endowed with enzymes that metabolize the unstable intermediates, prostaglandin endoperoxides, to various tissue-specific end-products, e.g. prostacyclin (PGI_2) for the vasculature, PGE_2 for transporting epithelia of the urinary and gastrointestinal tracts, and thromboxane A_2 (TxA_2) for aggregating platelets. The end-products of arachidonic acid metabolism, TxA_2, PGI_2, PGE_2, and $PGF_{2\alpha}$, differ greatly in their biological properties. Disease states, injury, and stress will influence the activity of enzymes that regulate the breakdown of prostaglandin en-

doperoxides and may favor the generation of products not usually associated with a tissue. Thus, TxA_2 may be produced by the kidney only after injury. Prostaglandins act as agents of defense and are released in response to stimuli that alter the resting state of the animal. For example, they are involved in intrinsic vascular mechanisms that adjust local blood flow to changing metabolic requirements of the tissue, and in those mechanisms effecting nutrient delivery not only by regulating tissue blood flow and vascular permeability but also by participating in local metabolic processes.

Studies on renal prostaglandin mechanisms serve as a paradigm for other organs. The interactions of renal prostaglandins with the renin-angiotensin and kallikrein-kinin systems are complex and involve prostaglandin-dependent mechanisms that regulate renin and kallikrein release, as well as those that modulate the actions of the effector hormones of each system, angiotensins and kinins. In the basal state the intrarenal activity of prostaglandins are regulated primarily by the kallikrein-kinin system. However, when the animal is stressed the renin-angiotensin system supersedes the kallikrein-kinin system in determining the level of activity of renal prostaglandins.

Platelet aggregation and adhesion to the blood vessel wall are influenced by interactions of PGI_2 and TxA_2. Prostacyclin may act as a circulating hormone; it is transformed by some tissues into an active metabolite, 6-keto-PGE_1, which may account for the prolonged platelet antiaggregatory activity and renin-releasing activities of PGI_2. The area of platelet-endothelial surface interaction holds great therapeutic promise through dietary intervention and drug development for modifying vascular disease, thromboembolism, and the initiation and evolution of atherosclerosis by manipulating arachidonic acid metabolism.

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