Cardiac and Renal Prostaglandin I$_2$

BIOSYNTHESIS AND BIOLOGICAL EFFECTS IN ISOLATED PERFUSED RABBIT TISSUES

PHILIP NEEDLEMAN, SUE D. BRONSON, ANGELA WYCHE, and MARK SIVAKOFF,
Department of Pharmacology, Washington University Medical School, St. Louis,
Missouri 63110
K. C. NICOLAOU, Department of Chemistry, University of Pennsylvania,
Philadelphia, Pennsylvania 19104

A B S T R A C T   Both the isolated perfused rabbit heart and kidney are capable of synthesizing prostaglandin (PG) I$_2$. The evidence that supports this finding includes: (a) radiochemical identification of the stable end-product of PGI$_2$, 6-keto-PGF$_{1\alpha}$, in the venous effluent after arachidonic acid administration; (b) biological identification of the labile product in the venous effluents which causes relaxation of the bovine coronary artery assay tissue and inhibition of platelet aggregation; and (c) confirmation that arachidonic acid and its endoperoxide PGH$_2$, but not dihomo-γ-linolenic acid and its endoperoxide PGH$_1$, serve as the precursor for the coronary vasodilator and the inhibitor of platelet aggregation. The rabbit heart and kidney are both capable of converting exogenous arachidonate into PGI$_2$ but the normal perfused rabbit kidney apparently primarily converts endogenous arachidonate (e.g., generated by stimulation with bradykinin, angiotensin, ATP, or ischemia) into PGE$_2$; while the heart converts endogenous arachidonate primarily into PGI$_2$. Indomethacin inhibition of the cyclo-oxygenase unmasks the continuous basal synthesis of PGI$_2$ by the heart, and of PGE$_2$ by the kidney. Cardiac PGI$_2$ administration causes a sharp transient reduction in coronary perfusion pressure, whereas the intracardiac injection of the PGH$_2$ causes an increase in coronary resistance without apparent cardiac conversion to PGI$_2$. The perfused heart rapidly degrades most of the exogenous endoperoxide probably into PGE$_2$, while exogenous PGI$_2$ traverses the heart without being metabolized. The coronary vasoconstriction produced by PGH$_2$ in the normal perfused rabbit heart suggests that the endoperoxide did not reach the PGI$_2$ synthetase, whereas

the more lipid soluble precursor arachidonic acid (exogenous or endogenous) penetrated to the cyclo-oxygenase, which apparently is tightly coupled to the PGI$_2$ synthetase.

INTRODUCTION

An endogenous prostaglandin (PG)[1]-like substance (measured by superfusion bioassay techniques) synthesized by the isolated perfused rabbit heart appeared to be the mediator of coronary vasodilation produced by several vasoactive substances. The evidence that supports this contention includes: (a) the concentration of PG-like-substance in the cardiac venous effluent was directly proportional the concentration of the coronary vasodilator stimulus (e.g., bradykinin, angiotensin II, or exogenous arachidonate); (b) the PG release and coronary dilation produced by the agonists was correlated both temporally and quantitatively; and (c) abolition of cardiac PG biosynthesis by indomethacin abolished the decrease in coronary resistance produced by these agonists (1-4).

An arachidonate metabolite, other than PGE$_2$ and PGF$_{2\alpha}$ may be responsible for the maintenance of coronary resistance. Exogenous arachidonic acid (AA) (the PG precursor) decreased coronary arterial resistance in the isolated perfused rabbit heart (4). Arachidonate also directly relaxed isolated spiral strips of bovine and human coronary arteries (5), which is in contrast to the primary prostaglandins PGE$_2$ or PGF$_{2\alpha}$ which contracted isolated coronary strips (5-8). In addition, inhibition of PG cyclooxygenase in isolated coronary artery strips led to a rapid and sustained increase in coronary tone (5, 7, 8). When the endogenous phos-

[1] Abbreviations used in this paper: AA, arachidonic acid; BK, bradykinin; PG, prostaglandin.

pholipids of the isolated rabbit heart or of isolated bovine coronary artery strips were labeled with [^{14}C]AA (9, 10), the primary metabolite of AA released had chemical and chromatographic properties identical with the biologically inactive 6-keto-PGF$_{1\alpha}$ (10, 12). Exogenous PGH$_2$ also relaxed bovine coronary artery strips, but the endoperoxide itself was rapidly degraded (13–15). A potent coronary relaxant was produced by incubating PGH$_2$ with microsomes from bovine coronary arteries (10, 15). The structure of the labile intermediate between the PG endoperoxides and 6-keto-PGF$_{1\alpha}$, has been chemically characterized to be 9-deoxy-6, 9α-epoxy-Δ^5-PGF$_{1\alpha}$ (termed prostacyclin or PGI$_2$) (16). PGI$_2$ has been discovered to be a potent inhibitor of platelet aggregation (17) and activator of platelet adenyl cyclase activity (18, 19).

The evidence that implicates PGI$_2$ biosynthesis as a major arachidonate metabolic pathway in the isolated rabbit heart is largely based on the appearance of the inactive metabolite 6-keto-PGF$_{1\alpha}$ in the cardiac venous effluent after stimulation (11). A number of additional criteria must be fulfilled to establish the identification and biological role of PGI$_2$ biosynthesis in heart. These criteria include the following: (a) Cardiac arterial resistance changes induced by hormones or changes in oxygen tension should be associated with the appearance of PGI$_2$ or its metabolite (6-keto-PGF$_{1\alpha}$, determined by radiochromatography) in the cardiac venous effluent. The presence of PGI$_2$ in the effluent is supported by its biological properties to relax blood vessel strips and to inhibit platelet aggregation. (b) The precursor fatty acid for PGI$_2$ must be AA and not dihomo-γ-linolenic acid. The latter fatty acid lacks the 5-6 double bond and thus administration of this material should not lead to the production of the (PGI$_2$) coronary relaxant or the platelet aggregation inhibitor. (c) Cardiac administration of cyclo-oxygenase inhibitors should abolish the presence of the coronary relaxant substance that inhibits platelet aggregation in the cardiac venous effluent. (d) The blood vessel relaxant substance (from intact hearts or coronary artery microsomes), should rapidly degrade to the stable end-product 6-keto-PGF$_{1\alpha}$. (e) Administration of exogenous PGI$_2$ should mimic the effect of hormone stimulation or the precursor AA.

The satisfaction of these criteria is the subject of this report. In addition, isolated perfused rabbit kidneys were utilized as a control tissue because its presumed primary arachidonate metabolic pathway led to PGE$_2$ synthesis and not to PGI$_2$.

METHODS

Organ perfusion. The hearts were removed from male New Zealand rabbits anesthetized with pentobarbital and perfused via the aorta at 30 ml/min with Krebs-Hanseleit buffer (37°C in 95% O$_2$/5% CO$_2$). The kidneys were perfused via the renal artery at 15 ml/min.

Superfusion bioassay. The venous effluent from either the perfused heart or kidney continuously bathed assay tissues hung in a vertical superfusion cascade (1–4). Bovine coronary artery was excised from freshly removed hearts and spiral strips were prepared (5). The bovine coronary artery strip is contracted by PGE$_2$ (5–7) and PGH$_1$ (12) and relaxed upon exposure to PGH$_2$, PGI$_2$, or PGE$_1$ (12, 14). 6-Keto-PGF$_{1\alpha}$ (0.1–5 μg) caused no detectable response on the bovine coronary strips. Direct application of arachidonic acid would relax the coronary artery strips (5), but the assay tissues were continuously treated with indomethacin (300–1,000 ng/ml) and therefore were unresponsive to exogenous or endogenous AA. The chick rectum and occasionally the rat fundal stomach strip were employed to indicate the presence of a PGE$_2$-like-substance in the venous effluent. The assay tissues were also continuously perfused with a mixture of antagonists to render the strips insensitive to catecholamines, serotonin, acetylcholine, or histamine.

Platelet aggregation bioassay for the presence of PGI$_2$ in cardiac or renal venous effluent. Citrated human platelet-rich-plasma was prepared as previously described (20). The infusion of indomethacin and the mixture of antagonists across the assay tissues in the superfusion cascade was interrupted 5 min before the collection of the venous effluent for testing on platelets. Thus, these inhibitors did not interfere with the platelet assay. The venous effluent from a perfused heart or kidney was collected below the assay tissues for 3 min after organ stimulation. The pooled effluent was well mixed and a 50-μl aliquot was taken and added to 400 μl of platelet-rich-plasma and incubated for 2 min with stirring at 37°C in a Payton Dual Channel aggregometer. Aggregation was initiated with AA.

Radiolabeling technique. The method of incorporation of radioactive AA into the heart or kidney lipids has been described in detail elsewhere (9). Briefly 5 μCi of [^{14}C]AA (Amersham Corp., Arlington Heights, Ill., 55 mCi/mmol) prepared as the sodium salt in saline (pH 9) was infused through the coronary or renal vascular bed of the isolated perfused organs for 20 min. The [^{14}C]arachidonate is incorporated into endogenous tissue lipids (primarily in phospholipids) which can be released in the form of ^{14}C-labeled PGs by agents which stimulate PG biosynthesis (9, 21). The venous effluent was collected and was acidified with 2 N formic acid to pH 3.5 and extracted with 2 vol of ethyl acetate. The combined organic extract was dried over anhydrous Na$_2$SO$_4$, vacuum concentrated, and applied with unlabeled standards to silica gel G (Brinkmann Instruments, Inc., Westbury, N. Y.) thin-layer chromatography plates. The solvent system (A-9) employed consisted of: ethyl acetate:acetic acid:2, 2, 4-trimethyl pentane:water-110:20:50:100 (organic phase). The PG standards were visualized by iodine staining, the radioactive peaks were detected on a Vanguard Scanner (Vanguard Instrument Corp., Melville, N. Y.).

Materials. PG standards were the gift of Dr. John Pike, (The Upjohn Co., Kalamazoo, Mich.). The prostaglandin endoperoxides PGH$_2$ and PGH$_1$ were synthesized with sheep seminal vesicle acetone-pentane powder and purified as previously described (22, 23). PGI$_2$ was chemically synthesized as previously described (24). Authentic 6-keto-PGF$_{1\alpha}$ and PGI$_2$ were also kindly supplied by Dr. Pike and the Upjohn Company.

Bovine artery microsomes were prepared from freshly dissected vessels. The arteries were homogenized (Polytron, Brinkmann Instruments, Inc.) and centrifuged for 10 min at 10,000 g. The supernate was centrifuged for 60 min

at 100,000 g. The pellet was resuspended in a 50-mM phosphate buffer (pH 7.4) and stored at −70°C. The 15 hydroperoxy AA (an in vitro PGI_2 synthetase inhibitor [25]) was synthesized enzymatically with soybean lipoxygenase and purified as previously described (26).

RESULTS

Determination of the cardiac release of a vasodilator substance with bioassay techniques. The isolated rabbit heart was perfused with Krebs-Hanseleit media and the venous effluent continuously bathed a series of isolated smooth muscle strips selected for their responsiveness to PG. Intracardiac injection of AA (5,8,11,14-eicosatetraenoic acid = C20:4) or bradykinin (BK) resulted in the appearance in the cardiac venous effluent of a substance which relaxed the bovine coronary artery assay strip (Fig. 1), associated with a concentration-dependent decrease in coronary perfusion pressure (1, 4). In contrast, intracardiac dihomo-γ-linolenic acid (8,11,14-eicosatrienoic acid=C20:3) resulted in the appearance of a substance in the venous effluent which contracted the chick rectum assay tissue (suggestive of PGE_1) and contracted the bovine coronary artery (suggestive of PGH_1). The lack of the 5-6 double bond in

dihomo-γ-linolenic acid precludes its conversion to PGI_2 (11). The infusion of indomethacin into the perfused rabbit heart resulted in the simultaneous contraction of the bovine coronary strip and relaxation of the chick rectum which were continuously bathed with the cardiac venous effluent (Fig. 1). Since the cyclo-oxygenase in the assay tissues was previously inhibited by indomethacin applied directly across the assay tissues but not into the heart, the contraction of the bovine coronary strip produced by the intracardiac indomethacin reflects the disappearance of the low concentration of continuously released PGI_2 from the cardiac effluent. The infusion of indomethacin through the heart inhibits the cardiac PG biosynthesis induced by BK (not shown), and the fatty acids (Fig. 1).

The intra-cardiac injection of the potent peptide vasoconstrictor (on isolated blood vessels and on blood pressure) angiotensin II actually caused a decrease in cardiac perfusion pressure in the constant flow perfused heart (as did BK or transient cardiac ischemia) and resulted in the appearance in the cardiac effluent of a substance which relaxed the bovine coronary artery strip (Fig. 1). However, when indomethacin was infused into the heart, intra-cardiac administration of
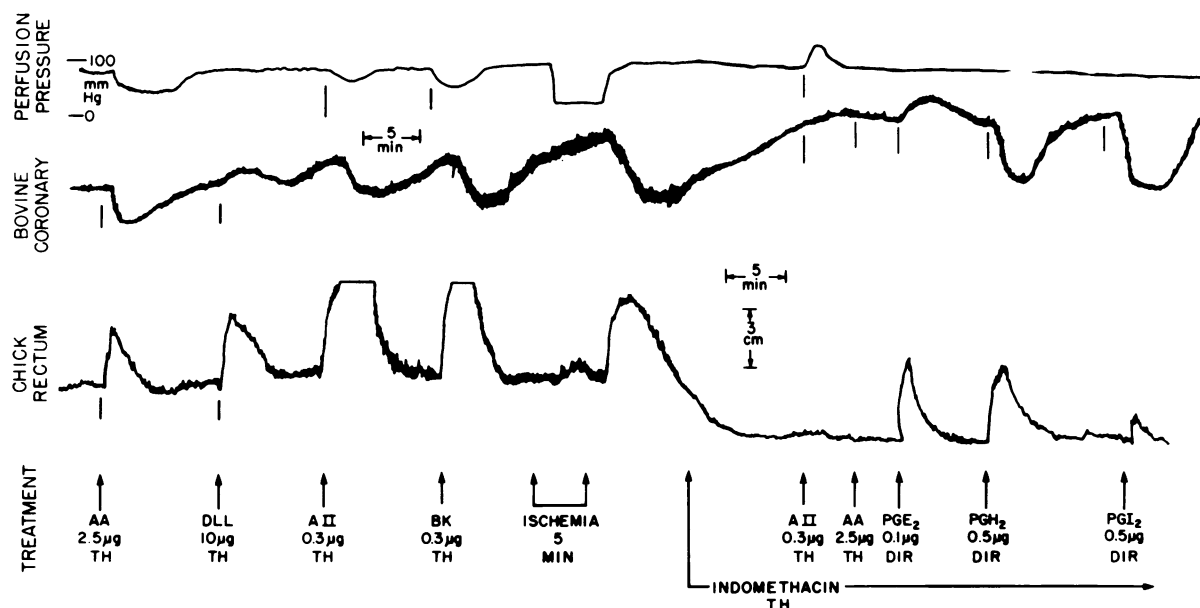

FIGURE 1   Response of the isolated perfused rabbit heart and the superfused assay tissues to fatty acid or hormonal stimulation and to changes in oxygen tension. The following abbreviations were employed: AA, arachidonic acid; DLL, dihomo-γ-linolenic acid; A II-angiotensin II; BK, bradykinin. The numbers in the treatment section indicate nanograms of agonist employed. DIR denotes direct application of available standards to the assay tissue and TH denotes injection through the heart. Ischemia was produced by diverting the flow of media away from the heart (by means of a three-way stopcock) and directly across the assay tissues. 20 min was allowed to elapse after intracardiac indomethacin (300 ng/ml) treatment. The bioassay experiments were each repeated five times with the same results. An angiotensin antagonist, [Sar[1], Ile[8]-A II] was continuously infused across the assay tissues to eliminate any direct effects of A II itself.

angiotensin resulted in increased vascular resistance in the perfused heart and no vasoactive substance was present in the effluent (Fig. 1).

Renal PGI$_2$ biosynthesis. For comparative purposes we studied PG biosynthesis in isolated perfused rabbit kidney. BK administration primarily induces renal PGE$_2$ (but not PGI$_2$) biosynthesis as evidenced by bioassay (contraction of both the chick rectum and bovine coronary artery strip) (Fig. 2 and [27]). Similarly, injection of angiotensin II or ATP through the kidney produced only PGE$_2$ in the effluent (27). Surprisingly, administration of exogenous AA (but not dihomo-γ-linolenate) to the perfused rabbit kidney resulted in (*a*) decrease in perfusion pressure (not shown), and (*b*) release of a substance which relaxed the bovine coronary strip which is suggestive of the PGI$_2$ (Fig. 2). Renal administration of indomethacin resulted in a simultaneous decrease in smooth muscle tone in the bovine coronary assay strip and the chick rectum (Fig. 2) which suggests that the kidney was continuously synthesizing and releasing mostly PGE$_2$.

Analysis of cardiac and renal effluents for a substance that inhibits platelet aggregation. Having established that the heart and kidney released substances with PG-like biological activity which was suggestive of the presence of PGI$_2$, we then sought to resolve if the vasodilator also inhibited platelet aggregation. The venous effluent from the perfused organs (obtained in the same experiments that indicated the presence of bioassayable PG, i.e., Figs. 1 and 2) was pooled and an aliquot was incubated with human platelet-rich-plasma and aggregation studies were performed (Fig. 3). Control samples were collected before hormone or fatty acid stimulation. The effluent obtained from the untreated heart did not contain an adequate basal level of PG to alter AA induced aggregation (Fig. 3, upper left-hand tracing). BK (500 ng but not 50 ng) injection into the perfused rabbit heart resulted in the presence in the cardiac venous effluent of a substance which inhibited platelet aggregation. Similar cardiac biosynthesis of an inhibitor of platelet aggregation was produced by AA and ischemia, but not by dihomo-γ-linolenic acid (Fig. 3, upper panel). Treatment of the heart with cyclo-oxygenase inhibitors (either indomethacin or aspirin) abolished the presence of the inhibitor of platelet aggregation in the cardiac effluent normally produced by BK, AA (Fig. 3), angiotensin II, or ischemia (not shown). Thus, treatment of the perfused rabbit heart with AA (but not dihomo-γ-linolenic acid), bradykinin, ischemia, or angiotensin II simultaneously resulted in (*a*) a decrease in cardiac perfusion pressure, (*b*) the release of a vasodilator substance, and (*c*) the release of an inhibitor of platelet aggregation. These three responses were blocked by indomethacin or aspirin treatment of the perfused heart.

In the kidney, (Fig. 2), bradykinin and dihomo-γ-linolenate caused the release of a coronary constrictor (Fig. 2) which did not inhibit platelet aggregation (Fig. 3, lower panel). On the other hand, exogenous AA: (*a*) decreased renal resistance, (*b*) released a coronary relaxant, (*c*) released a platelet aggregation inhibitory substance, and (*d*) all these AA responses were also blocked by indomethacin.
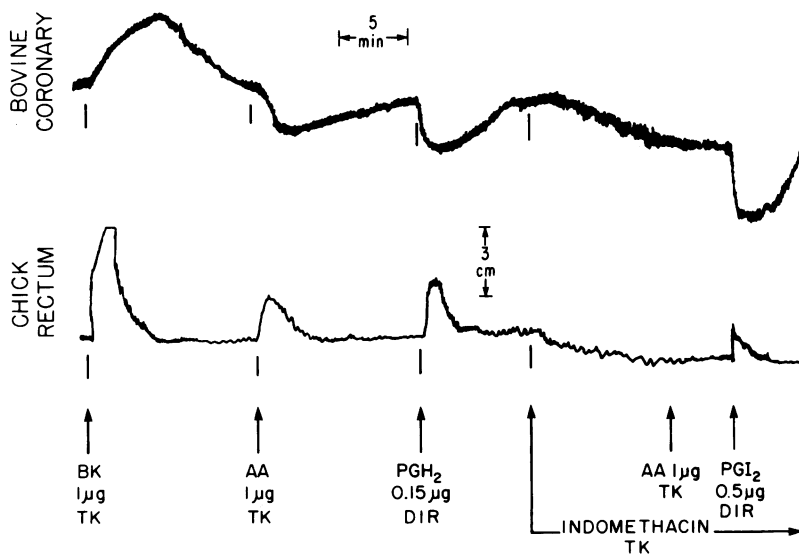

FIGURE 2 Prostaglandin bioassay of the renal venous effluent after BK or AA stimulation. The indomethacin concentration in the media perfused through the kidney was 600 ng/ml. TK denotes injections through the kidney and DIR denotes direct application of standards to the assay tissues.
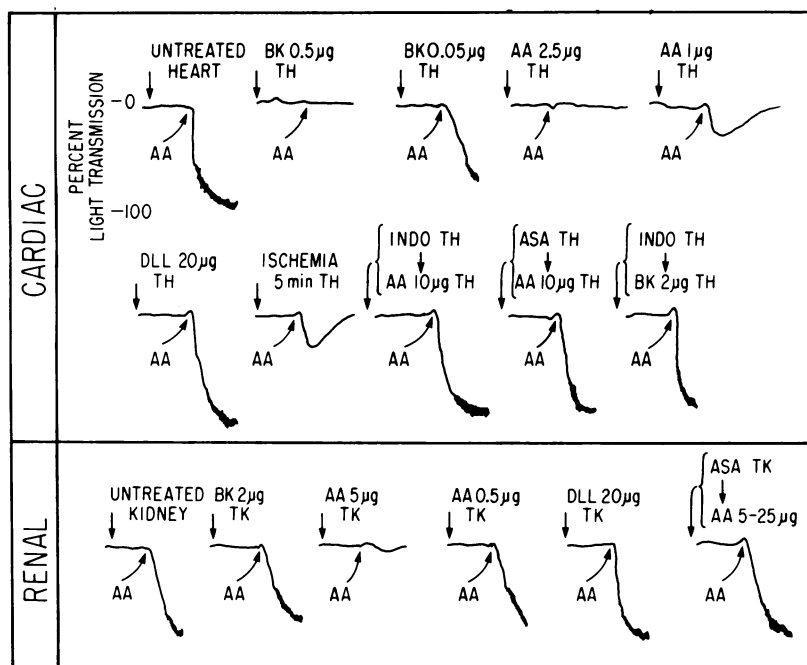
FIGURE 3   The influence of venous effluent obtained from the perfused rabbit heart or kidney on the aggregation of human platelet-rich-plasma. The following abbreviations were employed: AA, arachidonic acid; BK, bradykinin; DLL, dihomo-γ-linolenic acid; INDO, indomethacin; and ASA, aspirin. Untreated organs indicates collection of venous effluent of the perfused tissues before hormone stimulation. The arrow above the aggregometer tracing indicates the point of addition of the 50-μl venous effluent. 3 min later exogenous AA was added to the platelets (second arrow below the trace) to initiate aggregation. Platelet aggregation was completely inhibited by pretreatment with PGI_2 (0.2–10 ng). The metabolite 6-keto-$PGF_{1\alpha}$ had no effect on platelet aggregation in the doses tested (1–500 ng). Each experiment was repeated at least four times with the same results.

Radiochemical identification of the PG present in the cardiac and renal venous effluent. To further identify the PG metabolites synthesized we infused the perfused rabbit heart or kidney with [^{14}C]AA. The venous effluent was analyzed for radioactive products by thin layer chromatography after acid-lipid extraction. Approximately 90% of the infused label is incorporated into the cardiac phospholipids (9, 21). During the infusion of the exogenous [^{14}C]AA into both the kidney and the heart, the major detectable stable products present in the renal and cardiac effluent were 6-keto-$PGF_{1\alpha}$, with some PGE_2 and $PGF_{2\alpha}$ also present (Fig. 4, upper panel). In contrast, upon bradykinin stimulation of the perfused kidney the only detectable product was PGE_2, whereas the heart produced both 6-keto-$PGF_{1\alpha}$ and PGE_2 (Fig. 4, lower panels).

Administration of dihomo-γ-linolenic acid to the heart did not result in the release of either a substance which relaxed the coronary strip or which inhibited platelet aggregation. Furthermore, [^{14}C]PGE_1 was the primary radioactive product obtained after intra-cardiac infusion of [^{14}C]dihomo-γ-linolenate (11).

Thus, exogenous AA in the kidney or exogenous or endogenous AA in the heart release PGI_2 (determined by bioassay using blood vessels and platelets) and the stable PGI_2 metabolite, 6-keto-$PGF_{1\alpha}$ (determined by extraction and radiochromatography.

Microsomal generation of PGI_2. Direct application of PGH_2 to assay tissue caused an immediate contraction of the rabbit thoracic aorta strip and a slightly delayed relaxation of the dog (or bovine) coronary artery (Fig. 5). In contrast, when the same amount of PGH_2 was incubated (2 min, room temperature) with bovine coronary microsomes to enzymatically generate PGI_2, the PGH_2 activity (aorta constriction) disappeared and an immediate profound coronary relaxation was produced (Fig. 5). The relaxing activity disappeared if a 15-min delay was permitted between incubation and testing. The radioactive product obtained from long-term (15-min) incubations of [^{14}C]PGH_2 with blood vessel microsomes, was a mixture of 6-keto-$PGF_{1\alpha}$ and PGE_2 (Fig. 6). Incubation of PGH_1 (no 5-6 double bond) with bovine coronary artery microsomes did not result in disappearance of the PGH_1-induced aorta contraction nor an alteration in the contractile effect on the coronary assay tissue (Fig. 5). The incubation of bovine coronary microsomes with [^{14}C]PGH_1 resulted in the appearance of label that co-migrated with PGE_1 and
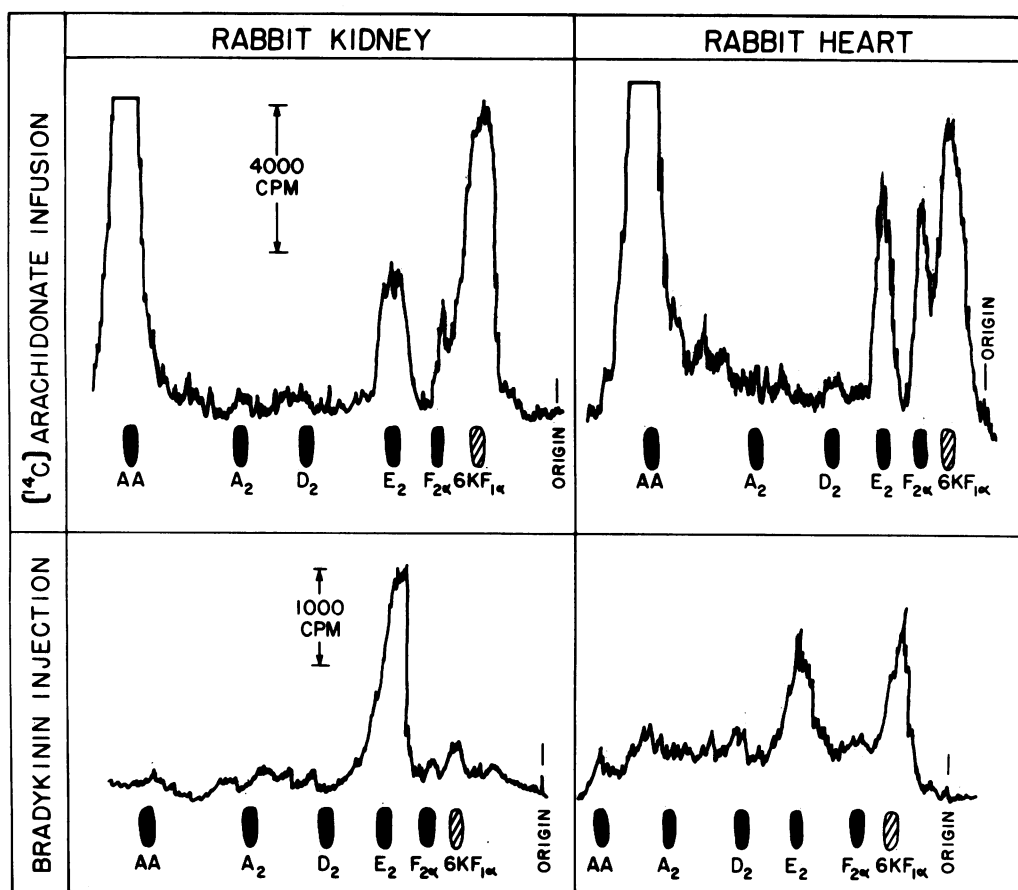
FIGURE 4 Radiochromatogram of the acid-lipid extract of the cardiac and renal venous effluent after [¹⁴C]arachidonate administration or BK injection. The primary PG standards added to the extract and visualized with I_2 vapors are indicated at the bottom of the figure. The upper panels show the chromatogram obtain during loading of the tissues with [¹⁴C]arachidonate. The lower panel represents the chromatogram obtained from the acid-lipid extract of the venous effluent of the prelabeled organs after injection of BK into the heart (0.5 μg) or the kidney (2 μg). $6\text{-Keto-PGF}_{1\alpha} = 6\text{KF}_{1\alpha}$.

PGD₁ and no radioactivity was detected in the 6-keto-PGF₁α zone (Fig. 6). Analogous results were obtained in the isolated perfused rabbit heart. [¹⁴C]AA was converted by the heart into 6-keto-PGF₁α and PGE₂; whereas [¹⁴C]dihomo-γ-linolenic acid (the precursor of PGH₁) was only converted to PGE₁ (11). Additional confirmation of the synthesis of PGI₂ is illustrated by the selective inhibition of 6-keto-PGF₁α by preincubations of the blood vessel enzyme with 15-hydroperoxy-arachidonic acid (Fig. 6 middle panel). PGH₂ was 10% as potent as PGI₂ in directly relaxing the bovine coronary artery assay tissue, whereas 6-keto-PGF₁α was inactive (0.1–10 μg).

Exogenous administration of PGI₂ and PGH₂. Cardiac administration of PGI₂ caused a sharp transient reduction in coronary perfusion pressure (Fig. 7). Myocardial degradation seemed minimal since com-

parable bovine coronary relaxation was produced when the PGI₂ was tested directly over the assay tissue or when injected into the heart (Fig. 7, Table I). On the other hand, rapid efficient renal destruction was suggested by the 90% loss in PGI₂ biological activity in one transit across the perfused rabbit kidney (Table I).

Whereas cardiac administration of PGI₂ or AA produced a reduction in coronary perfusion, PGH₂ produced a small increase. Low doses of PGH₂ injected as a bolus through the heart were rapidly converted to a PGE₂-like substance that contracted the bovine coronary artery assay tissue (Fig. 7). Higher doses of intracardiac endoperoxide were not completely degraded in the heart and the venous effluent contained a substance which contracted the rabbit aorta and caused a biphasic relaxation and contraction of coronary strip (Fig. 7). The dose response curves for rabbit aorta
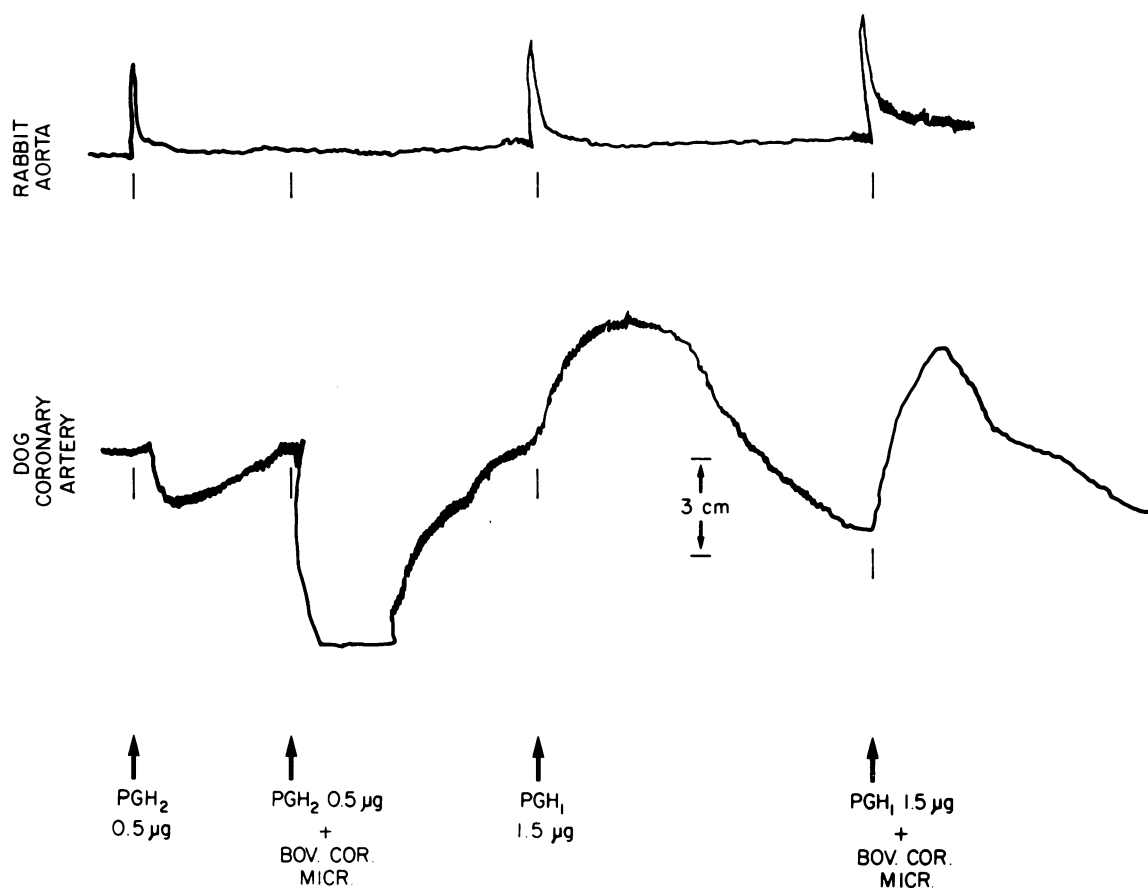
FIGURE 5 Comparative response of the rabbit aorta and dog coronary artery to endoperoxide, (PGH_2 or PGH_1), or the product of the incubation (2 min at 22°C) of the endoperoxide and bovine coronary artery microsomes (BOV. COR. MICR.) (100,000 g pellet). Rabbit thoracic aorta strips contract in response to PGG_2, PGH_2, and thromboxane A_2 but are nonresponsive to PGI_2 (14).

contractions produced by PGH_2 either applied directly or injected through the heart indicated that 86±5% ($n = 4$) of the endoperoxide was degraded in one transit across the heart.

Infusion of 15-hydroperoxy-arachidonic acid directly across the assay tissue blocked the conversion of PGH_2 to PGI_2 but did not affect bovine coronary artery relaxation from either directly applied or cardiac-produced PGI_2 (Fig. 7). However, intracardiac injection of high doses of PGH_2 did not release a substance that relaxed the 15-hydroperoxy-arachidonic acid-treated strips (Fig. 7). Therefore, bovine coronary relaxation after intracardiac administration of high doses of PGH_2 is due to the conversion by the assay tissue of spilled PGH_2 to PGI_2. Any conversion of the PGH_2 to PGI_2 by the rabbit heart, if it occurred, was below the sensitivity of the assay tissue for PGI_2.

The cardiac effluent from the PGH_2 (up to 2 μg) treated heart did not contain a substance that inhibited

platelet aggregation. Platelet aggregation could be inhibited by as little as 0.2 ng of exogenous PGI_2 (data not shown).

DISCUSSION

Both the isolated perfused rabbit heart and kidney are capable of synthesizing PGI_2. The evidence presented in support of this finding include: (a) radiochemical identification of the stable end product 6-keto-$PGF_{1\alpha}$ in the venous effluents (Fig. 1); (b) biological identification of the labile arachidonate product in the venous effluents which causes relaxation of bovine coronary artery and which inhibits platelet aggregation (Figs. 1–4); and (c) confirmation that AA (20:4) but not dihomo-γ-linolenic acid (C20:3) serves as the precursor for the coronary dilator, platelet inhibitory substance (Figs. 1, 2, and 6). We attempted to selectively abolish PGI_2 biosynthesis by infusing 15-hydroperoxy-
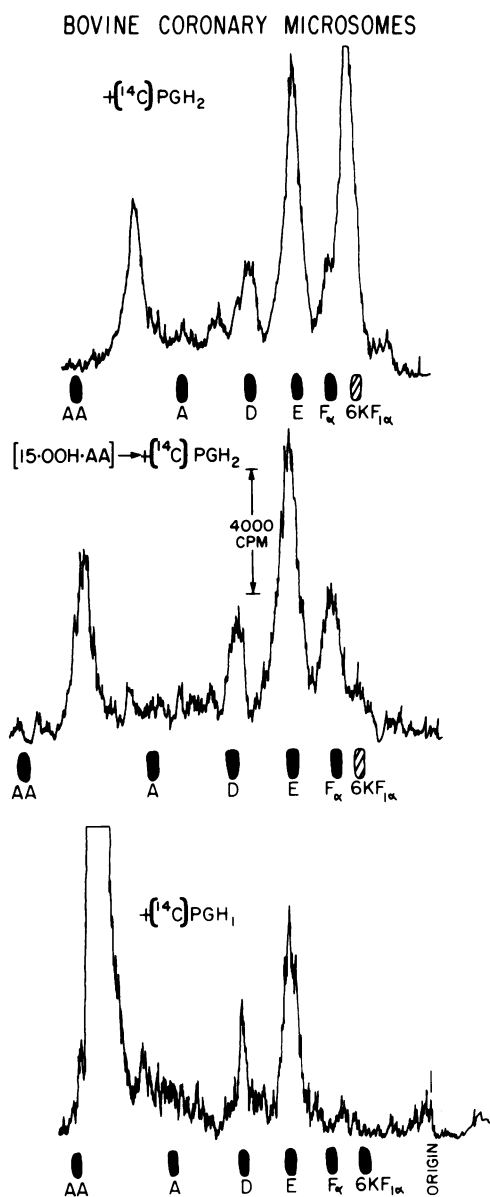
BOVINE CORONARY MICROSOMES

FIGURE 6 The enzymatic generation of PGI₂. Bovine coronary microsomes (10 mg protein) were incubated in a 100-mM phosphate buffer, pH 7.9, with 25 μg of [¹⁴C]PG endoperoxide (60,000 cpm) for 20 min at 37°C. The reaction mixture was acidified to pH 3.5 and extracted twice with ethyl acetate; concentrated and separated on a silica gel G plate in the A-9 solvent system. Note that the 6-keto-PGF$_{1\alpha}$ (6KF$_{1\alpha}$) runs just before the PGF$_{2\alpha}$ standard. There is no 6-keto-PGF$_{1\alpha}$ formed with microsomes that were preincubated with 15-hydroperoxy-arachidonic acid (15 · OOH · AA), a known in vitro antagonist of PGI₂ synthesis or when microsomes are incubated with [¹⁴C]PGH₁.

arachidonic acid) but the agent could block the in vitro enzymatic PGI₂ formation from PGH₂ by bovine aorta microsomes and bovine coronary artery (Fig. 6). Finally administration of PGI₂ mimicked the decrease in coronary perfusion pressure produced by exogenous or endogenous AA (Fig. 7).

Although, the heart and kidney are both capable of converting exogenous arachidonate into PGI₂, the normal kidney apparently primarily converts endogenous arachidonate (e.g., generated by stimulation with bradykinin, angiotensin, or ATP) into PGE₂ (Fig. 4 and [27]). In addition, the perfused heart and kidney appear to differ in regard to the basal PG (i.e., unstimulated) present in the effluent. Thus, the changes in the muscle tone of the coronary assay strip unmasked by the organ infusion of indomethacin suggest that the heart is continuously releasing PGI₂ and that the kidney is releasing PGE₂ (Figs. 1 and 4). Apparently the basal level of PGI₂ released by the heart into the effluent is at too low a concentration in the effluent to inhibit platelet aggregation. The high flow rate (30 ml/min), the dilution in the aggregation experiment (ninefold), and the rapid spontaneous decay of PGI₂ at pH 7.4 all tend to minimize the basal cardiac PGI₂ effect on platelets.

The differential PG biosynthesis (i.e., PGI₂ vs. PGE₂) by the perfused kidney is perplexing. In addition, the rabbit kidney can be induced to initiate thromboxane A₂ synthesis in a ureter obstruction model in response to either exogenous or endogenous arachidonate (27, 28). Furthermore, basal and stimulated PGE₂ biosynthesis is greatly exaggerated in perfused rabbit kidneys obtained from ureter obstructed animals (27). Thus, the isolated perfused rabbit kidney can be manipulated to synthesize PGE₂, PGI₂ or thromboxane A₂. There may be a differential distribution of enzymes for which PGH₂ serves as substrate throughout the kidney. The amount of PGE₂ synthesized (PGH₂ → PGE₂ isomerase) in the medulla is substantially greater than that in the cortex (29, 30). On the other hand, in the ureter obstructed rabbit kidney, total thromboxane A₂ (thromboxane synthetase; PGH₂ → T$_x$A₂) production is greater in the cortex than in the medulla (28). One possibility is that the kidney itself, (i.e., cortical or medullary tissue) does not contain PGI₂ synthetase (i.e., PGH₂ → PGI₂) but rather that the enzyme is localized in the renal blood vessels. Indeed, PGI₂ production has been demonstrated in every blood vessel tested (5, 14, 31, 32) and there is substantial evidence that cardiac synthesis merely reflects the presence of the enzyme in the coronary blood vessels (5, 10, 12). The resolution of the site and nature of PG biosynthesis is essential in understanding renal function since PGs are implicated in the regulation of renal resistance (33, 34), electrolyte excretion (35), and plasma renin synthesis and release (36).

The biological utility of local endogenous cardiac

arachidonic acid (up to 2 μg/ml) through the heart or kidney. Unfortunately, this agent did not appear to act on the intact organs (perhaps because of penetration problems or rapid enzymatic reduction to 15-hydroxy-

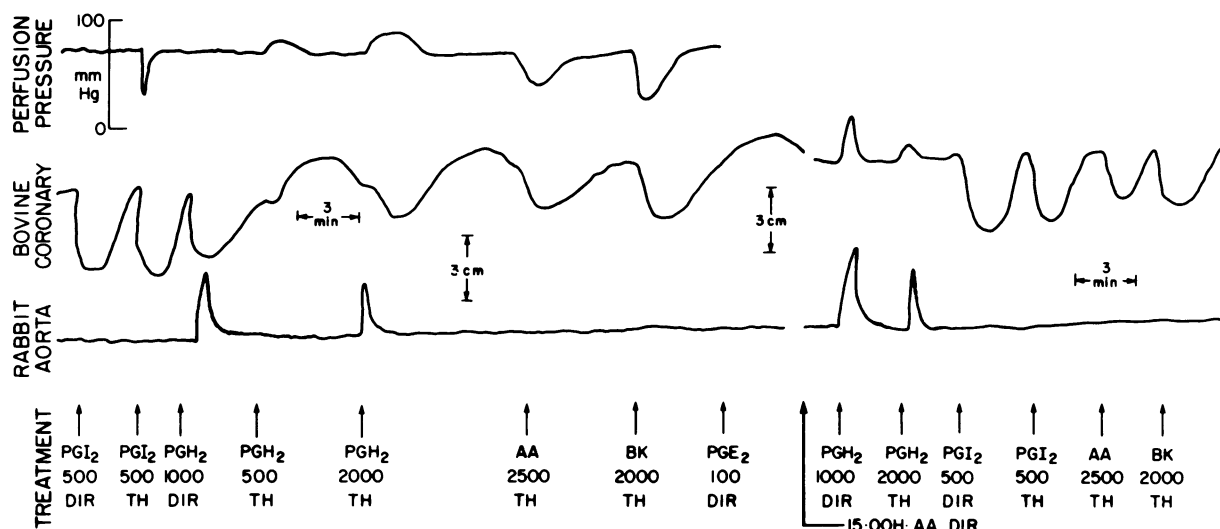*P. Needleman, S. D. Bronson, A. Wyche, M. Sivakoff, and K. C. Nicolaou*

FIGURE 7 Comparison of the effect of PGI_2 and PGH_2 on the isolated perfused rabbit heart. The PGI_2 was stored in an ethanol stock. The ethanol was evaporated in a stream of N_2 and was diluted as a 50-mM Tris buffer pH 9 solution just before use. The PGH_2 was stored as an acetone stock solution. The organic solvent was removed in a stream of N_2 and redissolved in a 50-mM phosphate buffer (pH 7.4) just before use. The number below each agonist indicates the amount in nanograms injected. DIR-denotes direct application of standards to the assay tissues, and TH-denotes injections through the heart. The 15-hydroperoxy-arachidonic acid (15 · OOH · AA) was dissolved in 0.1 M Tris pH 7.4 buffer and was infused over the assay tissues at a final concentration of 1 μg/ml for 15 min before testing.

PGI_2 production on coronary resistance is obvious. The impact on platelet aggregation of the PGI_2 released from the heart is less clear. The interaction of vascular PGI_2 production and platelet function has received a great deal of attention. It has been proposed that blood vessel PGI_2 synthetase "steals" the PG-endoperoxides from stimulated platelets and then the vascular enzyme manufactures PGI_2 which locally inhibits aggregation (37). There is a lack of definitive evidence to support this hypothesis. In the current and previous investigations we have demonstrated that the isolated perfused rabbit heart rapidly converts (in one transit across the coronary vascular bed) both endogenous and exogenous AA into PGI_2 (Fig. 1) which is temporally and quantitatively associated with a decrease in cardiac perfusion pressure. The isolated perfused rabbit heart does not appear to convert PGH_2 into PGI_2. Thus intracardiac administration of the endoperoxide: (a) causes an increase in coronary perfusion pressure, (b) does not lead to the release of a substance that relaxes bovine coronary artery strips; and (c) does not result in the appearance in the venous effluent of a substance which inhibits platelet aggregation. This suggests that the endoperoxide did not reach the PGI_2 synthetase whereas the more lipid soluble precursor AA penetrated to the cyclo-oxygenase which is apparently tightly coupled to the PGI_2 synthetase. Perhaps exogenous (i.e., nonvascular) PGH_2 could be converted

to PGI_2 in pathological situations involving coronary vascular injury. Endothelial damage could possibly result in enhanced endoperoxide penetration to the 6,9-oxycyclase resulting in PGI_2 synthesis. If experi-

TABLE I
Relaxation of Bovine Coronary Artery Strips by PGI_2 either Applied Directly (DIR) to the Assay Tissues or after Injection through the Heart (TH), or through the Kidney (TK)

Treatment	n	Dose of PGI_2 tested, ng				
		100	250	500	1,000	1,500
				mm		
PGI_2(DIR)	9	12.6	20.1	26.1	32.7	38.2
		±3.4	±5.1	±3.6	±3.8	±5.4
PGI_2(TH)	6	12.7	18.3	19.6	24.5	24.3
		±3.9	±3.3	±4.1	±8.3	±5.9
PGI_2(TK)	4	—	—	—	5.8*	14.0‡
					±1.7	±6

The values in the table are millimeters relaxation ± SEM; and n designates the number of experiments. The coronary relaxation produced by the renal venous effluent after intrarenal PGI_2 injection is significantly less than that produced by applying the PGI_2 directly to the coronary strip.
* $P < 0.001$.
‡ $P < 0.02$.

ments in the normal isolated perfused rabbit hearts serve as models of the in vivo situation, then it is difficult to visualize how the vascular enzyme could "steal" platelet endoperoxide for PGI$_2$ production. The more likely suggestion is that the blood vessel relies on endogenous substrates (i.e., AA and PGH$_2$) for its PGI$_2$ biosynthesis and therefore requires local stimulation (e.g., hormonal, mechanical, or changes in oxygen tension) to activate the process.

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