

A novel prostaglandin is the major product of arachidonic acid metabolism in rabbit heart

[hormonal stimulation/rat stomach homogenates/8,11,14-¹⁴C]eicosatrienoic acid/6(9)-oxy-11,15-dihydroxyprosta-7,13-dienoic acid/6-ketoprostaglandin F_{1α}]

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ABSTRACT The prostaglandins (PGs) released from the heart have generally been characterized as resembling PGE by bioassay techniques. The major PG formed from [¹⁴C]arachidonate (C_{20:4}) by the isolated perfused rabbit heart has chromatographic mobility similar to that of PGE₂ in most solvent systems. However, additional analysis of this radioactive "PGE" peak suggests that two substances are formed by the heart and migrate like PGE₂; one has chemical properties similar to those of authentic PGE₂ and the other is a novel PG. The unknown compound is the major PG formed by the heart from either exogenous arachidonate or hormonal stimulation of PG biosynthesis. The novel PG produced by the heart may be identical with either 6(9)-oxy-PGF or 6-keto-PGF_{1α}.

Synthesis and release of prostaglandins (PGs) by intact tissues occurs in response to various stimuli, both noxious and hormonal. The sequence of events leading to the release of PGs is thought (1, 2) to be initiated by the activation of phospholipase A₂ (phosphatide 2-acyl-hydrolase, EC 3.1.1.4) which liberates arachidonate (the major substrate for the cyclo-oxygenase) from the 2-position of phospholipids. The free arachidonate is then available for conversion to PGs (3). Various stimuli including decreased oxygen tension (4, 5), mechanical damage (6), hormones such as bradykinin (7) and angiotensin II (8), and arachidonate itself (9) release PGs from the isolated perfused rabbit heart. The PGs released have generally been characterized as PGE-like by bioassay. We recently described a method for studying the products of arachidonate metabolism (both exogenous and endogenous) in intact tissues by prelabeling the cardiac phospholipids with [¹⁴C]arachidonate (10). In the present work, in which we applied this method to the study of arachidonate metabolism in the intact rabbit heart, we found that the major PG formed by the heart is not PGE₂ but is, in fact, a novel PG.

METHODS

The hearts were removed from male New Zealand rabbits anesthetized with pentobarbital and perfused at 30 ml/min with Krebs-Henseleit buffer (37°; atmosphere, 95% O₂/5% CO₂) as previously described (5).

The method for incorporating radioactive arachidonate into heart lipids has been described in detail elsewhere (10). Briefly, 5 μCi of [¹⁴C]arachidonic acid (Amersham/Searle; specific activity 55 mCi/mmol) prepared as the Na salt in saline (pH 9.0) was infused through the coronary vascular bed for 20 min. The [¹⁴C]arachidonate is efficiently incorporated into endogenous tissue lipids and later can be released in the form of ¹⁴C-labeled PGs by agents which stimulate PG biosynthesis.

The coronary vasculature effluent was monitored for radioactive PGs by collection of 50- to 100-ml samples, conven-

tional acid-lipid extraction, and thin-layer chromatography as previously described (10). Solvent systems used for thin-layer chromatography are: system C, chloroform/methanol/acetic acid/water, 90:8:1:0.8 (vol/vol) (ref. 11); system BDA, benzene/dioxane/acetic acid, 60:30:3 (vol/vol) (ref. 12); system E, diethyl ether/methanol/acetic acid, 90:1.5:2 (vol/vol) (ref. 11); system SN, organic phase of ethyl acetate/methanol/acetic acid/2,2,4-trimethylpentane/water, 110:35:30:10:200 (vol/vol) (ref. 13). Solvent systems were made fresh daily; chromatography tanks were lined with filter paper and equilibrated before use. System SN was used with plates impregnated with 10% AgNO₃. Thin-layer plates used were silica gel G (Brinkman Instruments). ¹⁴C-Labeled PGE₂ was obtained from New England Nuclear and 8,11,14-¹⁴C]eicosatrienoic acid (specific activity, 55 mCi/mmol) was from Applied Science Labs. PG standards were a generous gift of Dr. John Pike, the Upjohn Co.

Preparation of rat gastric fundus homogenates and incubations with [¹⁴C]arachidonate were performed as described by Pace-Asciak and Wolfe (14), with 2.4 mM norepinephrine included in each incubation.

RESULTS

Products from Exogenous Arachidonic Acid. Infusion of arachidonic acid through the coronary vasculature of the isolated rabbit heart produces coronary vasodilation and concomitant appearance of a PG-like substance in the effluent (9). On analysis of the effluent from the coronary vasculature by thin-layer chromatography in system C, a characteristic pattern of products was obtained (Fig. 1B). A prominent peak of radioactivity was seen associated with PGE₂, and a lesser amount was associated with PGF_{2α}. Chromatography of another portion of the same coronary vasculature effluent sample in system BDA produced a radically different pattern; in this system, authentic ¹⁴C-labeled PGE₂ ran as a single peak (Fig. 1A, lower panel). In this solvent system, the major peak of radioactivity had a chromatographic mobility similar to that of PGF_{2α}. Chromatography in solvent system E and on AgNO₃-impregnated plates (system SN) produced patterns similar to those seen in system C (i.e., the major radioactive peak had mobility similar to that of PGE₂) (data not shown).

Chemical Characterization of "PGE" Peak. Several samples obtained during infusion of [¹⁴C]arachidonate were pooled, concentrated, and chromatographed in system C. The radioactive peak migrating with PGE₂ was scraped off and eluted twice with methanol to obtain material for further chemical characterization. Treatment of this "PGE" peak material with 2 M KOH in methanol at 65° for 60 min (15) resulted in only partial conversion to PGB₂ (Fig. 1C, upper panel). In contrast, standard ¹⁴C-labeled PGE₂ treated similarly was quantitatively converted to PGB₂ (Fig. 1C, lower panel). Furthermore,

Abbreviations: PG, prostaglandin; 6(9)-oxy-PGF, 6(9)-oxy-11,15-dihydroxyprosta-7,13-dienoic acid.

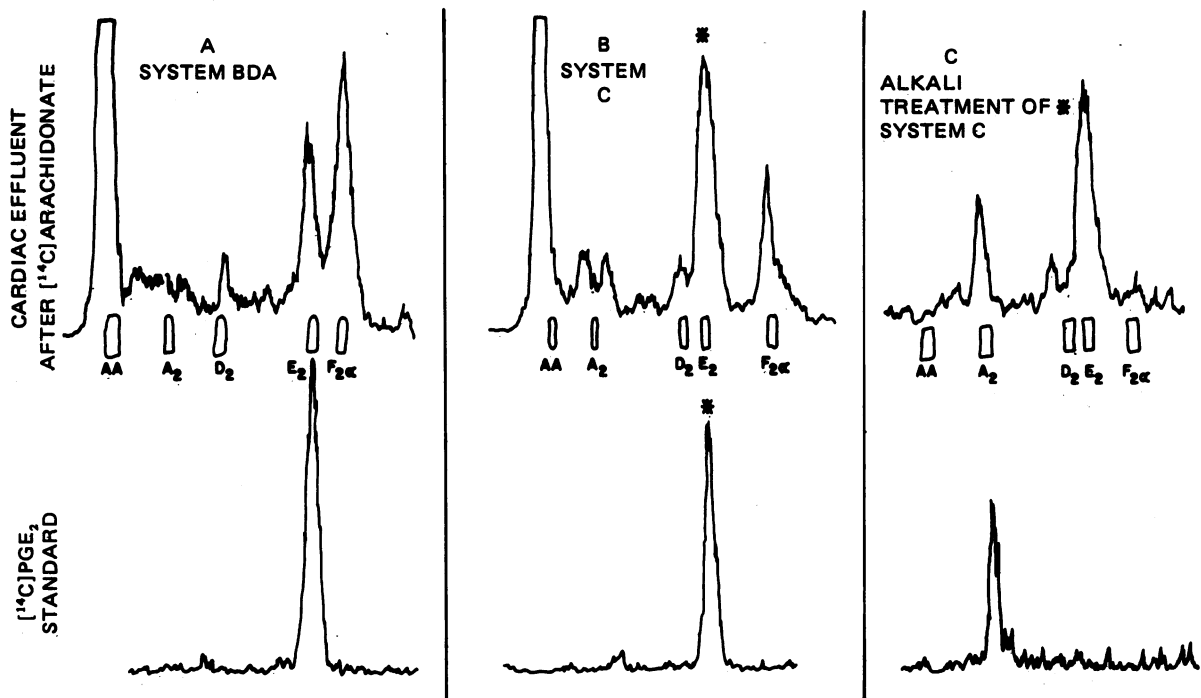


FIG. 1. *Upper panels.* Radio-scans (thin-layer chromatography) of coronary vasculature effluent after [^{14}C]arachidonate was infused through the heart at $1\ \mu\text{g}/\text{min}$ ($3.3 \times 10^5\ \text{cpm}/\text{min}$); effluent was extracted for PGs as described in the *Methods*. A. Chromatography in system BDA. B. Chromatography in system C. C. Radioactive peak from B (*) was eluted from the plate, treated with 2 M KOH in methanol at 65° for 60 min, extracted, and chromatographed in system C. *Lower panels.* Authentic [^{14}C]PGE₂ extracted and chromatographed in the same manner as the coronary vasculature effluent. The spots between the scans indicate the migration of unlabeled standards on the thin-layer chromatography plates.

treatment of the "PGE" peak with 1 M HCl resulted in only partial conversion to PGA₂, whereas authentic ^{14}C -labeled PGE₂ was completely converted (data not shown). These data suggest the presence of a PG (completely unlike PGE₂) that is resistant to alkali hydrolysis and acid conversion to PGA₂.

Products from [^{14}C]Eicosatrienoic Acid. Experiments were performed with 8,11,14- ^{14}C eicosatrienoic acid (C_{20:3}), the precursor of PGs of the 1-series, to determine if infusion of this fatty acid gave products similar to those from arachidonate. We found that 5-fold higher concentrations of the C_{20:3} acid were required for detectable PG synthesis. Analysis of the radioactive PG products extracted from the effluent in these experiments indicated that the PGE/PGF ratio was similar in both system C and system BDA (Fig. 2A and B). In contrast to the results

with [^{14}C]arachidonate, alkali treatment of the E zone obtained from the C_{20:3} acid resulted in essentially complete conversion to PGB (Fig. 2C). Thus, a PG comparable to the novel PG is not formed from the C_{20:3} acid (which lacks the 5–6 double bond).

Products Formed from Endogenous Arachidonic Acid. Hearts prelabeled with [^{14}C]arachidonate will release radioactive PGs when synthesis is stimulated by such agents as bradykinin and ATP (10). The predominant peak of radioactivity after such stimulation migrated with PGE₂ in system C (Fig. 3A and C), without demonstrable PGF_{2α} present. However, when similarly obtained samples were chromatographed in system BDA, two peaks were observed (Fig. 3B and D), one of which had chromatographic mobility similar to that of

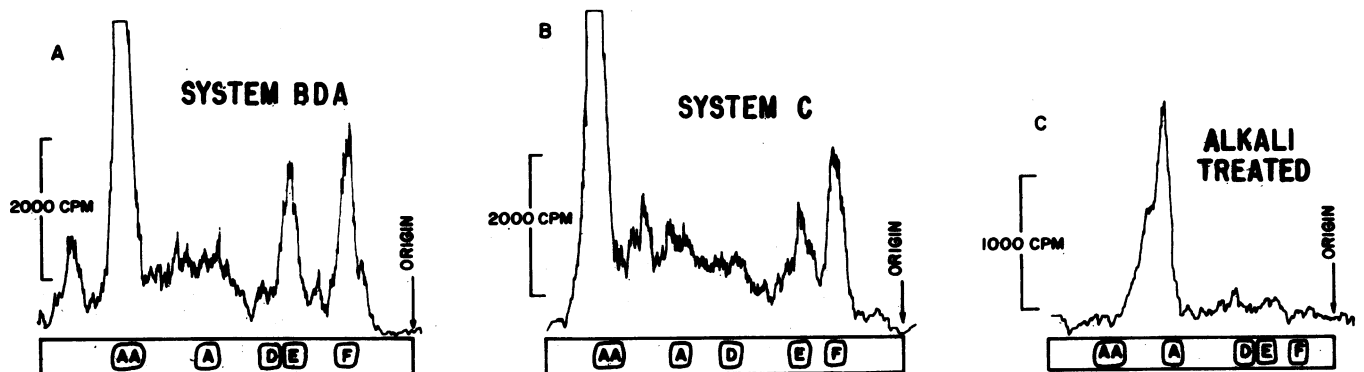


FIG. 2. Radio-scans (thin-layer chromatography) of coronary vasculature effluent after ^{14}C -labeled 8,11,14-eicosatrienoic was infused at $24\ \mu\text{g}/\text{min}$ ($6.7 \times 10^5\ \text{cpm}/\text{min}$). The effluent was collected during the infusion and extracted as described in the *Methods*. A. Chromatography in system BDA. B. Chromatography in system C. C. Radioactive peak corresponding to standard PGE₂ from B was eluted, treated with alkali (as described in legend to Fig. 1C), and chromatographed in system C. Unlabeled standards are indicated as spots on thin-layer chromatography plates below each radio-scan.

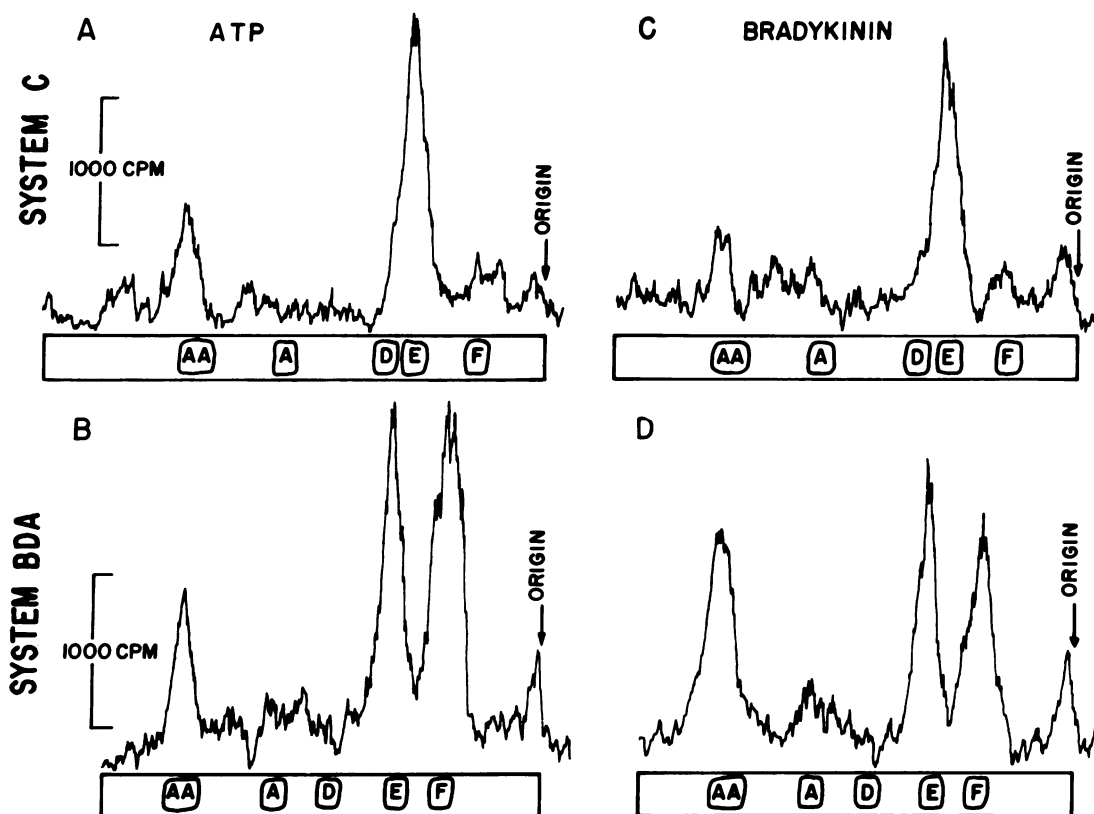


FIG. 3. Radio-scans (thin-layer chromatography) of $[^{14}\text{C}]$ PGs released from the heart by bradykinin ($5\ \mu\text{g}$) or ATP ($2\ \mu\text{mol}$). The hearts were pre-labeled with $[^{14}\text{C}]$ arachidonate. The stimulating agents were given as a 0.2-ml bolus; a 75-ml sample of coronary vasculature effluent was extracted and subjected to thin-layer chromatography.

$\text{PGF}_{2\alpha}$. Several samples from hormone-stimulated heart were pooled, and the PGE peak was isolated by chromatography in

system C and treated with alkali. Again, there was only partial conversion to PGB_2 (Fig. 4B). Thus, it appears that the products obtained with exogenous $[^{14}\text{C}]$ arachidonate are also produced from endogenous arachidonate upon hormone stimulation.

Treatment of hearts with indomethacin ($10\ \mu\text{g}/\text{min}$) completely abolished PG biosynthesis from both exogenous arachidonate and endogenous arachidonate after hormonal stimulation.

Chromatographic Characterization of Products from Rat Fundus Homogenates. Pace-Asciak and Wolfe (14, 16) have conclusively demonstrated, with gas chromatography-mass spectrometric methods, that the primary arachidonate products produced by homogenates of rat gastric fundus are 6(9)oxy-PGF and 6-keto-PGF $_{1\alpha}$. We used the rat stomach preparation to generate this material for comparison to the cardiac compound. After incubation of $[^{14}\text{C}]$ arachidonate with homogenates of rat fundus, extraction and chromatography in system C gave one major peak with chromatographic mobility similar to that of PGE $_2$ (Fig. 5A), confirming the results of Pace-Asciak and Wolfe (16). However, rechromatography of this "PGE" peak in system BDA produced a change in mobility such that the major peak of radioactivity moved with PGF $_{2\alpha}$ (Fig. 5B). This change was identical to that observed with the "PGE" peak isolated by system C from the coronary vasculature effluent (Fig. 5B, lower panel). Apparently, this novel PG is somewhat unstable: isolation of the radioactive "PGE" peak in system BDA followed by rechromatography in system C apparently decomposes the compound (Fig. 5C). When the system BDA "PGF" peak from rat fundus was so treated, a similar pattern of decomposition products was obtained (Fig. 5C). This provides an additional useful method for comparing the cardiac compound with the PGs formed by rat fundus.

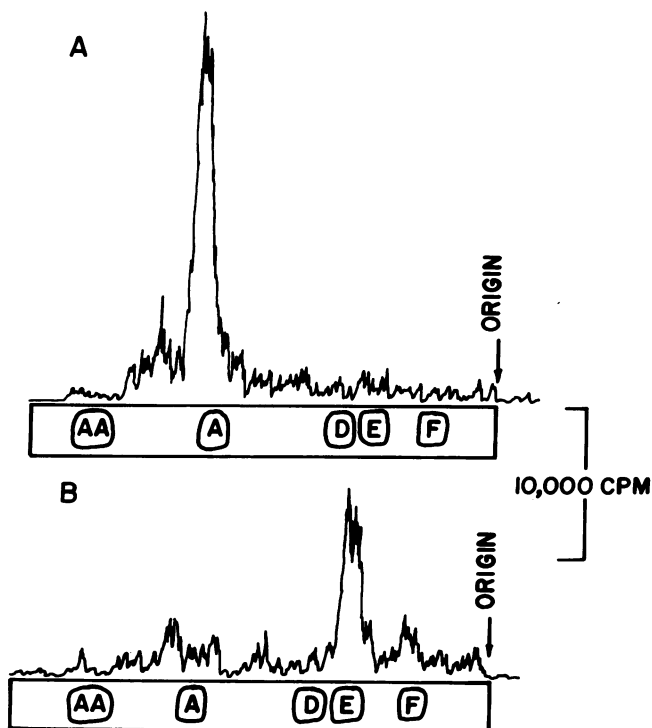


FIG. 4. Alkali treatment of $[^{14}\text{C}]$ PGE $_2$ (A) and the PGE-like substance (B) produced by the heart after hormonal stimulation.

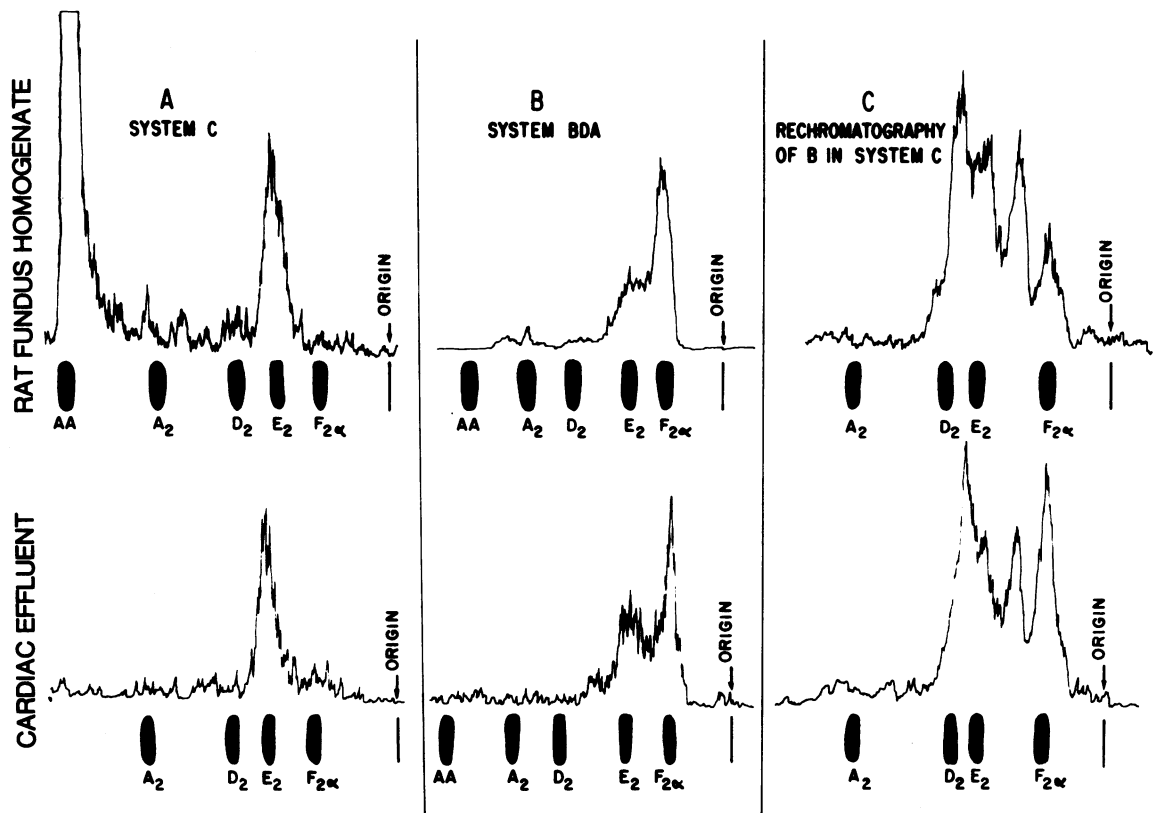


FIG. 5. Radio-scans (thin-layer chromatography) of products of [^{14}C]arachidonate metabolism. *Upper panels.* Homogenates of rat gastric fundus were incubated with 0.25 μCi of [^{14}C]arachidonate for 20 min in the presence of 2.4 mM norepinephrine and then extracted for PGs. A. Chromatography of incubation products in system C. B. Radioactive peak migrating with PGE_2 in system C was eluted and chromatographed in system BDA. C. Radioactive peak migrating with $\text{PGF}_{2\alpha}$ in system BDA was eluted and chromatographed in system C. *Lower panels.* The radioactive peak from [^{14}C]arachidonate infusion in rabbit heart and migrating with PGE_2 in system C was isolated as before. A. Rechromatography in system C. B. Chromatography in system BDA. C. Radioactive peak migrating with $\text{PGF}_{2\alpha}$ in system BDA was eluted and chromatographed in system C.

DISCUSSION

The release of PG-like material from the isolated perfused rabbit heart has been studied in several laboratories. The identity of this material has generally been described as PGE_2 -like on the basis of chromatographic mobility and bioassay characteristics. Thus, it was surprising to find that the major PG product obtained from exogenous [^{14}C]arachidonate and by hormone stimulation of endogenous arachidonate release had chemical and chromatographic properties distinct from those of PGE_2 . The assignment of a non- PGE_2 structure to the major PG produced by the heart is based on its chromatographic mobility, resistance to alkali conversion to PGB_2 , and resistance to acid conversion to PGA_2 . Although the data do not allow complete structural identification of the compound, they do suggest the lack of a β -OH-ketone structure (as in PGE_2 and PGD_2 , which are sensitive to alkali treatment).

Recently, Pace-Asciak and Wolfe (14, 16) have reported the structures (determined by gas chromatography-mass spectrometry) of two novel PGs formed by rat fundus homogenates: (i) 6(9)oxy-PGF, and (ii) 6-keto-PGF $_{1\alpha}$. Both of these PGs have certain properties in common with the unknown compound reported here—i.e., chromatographic mobility similar to that of PGE_2 in most solvent systems and resistance to alkali conversion to PGB_2 . The experiments with rat fundus homogenates reported here demonstrate two other striking similarities: (i) both the unknown compound formed by the heart and the products obtained from rat fundus homogenates have chromatographic mobility identical with that of $\text{PGF}_{2\alpha}$ in system

BDA, and (ii) the system BDA "F" peaks from both give coincident decomposition products.

The proposed mechanisms for the formation of both compounds by rat fundus involve oxygen attack at the C-6 position of the 5,6 double bond of arachidonate. Thus, a PG-precursor fatty acid which lacks the 5,6 double bond (e.g., 8,11,14-eicosatrienoic acid) would not be converted to either 6(9)oxy-PGF or 6-keto-PGF $_{1\alpha}$. We could find no evidence of the conversion of the $\text{C}_{20:3}$ acid in heart to the unknown compound formed from arachidonate, although PGE_1 and $\text{PGF}_{1\alpha}$ were produced from this acid. These results therefore suggest that the novel PG formed by rabbit heart may be identical with 6(9)oxy-PGF or 6-keto-PGF $_{1\alpha}$. Confirmation of this will require structural identification of this compound by mass spectrometry. Proposed structures of the compounds produced by rabbit heart are shown in Fig. 6.

It has been suggested that the coronary dilation produced by arachidonic acid or bradykinin in the rabbit heart is due to PGE_2 (1, 8). However, it has not been possible to induce this dilation with exogenous PGE_2 , even at very high concentrations. Similar results were obtained in experiments with uterus from pregnant animals (17). Two explanations for these findings were given: (i) the dilation is due to the endoperoxide intermediates, which rapidly decompose to PGE_2 ; and (ii) exogenous PGE_2 cannot match the high local concentration of endogenously produced (by arachidonate or bradykinin) PGE_2 , which may be synthesized very near the site of action. Our observation that the major PG produced by the heart is not PGE_2 suggests an-

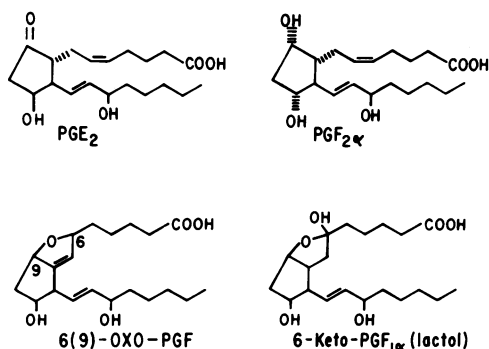


FIG. 6. Suggested structures of products of arachidonic acid metabolism in rabbit heart.

other alternative—namely, that this novel PG (or an intermediate in its synthesis), and not PGE₂, is responsible for the coronary dilation that occurs with arachidonate or bradykinin administration.

The implications of this work can be extended to other tissues. For example, the PGs produced by many organs have been identified as PGE₂ on the basis of chromatographic mobility in one solvent system. These results should be reevaluated in light of the present report. Furthermore, the biological activity of material believed to be PGE₂ should be compared to that of these novel PGs. The production of novel PGs in good yield from tissues as disparate as rat gastric fundus and rabbit heart justifies further search for these compounds in other tissues and extensive examination of their biological activity.

Note Added in Proof. We have recently found that isolated bovine coronary arteries convert arachidonic acid to a compound similar to that reported here (18). Moncada *et al.* (19) have shown that rabbit aorta microsomes convert PGH₂ to an unstable compound that inhibits platelet aggregation and relaxes vascular smooth muscle. We have found that microsomes from bovine coronary artery produce a similar compound from PGH₂ that is a potent coronary artery relaxant. Furthermore, in recent experiments we have found that the isolated rabbit heart, upon stimulation of PG biosynthesis (by arachidonate or bra-

dykinin), releases a substance which relaxes bovine and canine coronary arteries and its synthesis is inhibited by indomethacin. These results suggest that an intermediate in the synthesis of the novel PG reported here is a potent coronary vasodilator and may be important in the regulation of coronary vascular resistance.

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