Characterization of a Novel Metabolic Pathway of Arachidonate in Coronary Arteries which Generates a Potent Endogenous Coronary Vasodilator*

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SUMMARY

Bovine coronary artery strips were incubated with [1-14C]arachidonic acid and the chemical properties of the various prostaglandins (PG) formed were studied. Arachidonic acid was converted to two major prostaglandin products, PGE_2 and a novel prostaglandin having chemical (i.e. base hydrolysis and borohydride reduction) and chromatographic properties identical with 6-keto-PGF_10. This final compound was inactive on coronary artery strips. The endoperoxide intermediates, PGG_2 or PGH_2, previously shown to induce coronary relaxation, were not released into the medium from the isolated bovine coronary arteries. The arachidonic acid-induced dilation may have been due to an intracellular action of PGH_2 (or PGG_2) or to the action of another, yet unidentified, labile intermediate formed in the enzymatic conversion of endoperoxides to 6-keto-PGF_10. When PGH_2 was incubated with bovine coronary microsomes, the PGH_2 was completely metabolized (i.e. loss of rabbit aorta contraction) but a compound was generated which was a much more potent coronary relaxant. We suggest that this major novel metabolic pathway of arachidonate generates a substance, intermediate between PGH_2 and the final 6-keto PGF_10-like product, which is a potent coronary vasodilator.

Bovine coronary arteries maintained in vitro continuously release a PGE_2-like substance (determined by a biological assay) (1). Treatment with PG-cyclooxygenase inhibitors (indomethacin, meclofenamate, or aspirin) increased basal coronary artery tone (1, 2). Low oxygen tension increased the rate of prostaglandin release and simultaneously reduced coronary artery tone; these responses were also inhibited by indomethacin (3).

Exogenous PGE_2 or PGF_2α contracted bovine and human coronary arteries but the precursor arachidonic acid caused relaxation; the latter effect was abolished by prostaglandin synthetase inhibitors (2). These results suggest that arachidonate was converted by coronary cyclooxygenase to a relaxing substance which was different from PGE_2 or PGF_2α. Administration of the endoperoxides, PGH_2 or PGG_2, also resulted in relaxation of bovine coronary arterial strips (4). The paradoxical relaxation of bovine or human coronary arteries produced by arachidonate or PGH_2 suggests that either (a) PGH_2 was the active relaxing substance synthesized from arachidonate and that its subsequent enzymatic conversion to PGE_2 (by PGH_2 → PGE_2 isomerase) was slow, thus allowing the endoperoxide to exert its relaxant effect, or (b) that a novel dilating substance was produced by the isolated coronary arteries.

In the present investigation we studied the metabolism of [1-14C]arachidonic acid by bovine coronary artery strips. We identify a novel pathway of arachidonate metabolism which has as its final product a compound similar to 6-keto-PGF_10. In addition we find that bovine coronary microsomes convert PGH_2 to a substance which is a potent coronary relaxant. We suggest that arachidonic acid is converted to a labile intermediate in the biosynthesis of 6-keto-PGF_10 and that this intermediate is responsible for the coronary relaxation induced by both arachidonate and PGH_2.

MATERIALS AND METHODS

Bovine coronary arteries were excised from freshly removed hearts and cut into spiral strips (2). The coronary spirals were suspended in 10-ml chambers (37°C) containing Krebs-Henseleit medium continuously bubbled with O_2-CO_2 (95:5%). [1-14C]Arachidonic acid (2 μCi, 19 μM, prepared as the sodium salt) was incubated with bovine coronary artery strips (1.5 to 2 g) for 1 h. The medium was removed and the coronaries were washed twice with Krebs-Henseleit medium, and then incubated for 4 to 5 h in fresh medium. Aliquots of the medium were removed periodically and tested for biological activity over a cascade of prostaglandin-sensitive smooth muscles (rat stomach strips, chick rectum) and compared to PGE_2 standards (5). At the end of the 4- to 6-h incubation, the medium was acidified with 0.5 M citric acid to pH 3.5 and extracted twice with 15 ml of ethyl acetate. The combined extract was dried over anhydrous Na_2SO_4 and aliquots were analyzed by thin layer chromatography. The thin layer zones corresponding to PGE_2 were subjected to alkali treatment (6) and to NaBH_4 reduction (7). The tissues were weighed and extracted and analyzed for their total lipid content. The coronaries were homogenized in 20 volumes of chloroform/methanol (2:1) of tissue followed by two washings with 0.1 M KCI. The extracts were evaporated to dryness and subjected to thin layer chromatography. The radioactivity of the lipid extract (93 ± 2% of the original arachidonic acid) was determined by a bioassay (8). [1-14C]PGH_2, [1-14C]PGF_2α (specific activity 55 mCi/mmol), and 6-keto-PGF_1α were obtained from New England Nuclear. Prostaglandin standards were a generous gift of Dr. John Pike of the Upjohn Co.

RESULTS AND DISCUSSION

Incubation of the [1-14C]arachidonate-labeled coronaries for 4 to 6 h resulted in a gradual release of PGE_2-like substance (0.4 ± 0.1 μg/g of tissue/h, n = 10) into the medium (as determined by bioassay). Acid-lipid extraction of the medium and thin layer chromatography (Fig. 1A) indicated the pres-
A Prostaglandin Vasodilator from Arachidonate by a Novel Pathway

We previously observed that isolated perfused rabbit hearts convert arachidonic acid into a novel prostaglandin (10). A similar situation appears to exist with bovine coronary arteries (Fig. 2). When the extract of the coronary medium was chromatographed in a solvent system of benzene:diethylether:acetonic acid:water, 60:30:1:0.5 (System B/D/A), the "PGE" peak separated into two compounds, one with chromatographic mobility identical to PGE₂ and the other with chromatographic mobility similar to PGF₁₅ (Fig. 2, Panel 1). The lack of a radioactive peak co-migrating with PGF₁₅ (Fig. 2, Panel 2) in chromatography System C suggests that coronary arteries produce a substance different from PGE₂ or PGF₁₅. In six experiments the ratio of PGE/unknown substance (as determined from chromatography in System B/D/A) was 0.8 to 1.5. Alkaline treatment (0.5 N KOH in methanol) of the total PGE peak isolated from System C resulted in only partial conversion to PGB₂ (Fig. 2, Panel 3), whereas authentic PGE₂ was completely (>95%) converted (Fig. 2, lower Panel 3). NaBH₄ reduction of the PGE peak from the coronary also gave only partial conversion to PGF₂₅ + PGF₃₅ (Fig. 2, Panel 4). In each case, the percentage of radioactivity converted by the treatment was equivalent to the percentage of PGE in the total peak. The apparent resistance of the unknown substance to NaBH₄ reduction suggests the lack of a ketone group, while the resistance to alkaline treatment suggests the lack of a β-OH ketone structure (as is present in prostaglandins E₂ and D₂).

The chemical and chromatographic properties of the unknown substance are similar to those of the major prostaglandin produced by the rat fundus homogenate which has been identified as 6-keto-PGF₁₅ (11, 12).

Arachidonic acid and the prostaglandin endoperoxides caused coronary relaxation (4) while the primary prostaglandins (PGE₂ and PGF₁₅) caused coronary constriction (2); the novel prostaglandin produced by the rabbit heart (10) and that produced by bovine coronary arteries were inactive on coronaries at the doses tested (up to 10 μg) (Fig. 3). These results suggest that either the endoperoxides directly relax coronary arteries or are converted to a labile precursor of 6-keto-PGF₁₅. Therefore we incubated microsomes from bovine coronary arteries with PGH₂ for short times (2 min) and tested the products formed on bovine coronary arteries to determine if the endoperoxides are converted to a coronary relaxant. As shown in Fig. 4, coronary microsomes convert PGH₂ to a potent coronary relaxant. This substance has no effect on rabbit aorta but is an extremely potent inhibitor of platelet aggregation (data not shown); these properties are very similar to those of the unstable prostaglandin recently discovered by Moncada et al. (13). The latter compound was formed by incubating rabbit aorta microsomes with PGH₂ but not with arachidonate. One apparent difference from the rabbit aorta experiments is that the isolated coronary can continuously convert arachidonate to the relaxant as evidenced by the vasoconstriction produced by indomethacin. We feel this newly described coronary metabolic pathway can function independently of circulating platelets and must continuously modulate coronary (and probably other tissues) vascular resistance.

Arachidonic acid infused through an isolated perfused rabbit heart produced coronary vasodilation and concomitant ap-
Fig. 2. Separation of the major prostaglandin products released from coronary strips. Coronaries were labeled with radioactive arachidonate and then incubated for 4 h. The coronary incubation medium extracts and [3H]PGE₂ standard were subjected to thin layer chromatography in Panel 1 in B/D/A (benzene:dioxane:glacial acetic acid, 60:30:3) and in Solvent System C, i.e. chloroform:methanol:acetic acid:water, 90:8:1:0.8 (in all the remaining panels). In Panels 1 and 2 the entire extract was tested in both the solvent systems. Following separation in System C, the PGE₂ spot (*) was scraped from the plate and eluted from the silica gel and treated either with KOH and rechromatographed (Panel 3) or treated with NaBH₄ and rechromatographed (Panel 4).

Fig. 3. Metabolic pathway of arachidonate conversion and the biological effectiveness of the products on isolated bovine coronary arteries. A tracing of the contractile response of bovine coronary artery strips superfused with Krebs-Henseleit solution as previously described (2, 4) is presented adjacent to the various arachidonate products. The question mark designates the labile intermediate between PGH₂ and 6-keto-PGF₁α which is the potent coronary relaxing substance (Fig. 4).

Fig. 4. The coronary relaxant effect of PGH₂. The upper panel illustrates a typical recording of the coronary relaxation produced by (a) direct PGH₂ addition to a superfused (10 ml/min, Krebs-Henseleit medium) bovine coronary artery strip or (b) by addition of the reaction mixture of the same amount of PGH₂ incubated with bovine coronary artery microsomes (2 min at 22°C). The microsomes themselves have no direct effect. The lower panel presents a dose-response curve comparing the potency of PGH₂ alone versus the "activated" product produced by the coronary microsomes.

We studied the products of arachidonate metabolism by prelabeling the cardiac phospholipids with [1-¹⁴C]arachidonate (14). Surprisingly, we found that the major product formed by the heart was not PGE₂ but was, in fact, a novel prostaglandin (10). This substance, like the coronary compound described here, shared all the chemical and chromatographic properties of 6-keto-PGF₁α. Thus, a major possibility which arises from this work is that the biosynthesis of prostaglandin in the heart is largely restricted to the coronary vascular smooth muscle. It has been suggested that the coronary dilation produced by arachidonic acid or hormone stimulation with bradykinin or angiotensin in the rabbit heart was due to PGE₂; however, it has not been possible to match this dilation with exogenous PGE₂, even at very high concentrations (15). Two explanations for these findings were given: (a) the dilation was due to the endoperoxide intermediates, which decomposed to PGE₂ and...
(b) exogenous PGE cannot match the high, local concentration of endogenously produced PGE, which may be synthesized very near the site of action. Our observation that the major product synthesized by both the isolated perfused heart and the coronary artery strips is not PGE, suggests another alternative, namely that this novel prostaglandin intermediate (Fig. 3) is responsible for the coronary dilation which occurs with arachidonate or bradykinin administration to an intact heart. Indeed, there are numerous demonstrations that isolated or cultured vascular smooth muscles intrinsically synthesize prostaglandins and therefore possess the potential for the local regulation of blood vessel tone (16–19).

The interaction between the arachidonate metabolites synthesized in blood vessels and platelets is of considerable pathophysiological significance. The two synthetic pathways appear to be physiologically antagonistic and the net response may reflect the algebraic sum of the two systems. Thus, stimulated platelets release thromboxane A, which induces aggregation and vasoconstriction, whereas blood vessels synthesize a vasodilator that inhibits aggregation. Both substances are highly labile and therefore would be much more critical for local vascular regulation rather than systemic responses.

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