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Identification of 13,14-dihydro-15-oxo-prostaglandin $F_{2\alpha}$ in the circulation during infusions of bradykinin and prostaglandin E_2 in man

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1 Bradykinin, prostaglandin E_2 (PGE_2), PGD_2 and vehicle (saline) were each administered intravenously on separate occasions to 6 healthy men for a period of 60 min.

2 13,14-Dihydro-15-oxo-$PGF_{2\alpha}$ was identified in plasma samples obtained during intravenous infusions of bradykinin and PGE_2 but not during infusions of PGD_2 or saline.

3 The structure of this metabolite was verified by comparison of three different derivatives with authentic standards, using gas chromatography/electron capture mass spectrometry.

4 Bradykinin increased plasma concentrations of 13,14-dihydro-15-oxo-$PGF_{2\alpha}$ from baseline values in the range $< 5-10$ pg ml^{-1} to 28–403 pg ml^{-1}. PGE_2 increased plasma concentrations of 13,14-dihydro-15-oxo-$PGF_{2\alpha}$ from baseline values in the range $< 5-17$ pg ml^{-1} to 160–603 pg ml^{-1}. Neither PGD_2 nor the vehicle affected 13,14-dihydro-15-oxo-$PGF_{2\alpha}$ concentrations.

5 We conclude that bradykinin-stimulated 13,14-dihydro-15-oxo-$PGF_{2\alpha}$ may be derived from PGE_2 or $PGF_{2\alpha}$. The possibility that these prostaglandins are synthesized by stimulation of microvascular endothelium during bradykinin infusion is discussed.

Introduction

Bradykinin stimulates prostaglandin synthesis by activation of phospholipase and liberation of arachidonic acid (Hong & Levine, 1976; Hong, 1980; Crutchley *et al.*, 1983; Bareis *et al.*, 1983). We recently showed that in man, intravenous bradykinin increases the plasma concentration of 6-oxo-prostaglandin $F_{1\alpha}$ (6-oxo-$PGF_{1\alpha}$), the stable hydrolysis product of prostacyclin (PGI_2) (Barrow *et al.*, 1986). We subsequently investigated the possibility that other prostaglandins are also synthesized in response to bradykinin *in vivo*. Here, we present evidence for this by identification, characterization and quantification of 13,14-dihydro-15-oxo-$PGF_{2\alpha}$, a metabolite of $PGF_{2\alpha}$ (Granström, 1972; Granström *et al.*, 1982), which may also be derived from PGE_2 (Hamberg & Israelsson, 1970; Granström & Kindahl, 1982) or possibly PGD_2 (Ellis *et al.*, 1979; Watanabe *et al.*, 1985; Liston & Roberts, 1985).

Methods

Six healthy drug-free men aged 28–41 years, weight 70–86 kg participated in these studies. Protocols were approved by the Research Ethics Committee of the Royal Postgraduate Medical School and Hammersmith Hospital.

Protocols

Bradykinin (Sigma Chemical Company, Poole, Dorset), PGE_2 (Upjohn Ltd, Fleming Way, Crawley, Sussex), PGD_2 (a generous gift from Dr P. Humphreys, Glaxo, Ware) or vehicle (NaCl 0.15 M) were administered using a Braun constant infusion pump via an indwelling venous cannula in the forearm. They were infused in random order with at least 2 days between infusions. Doses of bradykinin, PGE_2 and PGD_2 were selected on the basis of individual tolerability: 12–42 μg kg^{-1}, 6–12 μg kg^{-1} and 1.4–9.1 μg kg^{-1} respectively. Each infusion lasted 60 min. Blood was sampled from a contralateral vein before and during the final minute of each infusion.

Sample collection

Blood (20 ml) was collected into ice-cold lithium heparin tubes and centrifuged immediately (1000 *g*; 4°C; 10 min). Plasma was separated and internal standards of [$^2H_4$]-6-oxo-$PGF_{1\alpha}$, [$^2H_4$]-$PGF_{2\alpha}$, [$^2H_4$]-

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PGE_2, $[^2H_4]$-13,14-dihydro-15-oxo-$PGF_{2\alpha}$ and $[^2H_6]$-PGD_2 (5 ng each) were added. The deuterated standards were generous gifts from Dr J. Pike (Upjohn Company, Kalamazoo, M.I., U.S.A.).

Prostaglandin analysis

Plasma samples were prepared for analysis by capillary column gas chromatography (g.c.)/electron capture mass spectrometry (e.c.m.s.) using methods described previously (Blair *et al.*, 1982; Barrow *et al.*, 1984), but with minor modifications. Briefly, fresh plasma was acidified with dilute hydrochloric acid to pH 3.5 immediately following separation, and applied to a μBondapack C_{18} Sep-Pak, which was then washed with water (10 ml). Prostaglandins were eluted from the C_{18} Sep-Pak with ethyl acetate (7 ml), which was applied directly to a silica Sep-Pak. Prostaglandins were eluted from the silica Sep-Pak with methanol (5 ml). Samples were routinely converted to methoxime (MO), 3,5-*bis*-trifluoro-methyl benzyl (TFMB) ester, trimethylsilyl (TMS) ether derivatives. They were stored in *n*-dodecane (10 μl) at −20°C. Aliquots (2.5 μl) were analysed by g.c./e.c.m.s. The detection limit was 2 pg ml^{-1} for 6-oxo-$PGF_{1\alpha}$ and 5 pg ml^{-1} for PGD_2, PGE_2 and 13,14-dihydro-15-oxo-$PGF_{2\alpha}$ when 10 ml plasma samples were assayed.

In some cases, 13,14-dihydro-15-oxo-$PGF_{2\alpha}$ was converted to a MO, TFMB ester, *bis*-(*tert*-butyl-dimethyl-silyl) (BDMS) ether derivative or to a MO, TFMB ester, *n*-butyl boronate derivative. The latter derivative, which is not described in our previous publications, was prepared by treatment of the methoxime with 30 μl *n*-butyl boronic acid (5 mg ml^{-1}; Sigma Chemical Company) in 2,2-dimethoxypropane. The solution was allowed to stand at ambient temperature for 3 h, and overnight at −20°C. Because the derivative is rapidly hydrolysed, aliquots (2.5 μl) were assayed without removal of solvent or reagent. The temperature of the capillary column was programmed from 80 to 325°C at 20°C min^{-1}.

Results

13,14-Dihydro-15-oxo-$PGF_{2\alpha}$ was identified in plasma obtained during bradykinin infusion. The structure of this metabolite was verified by comparison of the e.c. mass spectra and g.c. retention times of 3 different derivatives with authentic standards: the MO, TFMB ester, *bis*-TMS ether derivative (Figure 1); the MO, TFMB ester, *bis*-BDMS ether derivative (Figure 2) and the MO, TFMB ester, 9,11-*n*-butyl boronate derivative (Figure 3). Quantitative conversion to the latter derivative confirmed the 9,11-*cis* configuration of the hydroxyl groups on the cyclopentane ring.

Figure 4 shows the concentrations of 13,14-

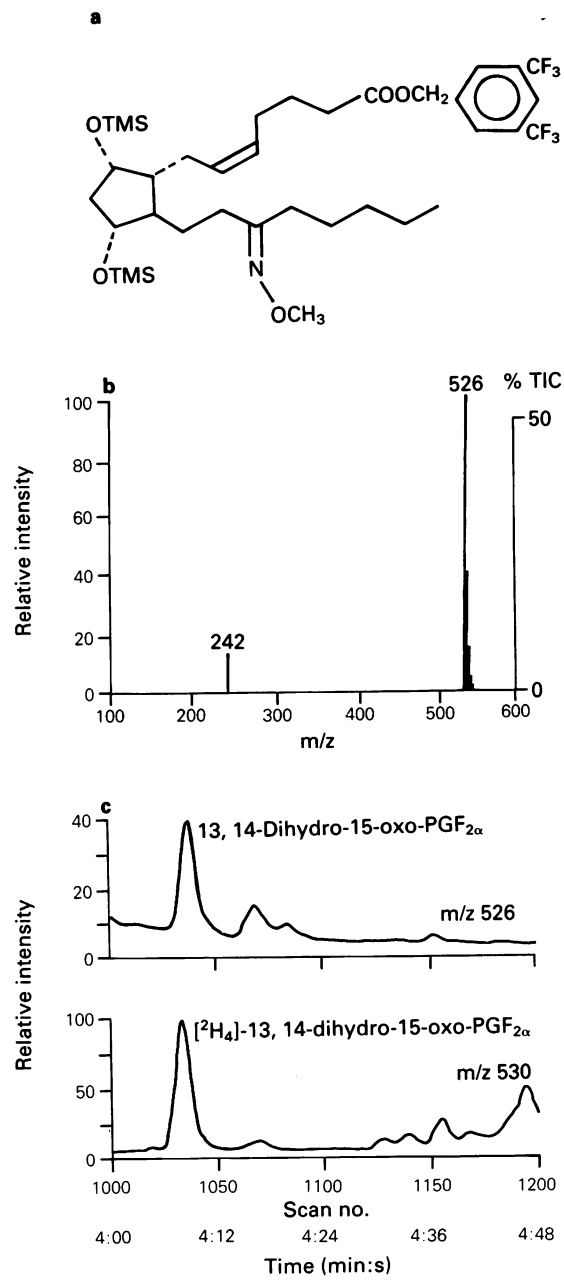

Figure 1 (a) The methoxime, 3,5-*bis*-trifluoromethyl benzyl ester, *bis*-trimethylsilyl (TMS) ether derivative of 13,14-dihydro-15-oxo-prostaglandin $F_{2\alpha}$ (13,14-dihydro-15-oxo-$PGF_{2\alpha}$). (b) Electron capture mass spectrum. (c) Selected ion chromatogram. An extracted plasma sample from blood taken during infusion of bradykinin to which $[^2H_4]$-13,14-dihydro-15-oxo-$PGF_{2\alpha}$ (5 ng) had been added as internal standard.

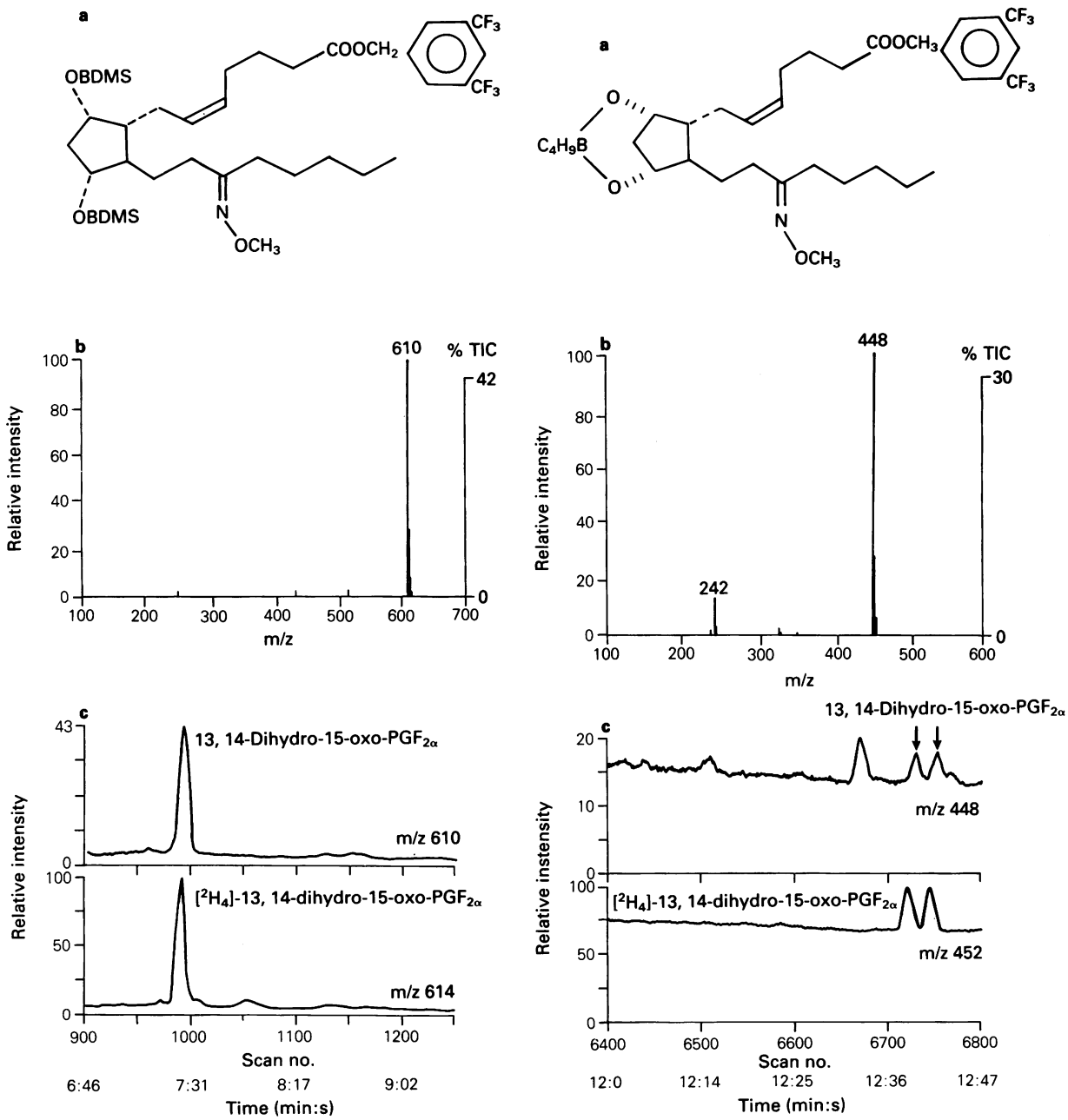

Figure 2 (a) The methoxime, 3,5-*bis*-trifluoromethyl, *bis* (*tert*-butyl dimethylsilyl) (BDMS) ether derivative of 13,14-dihydro-15-oxo-PGF$_{2\alpha}$. (b) Electron capture mass spectrum. (c) Selected ion chromatogram. An extracted plasma sample from blood taken during infusion of bradykinin to which [^{2}H$_4$]-13,14-dihydro-15-oxo-PGF$_{2\alpha}$ (5 ng) had been added.

Figure 3 (a) The methoxime, 3,5-*bis*-trifluoromethyl benzyl ester, *n*-butyl boronate derivative of 13,14-dihydro-15-oxo-PGF$_{2\alpha}$. (b) Electron capture mass spectrum. (c) Selected ion chromatogram. An extracted plasma sample from blood taken during bradykinin infusion to which [^{2}H$_4$]-13,14-dihydro-15-oxo-PGF$_{2\alpha}$ (5 ng) had been added.

dihydro-15-oxo-$PGF_{2\alpha}$ and 6-oxo-$PGF_{1\alpha}$ in samples obtained immediately before and at the end of bradykinin infusions. Concentrations of 13,14-dihydro-15-oxo-$PGF_{2\alpha}$ rose from baseline values in the range <5–10 pg ml^{-1} to 28–403 pg ml^{-1}. During these low dose bradykinin infusions, elevations in 6-oxo-$PGF_{1\alpha}$ concentrations were small and variable. In the same samples, no changes in PGE_2, PGD_2 or $PGF_{2\alpha}$ were found. Infusions of saline did not increase plasma concentrations of 6-oxo-$PGF_{1\alpha}$ or 13,14-dihydro-15-oxo-$PGF_{2\alpha}$.

Figure 5 shows concentrations of 13,14-dihydro-15-oxo-$PGF_{2\alpha}$ in plasma sampled immediately before and at the end of 60 min infusions of PGE_2. These increased from baseline values in the range <5–17 pg ml^{-1} to 160–603 pg ml^{-1}. The structure of the PGE_2-derived metabolite was verified in 4 samples by quantitative conversion to BDMS and *n*-butyl boronate derivatives. No PGE_2 was detected in plasma sampled during the infusions and, as expected, there was no increase in 6-oxo-$PGF_{1\alpha}$.

During PGD_2 infusions, plasma concentrations of PGD_2 increased from baseline values in the range <5–22 pg ml^{-1} to 612–2182 pg ml^{-1} (Figure 6). There was no elevation in plasma 13,14-dihydro-15-oxo-$PGF_{2\alpha}$ concentration.

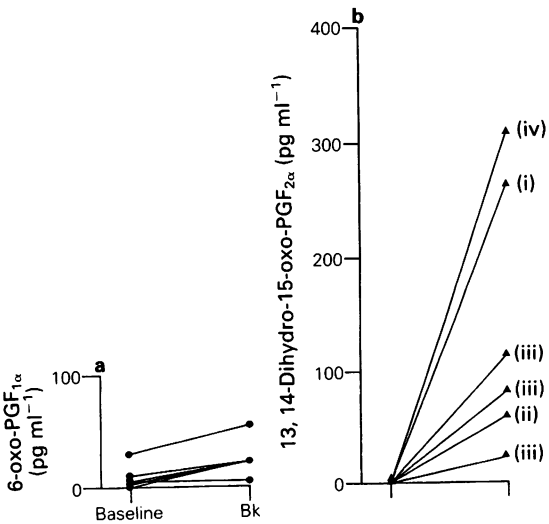

Figure 4 Plasma concentrations (pg ml^{-1}) of (a) 6-oxo-prostaglandin $F_{1\alpha}$ (6-oxo-$PGF_{1\alpha}$, ●) and (b) 13,14-dihydro-15-oxo-$PGF_{2\alpha}$ (▲) before and during bradykinin (Bk) infusions. Bradykinin doses: (i) 12 μg kg^{-1}, (ii) 18 μg kg^{-1}, (iii) 24 μg kg^{-1}, (iv) 42 μg kg^{-1}.

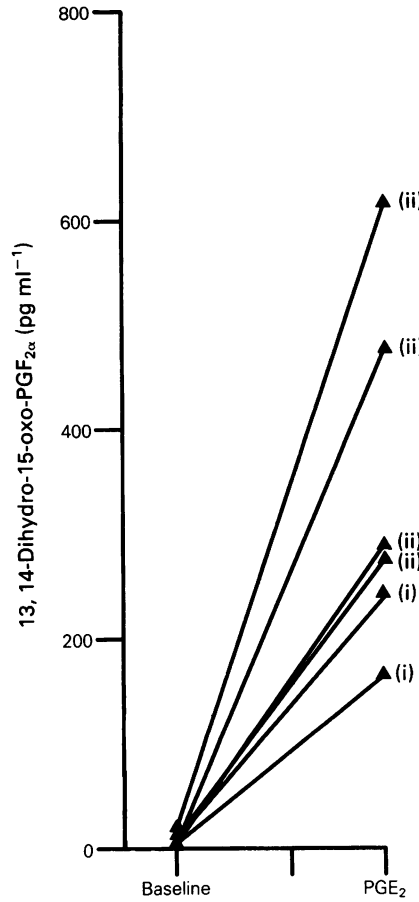

Figure 5 Plasma concentrations (pg ml^{-1}) of 13,14-dihydro-15-oxo-$PGF_{2\alpha}$ (▲) before and during prostaglandin E_2 (PGE_2) infusions. PGE_2 doses: (i) 6 μg kg^{-1}, (ii) 12 μg kg^{-1}.

Discussion

The principle finding is that, in man, intravenous bradykinin increases the plasma concentration of 13,14-dihydro-15-oxo-$PGF_{2\alpha}$ (Figures 1–4). There was no correlation between the dose of bradykinin and the magnitude of the increase in plasma concentration of the metabolite (Figure 4). This implies that there is considerable individual variation in this response to bradykinin, either at the level of stimulation of prostaglandin synthesis or of subsequent prostaglandin distribution and metabolism. The origin of the 13,14-dihydro-15-oxo-$PGF_{2\alpha}$ formed during bradykinin infusion is of considerable interest. $PGF_{2\alpha}$ is an

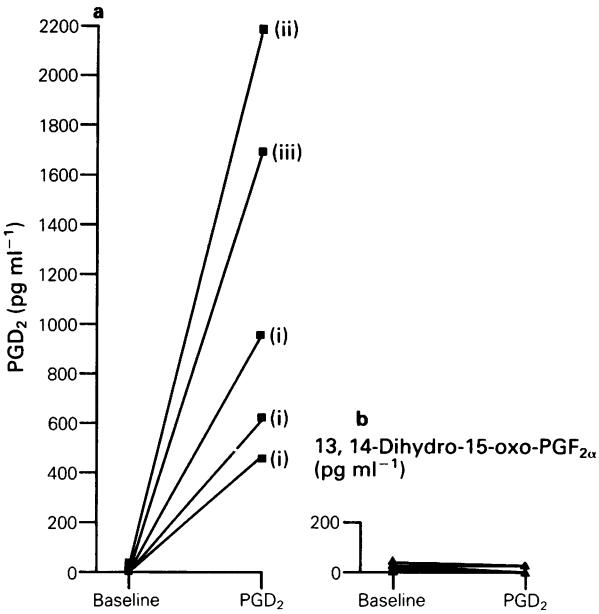

Figure 6 Plasma concentrations (pg ml^{-1}) of (a) prostaglandin D_2 (PGD_2, ■) and (b) 13,14-dihydro-15-oxo-$PGF_{2\alpha}$ (▲) before and during PGD_2 infusions. PGD_2 doses: (i) 1.4 μg kg^{-1}, (ii) 6.2 μg kg^{-1}, (iii) 9.1 μg kg^{-1}.

obvious possible source, since during intravenous infusion in man it is metabolised to 13,14-dihydro-15-oxo-$PGF_{2\alpha}$ (Granström, 1972; Granström *et al.*, 1982). Hamberg & Israelsson (1970) showed that guinea-pig liver can form $PGF_{2\alpha}$ from PGE_2 and Granström & Kindahl (1982) used two dimensional thin layer chromatography to show that a bolus injection of PGE_2 is converted to 13,14-dihydro-15-oxo-$PGF_{2\alpha}$ in the rabbit. Our findings, based on more rigorous analytical techniques, show that intravenous PGE_2 is also converted to 13,14-dihydro-15-oxo-$PGF_{2\alpha}$ in man, indicating that PGE_2, as well as $PGF_{2\alpha}$ could be the source of this metabolite during bradykinin infusion. We did not detect PGE_2 in the circulation during infusions of PGE_2 that caused marked accumulation of 13,14-dihydro-15-oxo-$PGF_{2\alpha}$. This is presumably due to the efficiency of the uptake mechanism for PGE_2 in the lung (Ferreira & Vane, 1967). Consequently, failure to detect PGE_2 in blood sampled during bradykinin infusion does not rule out the possibility that this is the origin of the 13,14-dihydro-15-oxo-$PGF_{2\alpha}$. Our earlier tentative observation that 13,14-dihydro-15-oxo-$PGF_{2\alpha}$ might be a metabolite of intravenous PGD_2 (Barrow *et al.*, 1984), was not confirmed, indicating that circulating PGD_2 is not the origin of this metabolite during bradykinin infusion. PGD_2 itself was detected during PGD_2 infusions, with some individual variation between dose and plasma concentration (Figure 6), in keeping with inter-subject variation in prostaglandin uptake and/or metabolism (Barrow *et al.*, 1984).

These experiments do not determine the cellular origin of the bradykinin-stimulated 13,14-dihydro-15-oxo-$PGF_{2\alpha}$. One possibility is that, in contrast to bradykinin-stimulated PGI_2 synthesis by human endothelial cells from large blood vessels (Hong, 1980), it is derived from PGE_2 and $PGF_{2\alpha}$ produced by endothelium in the microcirculation.

Cultured endothelial cells from the microvasculature synthesize predominantly PGE_2, together with some $PGF_{2\alpha}$ and little PGI_2 (Charo *et al.*, 1984). Similarly, coronary microvessels produce PGE_2 rather than PGI_2 (Gerritson & Printz, 1981; Gerritson & Cheli, 1983). Since endothelial cells from large blood vessels synthesize PGI_2 in response to bradykinin, it is plausible that microvascular endothelial cells would produce PGE_2 and $PGF_{2\alpha}$ in response to this stimulus. The ability of endothelium from different regions of the circulation to produce different prostaglandins could have important functional consequences.

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