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Bradykinin-stimulated prostaglandin synthesis in conscious rabbits

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1 Bradykinin was infused intravenously into conscious rabbits to determine its effect on the concentration of prostaglandins in plasma. 6-Oxo-prostaglandin (PG) $F_{1\alpha}$, the stable hydrolysis product of prostacyclin, and 13,14-dihydro-15-oxo-$PGF_{2\alpha}$, a metabolite derived from PGE_2 and $PGF_{2\alpha}$, were measured by gas chromatography-electron capture mass spectrometry.

2 Incremental infusions of bradykinin ($0.4-3.2\,\mu g\,kg^{-1}\,min^{-1}$) increased plasma concentrations of both 6-oxo-$PGF_{1\alpha}$ and 13,14-dihydro-15-oxo-$PGF_{2\alpha}$.

3 Aspirin ($10\,mg\,kg^{-1}$, i.v.) inhibited bradykinin-stimulated 6-oxo-$PGF_{1\alpha}$ and 13,14-dihydro-15-oxo-$PGF_{2\alpha}$ synthesis at 30 min and 6 h. At 24 h, the mean bradykinin-stimulated 13,14-dihydro-15-oxo-$PGF_{2\alpha}$ concentration was 66% of its original value, whilst 6-oxo-$PGF_{1\alpha}$ remained substantially inhibited.

4 The different rates of recovery of bradykinin-stimulated production of the two prostaglandins after inhibition by aspirin suggests that intravenous bradykinin stimulates prostacyclin and $PGE_2/PGF_{2\alpha}$ production in distinct cell populations which synthesize cyclo-oxygenase at different rates.

Introduction

Intravenous administration of bradykinin stimulates prostacyclin synthesis in man, causing a dose-dependent elevation in the plasma concentration of its hydrolysis product, 6-oxo-prostaglandin (PG) $F_{1\alpha}$ (Barrow *et al.*, 1986). This response is inhibited by aspirin but recovery is rapid, being complete in 6 h (Heavey *et al.*, 1985). Bradykinin also increases the plasma concentration of 13,14-dihydro-15-oxo-$PGF_{2\alpha}$ in man (Barrow *et al.*, 1987). This metabolite may originate from PGE_2 (Hamberg & Israelsson, 1970; Granström & Kindahl, 1982; Barrow *et al.*, 1987) as well as from $PGF_{2\alpha}$ (Granström & Kindahl, 1982). The objective of the present study was to determine the effects of bradykinin and of aspirin on plasma concentrations of 6-oxo-$PGF_{1\alpha}$ and 13,14-dihydro-15-oxo-$PGF_{2\alpha}$ in rabbits. We chose rabbits because they are large enough for repeated blood sampling. Moreover, they may possess two types of bradykinin receptor (Regoli & Barabé, 1980), and some of the vascular responses to bradykinin are mediated by cyclo-oxygenase products in this species (Cherry *et al.*, 1982; Förstermann *et al.*, 1986).

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Methods

New Zealand white male rabbits, 3.2–4.5 kg, were used in these studies. They were loosely restrained in a box whilst procedures were carried out. Bradykinin (UCB Bioproducts, Brussels, Belgium) was dissolved in 0.9% w/v NaCl in sterile water (saline). Aspirin (Sigma Chemical Co., Poole), was prepared by dissolving 160 mg in 2 ml 1 M Tris buffer (pH 8.4) and diluting with 14 ml saline.

Effect of bradykinin on plasma prostaglandins

Six rabbits were infused with bradykinin and three infused with saline as controls as follows. A 22 gauge cannula (Abbocath, Sligo, Ireland) was inserted into the posterior marginal ear vein and 4 ml of blood allowed to drip into a bottle containing 40 units of heparin. Subsequent samples were collected in the same way to minimize trauma to the vein. The cannula was stoppered and kept patent by bolus injections of heparin in saline ($10\,units\,ml^{-1}$) every 10 min. One hour later a second blood sample (4 ml) was taken and an intravenous cannula, connected to a syringe driven by an infusion pump (Braun, Melsungen, W. Germany), was inserted in the opposite ear. Bradykinin

was then infused at rates of 0.4, 0.8, 1.6 and 3.2 $\mu g\ kg^{-1}\ min^{-1}$. After 10 min at each rate, a blood sample (2 ml) was taken and the next rate of infusion started. A further blood sample (4 ml) was taken 1 h after the end of the infusion.

Effect of aspirin on bradykinin-stimulated prostaglandin synthesis

Six rabbits were studied on three occasions each 2–4 weeks apart. In a crossover design they received a saline infusion with Tris-saline vehicle, a bradykinin infusion with Tris-saline vehicle, and a bradykinin infusion with aspirin (10 $mg\ kg^{-1}$). After insertion of an intravenous cannula, a blood sample (4 ml) was collected as described above and the cannula then used to infuse either bradykinin (0.8 $\mu g\ kg^{-1}\ min^{-1}$ for 5 min followed by 1.6 $\mu g\ kg^{-1}\ min^{-1}$ for 5 min) or saline. At the end of the infusion a second intravenous cannula was inserted into the opposite ear and a further blood sample (4 ml) taken. Aspirin or Tris-saline was injected intravenously as a bolus and both cannulae removed. Infusion of bradykinin or saline and blood sampling were repeated with fresh cannulae 30 min and 6 h after injection of the aspirin or Tris-saline. Bradykinin infusion was performed in the same manner in a further 6 rabbits before and 24 h after the intravenous injection of aspirin.

Sample preparation and analysis

Blood samples were placed immediately on ice. Plasma was separated at 4°C within 20 min by centrifugation and added to 20 ml water containing 5 ng [$^2H_4$]-6-oxo-$PGF_{1\alpha}$ and 5 ng [$^2H_4$]-13,14-dihydro-15-oxo-$PGF_{2\alpha}$ as internal standards. Prostaglandin standards were the generous gifts from Dr J. Pike (Upjohn Co., Kalamazoo, U.S.A.). Samples were stored at −20°C and processed and extracted within 5 days of collection as described previously (Blair *et al.*, 1982; Barrow *et al.*, 1987). The derivatives were stored in n-dodecane (10 µl) at −20°C and analysed by capillary column gas chromatography/electron capture mass spectrometry. The detection limit for 6-oxo-$PGF_{1\alpha}$ was 20 pg per sample and for 13,14-dihydro-15-oxo-$PGF_{2\alpha}$ 50 pg per sample (e.g. 10 $pg\ ml^{-1}$ and 25 $pg\ ml^{-1}$ respectively when a 2 ml sample was assayed).

Statistical analysis

Student's *t* test was used to determine if there was a significant effect of increasing doses of bradykinin, compared with placebo, on plasma 6-oxo-$PGF_{1\alpha}$ and 13,14-dihydro-15-oxo-$PGF_{2\alpha}$ concentrations. In the aspirin experiment differences were analysed by the Wilcoxon test for paired differences, thereby avoiding assumptions as to the distributions of the variables involved, and considered significant if $P<0.05$. Results are presented as the mean ± s.e.mean.

Results

Animals sat quietly during the study without apparent discomfort. During infusion of bradykinin (0.4–3.2 $\mu g\ kg^{-1}\ min^{-1}$) the mean plasma 6-oxo-$PGF_{1\alpha}$ concentration increased from 112 ± 29 $pg\ ml^{-1}$ to 603 ± 49 $pg\ ml^{-1}$ ($P<0.02$) and the 13,14-dihydro-15-oxo-$PGF_{2\alpha}$ concentration increased from 34 ± 6 $pg\ ml^{-1}$ to 551 ± 190 $pg\ ml^{-1}$ ($P<0.05$) (Figures 1 and 2). One hour after infusion, the concentrations had fallen to 156 ± 50 $pg\ ml^{-1}$ and 40 ± 13 $pg\ ml^{-1}$ respectively. Control infusions of saline were accompanied by small increases in plasma concentrations of 6-oxo-$PGF_{1\alpha}$ from 145 ± 31 to 196 ± 52 $pg\ ml^{-1}$ and of 13,14-dihydro-15-oxo-$PGF_{2\alpha}$ from 60 ± 9 to 89 ± 20 $pg\ ml^{-1}$. One hour after the control infusion, the mean concentrations of the two prostaglandins were 287 ± 47 and 60 ± 4 $pg\ ml^{-1}$ respectively.

A different method of sampling was adopted in the

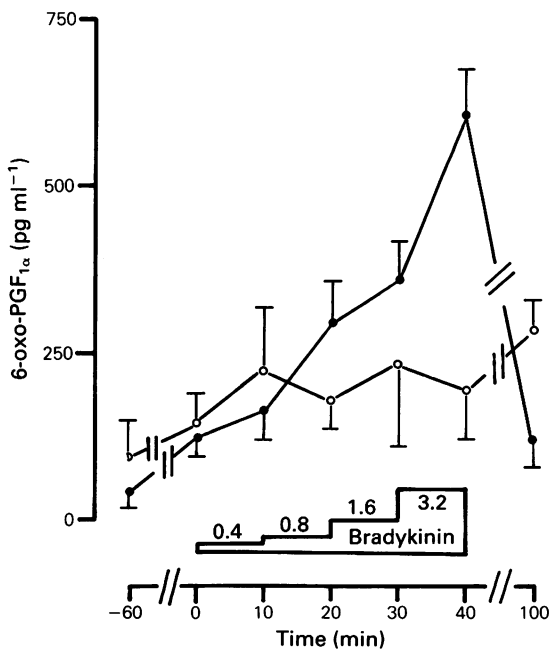

Figure 1 Effect of bradykinin infusion on mean plasma concentrations of 6-oxo-$PGF_{1\alpha}$ in the rabbit; vertical lines show s.e.mean. Bradykinin was given as a stepwise infusion from 0.4–3.2 $\mu g\ kg^{-1}\ min^{-1}$ (●, $n=6$) or equivalent volumes of saline (○, $n=3$). The effect of bradykinin infusion was significantly different from saline, $P<0.05$.

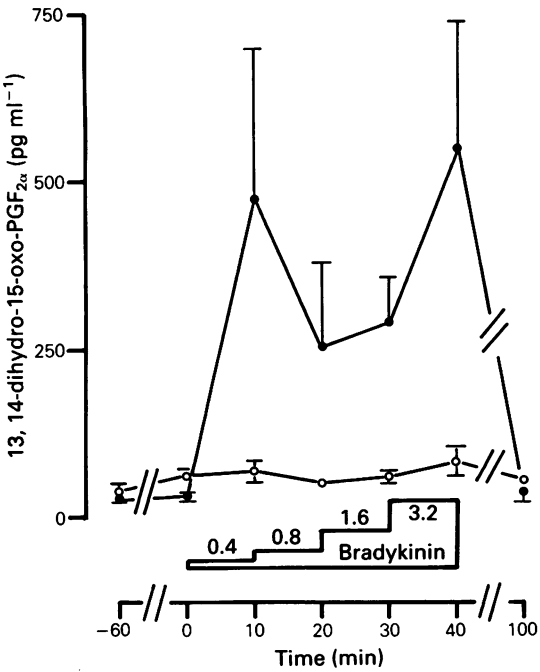

Figure 2 Effect of bradykinin on mean plasma concentrations of 13,14-dihydro-15-oxo-$PGF_{2\alpha}$ in the rabbit; vertical lines show s.e.mean Bradykinin infusion was given as a stepwise infusion from 0.4–3.2 ug kg^{-1} min^{-1} (●, $n = 6$) or equivalent volumes of saline (○, $n = 3$). The effect of bradykinin infusion was significantly different from that of saline, $P < 0.05$.

aspirin study because of the increase in plasma 6-oxo-$PGF_{1\alpha}$ concentration that occurred in saline-infused control animals when blood was sampled from an indwelling line. All blood samples were obtained immediately after inserting a fresh cannula into the ear vein. Blood sampled before and during saline infusion following Tris-saline vehicle showed that this protocol resulted in some elevation of 6-oxo-$PGF_{1\alpha}$ concentrations at 30 min but had little effect on 6-oxo-$PGF_{1\alpha}$ at 6 h (Figure 3a) or on 13,14-dihydro-15-oxo-$PGF_{2\alpha}$ at 30 min or 6 h (Figure 4a). Ten minute infusions of bradykinin before and after aspirin vehicle caused an increase in the plasma concentrations of both 6-oxo-$PGF_{1\alpha}$ (Figure 3b) and 13,14-dihydro-15-oxo-$PGF_{2\alpha}$ (Figure 4b) ($P < 0.001$, $n = 18$ in each case). Aspirin inhibited the bradykinin-stimulated rise in 6-oxo-$PGF_{1\alpha}$ at 30 min ($P < 0.05$) and at 6 h ($P < 0.05$) (Figure 3c). It also inhibited the effect of bradykinin on 13,14-dihydro-15-oxo-$PGF_{2\alpha}$ at 30 min and at 6 h ($P < 0.05$) (Figure 4c).

When a separate group of 6 rabbits was infused with bradykinin before and 24 h after injection of aspirin, the rise in plasma 6-oxo-$PGF_{1\alpha}$ concentration was inhibited by aspirin to 6% of control (17 ± 7 pg ml^{-1} compared with 309 ± 67 pg ml^{-1} before aspirin, $P < 0.05$, Figure 5). In contrast, the mean bradykinin-stimulated plasma 13,14-dihydro-15-oxo-$PGF_{2\alpha}$ concentration after aspirin was 66% of control (624 ± 241 pg ml^{-1} after aspirin compared to 944 ± 312 pg ml^{-1} before, $P > 0.05$, Figure 5).

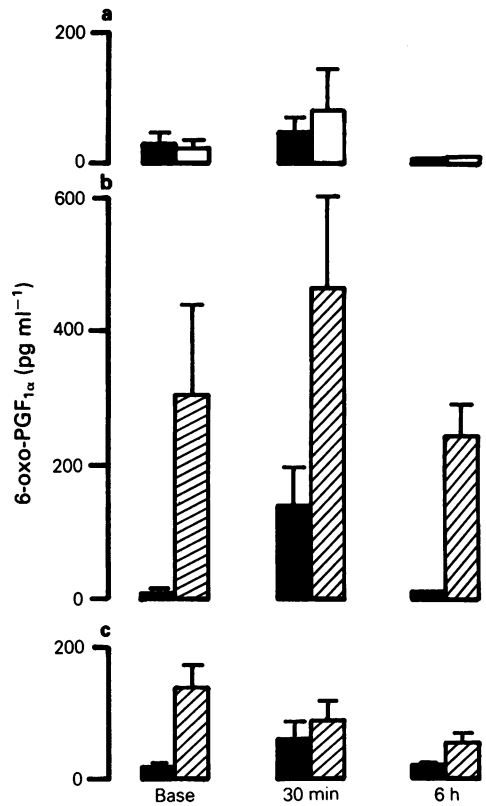

Figure 3 Effect of aspirin on bradykinin-stimulated 6-oxo-$PGF_{1\alpha}$ plasma concentrations. The columns indicate mean 6-oxo-$PGF_{1\alpha}$ plasma concentrations in samples obtained before and at the end of saline or bradykinin infusions in 6 rabbits; vertical lines show s.e.mean. The infusions of saline (10 min) or bradykinin (0.8 μg kg^{-1} min^{-1} for 5 min and 1.6 μg kg^{-1} min^{-1} for 5 min) were given before and at 30 min and 6 h after either aspirin 10 mg kg^{-1} or Tris-saline vehicle at time 0. (a) Saline infusion before and after Tris-saline vehicle: solid column = sample taken before infusion; open column = sample taken at the end of saline infusion. (b) Bradykinin infusion before and after Tris-saline vehicle: solid column = sample taken before infusion; hatched column = sample taken at the end of bradykinin infusion. (c) Bradykinin infusion after aspirin: solid column = sample taken before infusion; hatched column = sample taken at the end of bradykinin infusion.

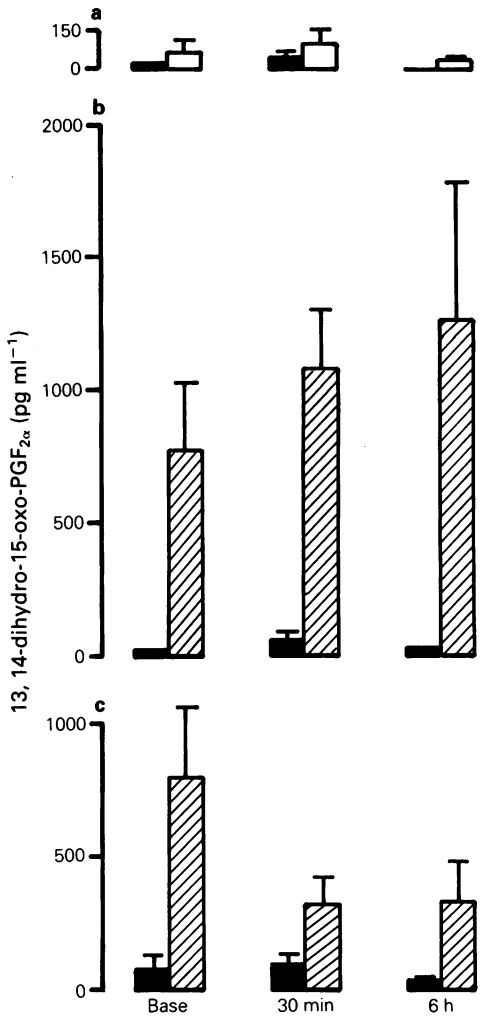

Figure 4 Effect of aspirin on bradykinin-stimulated 13,14-dihydro-15-oxo-$PGF_{2\alpha}$ plasma concentrations. The columns indicate mean 13,14-dihydro-15-oxo-$PGF_{2\alpha}$ plasma concentrations in samples obtained before and at the end of saline or bradykinin infusions in 6 rabbits; s.e.mean shown by vertical lines. The infusions of saline (10 min) or bradykinin (0.8 $\mu g\,kg^{-1}\,min^{-1}$ for 5 min and 1.6 $\mu g\,kg^{-1}\,min^{-1}$ for 5 min) were given before and at 30 min and 6 h after either aspirin 10 $mg\,kg^{-1}$ or Tris-saline vehicle at time 0. (a) Saline infusion before and after Tris-saline vehicle: solid column = sample taken before infusion; open column = sample taken at the end of saline infusion. (b) Bradykinin infusion before and after Tris-saline vehicle: solid column = sample taken before infusion; hatched column = sample taken at the end of bradykinin infusion. (c) Bradykinin infusion after aspirin: solid column = sample taken before infusion; hatched column = sample taken at the end of bradykinin infusion.

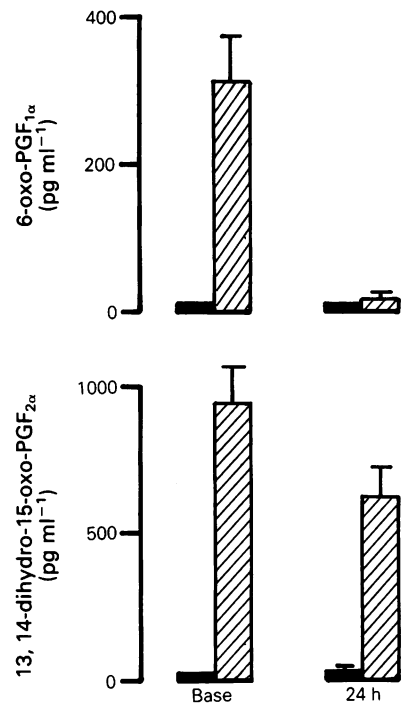

Figure 5 Effect of aspirin on bradykinin-stimulated prostaglandins at 24 h. Results are shown as mean with s.e.mean shown by vertical lines ($n = 6$); solid columns = before bradykinin infusion; hatched columns = at the end of bradykinin infusion. Base = results before aspirin; 24 h = results 24 h after aspirin (10 $mg\,kg^{-1}$).

Discussion

This study shows that intravenous administration of bradykinin causes an increase in the plasma concentrations of 6-oxo-$PGF_{1\alpha}$ and of 13,14-dihydro-15-oxo-$PGF_{2\alpha}$ in conscious rabbits. However, this species differs quantitatively from man both in the basal plasma concentrations of these prostaglandins and in their response to bradykinin. Baseline values of 6-oxo-$PGF_{1\alpha}$ are tenfold higher than in man (Blair *et al.*, 1982), perhaps because of trauma to the rabbit ear vein caused by the cannula, which can stimulate local synthesis of prostacyclin (Ritter *et al.*, 1983). This trauma is inevitably greater than when sampling from human forearm veins which are larger and can be kept motionless during sampling. The magnitude of the response of 6-oxo-$PGF_{1\alpha}$ to bradykinin is also approximately tenfold that which occurs in man (Barrow *et al.*, 1986).

13,14-Dihydro-15-oxo-$PGF_{2\alpha}$ is formed enzy-

matically in the lung (Granström & Kindahl, 1982), and prostaglandin delta 13 reductase and 15-hydroxyprostaglandin dehydrogenase (Hansen, 1976) are present in a variety of tissues. However, blood from skin incisions in man contain PGE_2 but little or no 13,14-dihydro-15-oxo-$PGF_{2\alpha}$ (Ritter *et al.*, 1987), so it is unlikely that this metabolite is formed locally at sites of vascular trauma. Baseline plasma concentrations of this metabolite in the rabbit are greater than in man (Barrow *et al.*, 1987), but values after bradykinin stimulation are similar in both species. These species differences may be caused by different rates of prostaglandin synthesis or of elimination.

Pretreatment of rabbits with aspirin inhibited the bradykinin-induced elevations in plasma concentrations of 6-oxo-$PGF_{1\alpha}$ and of 13,14-dihydro-15-oxo-$PGF_{2\alpha}$, presumably by inhibition of cyclo-oxygenase (Vane, 1971). The half life of aspirin in male rabbits is 30 min (Buchanan *et al.*, 1983), while inhibition of bradykinin-stimulated prostaglandin synthesis persisted for many hours, consistent with irreversible acetylation of the enzyme (Roth *et al.*, 1975). In contrast to the effect of aspirin on bradykinin-stimulated plasma concentrations of 6-oxo-$PGF_{1\alpha}$ in man, which recover in 6 h (Heavey *et al.*, 1985), the response to bradykinin in the rabbit remained inhibited both at 6 and 24 h.

Bradykinin-stimulated 13,14-dihydro-15-oxo-$PGF_{2\alpha}$ was also inhibited by aspirin but recovered substantially within 24 h. The more rapid recovery of the response of 13,14-dihydro-15-oxo-$PGF_{2\alpha}$ to bradykinin after inhibition of aspirin, compared with that of 6-oxo-$PGF_{1\alpha}$, suggests that bradykinin stimulates prostaglandin synthesis by more than one functionally distinct pool of cyclo-oxygenase within the body. Since recovery of prostaglandin synthesis after inhibition by aspirin depends on new protein synthesis (Czervionke *et al.*, 1979; Bailey *et al.*, 1985; Frazer & Ritter, 1987), the implication is that bradykinin-stimulated 13,14-dihydro-15-oxo-$PGF_{2\alpha}$ may originate from cells in which cyclo-oxygenase synthesis is more rapid than in those responding to bradykinin with prostacyclin synthesis.

The tissue of origin of bradykinin-stimulated prostaglandin synthesis is unknown. Bradykinin stimulates prostacyclin synthesis by large vessel endothelial cells *in vitro* (Hong, 1980), and PGE_2, which can be metabolised to 13,14-dihydro-15-oxo-$PGF_{2\alpha}$ (Granström & Kindahl, 1982; Barrow *et al.*, 1987), has been reported to be the principal cyclo-oxygenase product of microvascular endothelial cells (Gerritsen & Cheli, 1983; Charo *et al.*, 1984). It is possible that intravenous bradykinin stimulates prostacyclin synthesis by endothelium from large vessels and PGE_2 synthesis by endothelium of the microcirculation.

We have previously shown in an *ex vivo* study that prostacyclin synthesis in the rabbit recovers from inhibition by aspirin more rapidly in aortic endothelium than in other tissues (Frazer & Ritter, 1987). However, it remains inhibited at approximately 64% of control 24 h after aspirin. This contrasts with the present finding that the bradykinin-stimulated plasma 6-oxo-$PGF_{1\alpha}$ concentration is less than 10% of control, 24 h after aspirin. This raises the question whether there are regional differences in the rate of endothelial cyclo-oxygenase synthesis in different vascular beds.

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