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Reciprocal inhibition of nitric oxide and prostacyclin synthesis in human saphenous vein

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1 Angiotensin II (AII) causes contraction of isolated rings of human saphenous vein, responses that are attenuated by the presence of functional endothelium. In this study, we have investigated the mechanisms controlling the release by AII of two endothelial-derived vasorelaxants, prostacyclin (PGI_2) and nitric oxide (NO).

2 Myotropic and biochemical changes were measured in response to AII. The biochemical responses measured were the output of PGI_2 (as 6-oxo-$PGF_{1\alpha}$) and of NO (as cyclic GMP). Inhibitors of cyclo-oxygenase (COX; piroxicam) or NO synthase (NOS; L-NAME), were added to the system to determine the influence of endogenous prostaglandins and NO on both myotropic and biochemical responses. Furthermore, to mimic the effects of endogenous, PGI_2 or NO, exogenous forms of these relaxants were added, during inhibition of their endogenous release.

3 Contractions of the rings of saphenous vein in response to AII (1-100 nM) were unaffected by treatment with either piroxicam (5 μM) or L-NAME (200 μM) individually. However, when these two inhibitors were used together, there was an increase in the contractions in response to AII.

4 Biochemical analyses revealed that during stimulation by AII, levels of PGI_2 and NO were enhanced when synthesis of the other vasodilator was inhibited, suggesting that endogenous NO inhibits PGI_2 synthesis and endogenous, PGI_2 or another vasorelaxant PG can inhibit NO synthesis.

5 Exogenous PGI_2 (as iloprost) or NO (from glyceryl trinitrate) inhibited the increased output of endogenous NO or PGI_2 respectively.

6 These results demonstrate the presence, in human saphenous vein, of a mechanism which ensures that levels of vasodilatation are maintained through a compensatory increase in one relaxant agonist when output of the other is decreased. If present *in vivo* such a mechanism would be important in maintaining saphenous vein graft patency as both PGI_2 and NO are not only vasodilators, but inhibit platelet aggregation and myoinitimal hyperplasia, processes implicated in degeneration of graft function.

Keywords: Nitric oxide; prostacyclin; angiotensin II; human saphenous vein

Introduction

Over the last three decades, the endothelium has become recognised as a cell with extensive metabolic activity of great importance to vascular function. This has encouraged correlation of the response of the vascular smooth muscle with the metabolic reactions taking place in the associated endothelium. The clearest example of this correlation was the description of the endothelial derived relaxing factor, eventually characterized as nitric oxide (NO) (Moncada *et al.*, 1991). Another example is prostacyclin (PGI_2), also synthesized by endothelium and a vasodilator and anti-aggregatory agent for platelets, as is NO itself (Moncada & Vane, 1979; Moncada *et al.*, 1991). Thus there are at least two endothelial derived factors that are capable of modifying significantly the constrictor responses of vascular smooth muscle. An important practical corollary of this interplay between endothelium and smooth muscle is the belief that the endothelial function of the human saphenous vein, the conduit used most commonly for coronary artery bypass surgery (CABS), is a determining factor in the long term patency of the blood vessel after grafting. This is attributed largely to the ability of NO and PGI_2 to modulate smooth muscle tone and to prevent thrombosis and smooth muscle proliferation (Angelini *et al.*, 1987; 1989; 1991; Painter, 1990). In the saphenous vein, release of these endothelium-derived agonists can be stimulated by, and therefore can modify the actions of, a variety of endogenous vasoactive substances including leukotriene C_4 (LTC_4), leukotriene D_4 (LTD_4) and histamine (Yang *et al.*, 1989; Allen *et al.*, 1992).

Another important endogenous vasoactive agonist of interest in this context is angiotensin II (AII). This peptide is a potent vasoconstrictor, circulates at elevated levels during and after the CABS procedure and has been implicated in the post-operative hypertension observed in 30-60% of patients after CABS (Taylor *et al.*, 1977). We have recently reported that while AII is a potent constrictor of human saphenous vein, the responses of human saphenous vein to AII were attenuated by the presence of functioning endothelium (Barker *et al.*, 1994a), suggesting that AII can stimulate the release of endothelium-derived relaxing factors. Release of PGI_2 or NO stimulated by AII has been reported in a number of vascular preparations; however, which modulating factor is released appears to be tissue and/or species-dependent (Toda & Miyazaki, 1981; Yamazaki & Toda, 1981; Greutter *et al.*, 1987; Vallotton *et al.*, 1990). In order to assess the relative roles of PGI_2 and NO in modulating contractions due to AII in human saphenous vein, we have measured myotropic responses to AII in the presence of nitric oxide synthase (NOS) and/or cyclo-oxygenase (COX) inhibitors. These responses were correlated with biochemical changes induced at the same time. The biochemical variables chosen for measurement were 6-oxo-$PGF_{1\alpha}$ (as an indicator of PGI_2 release) and guanosine 3' : 5'-cyclic monophoshate (cyclic GMP, as an indicator of NO release). Some of these results have been communicated to the British Pharmacological Society (Barker *et al.*, 1994b).

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Methods

Preparation of vascular rings

Samples of saphenous vein were obtained from patients who were undergoing elective myocardial revascularisation. In order to maximize the amount of functional endothelium and reduce damage to smooth muscle of the sample, they were obtained as soon as possible after the first excision and not subjected to any further surgical procedures. Samples were stored at 4°C in Krebs Henseleit (KH) buffer and used within 12 h (Barker *et al.*, 1994a). In the laboratory, transverse sections (2–4 mm rings) of saphenous vein were mounted on parallel wires, one of which was fixed and the other attached to a force transducer (Grass model FT03D) to allow changes in tension to be recorded isometrically. The rings were bathed in KH buffer, maintained at 37°C, aerated with 95% O_2 and 5% CO_2. Each ring was given an initial tension of 30–40 mN and allowed to equilibrate until a stable baseline was attained. In order to assess the viability of each ring, the maximum direct contractile response was measured and the presence of functional endothelium was checked.

To obtain the maximum direct contractile response of each ring, KCl (to give a final organ bath concentration of 90 mM) was added to the organ bath and left in the organ bath until a plateau was attained (usually about 5 min); this was repeated until a consistent response was obtained. The responses induced by KCl were taken to be the maximal (100%) response of the ring. Only those rings which gave a KCl response of greater than 10 mN were tested further for functional integrity of the endothelium.

Endothelial function was assessed by precontracting the rings with phenylephrine (PE, 10 μM) and then adding bradykinin (BK, 10 μM) an endothelium-dependent vasodilator of this tissue) to the organ bath (Barker *et al.*, 1994a). Only those rings which relaxed in response to BK (median relaxation; 26% CI 20,31 of the PE contraction) were used further in the study.

Following these two preliminary tests, rings were exposed to AII and either their myotropic or biochemical responses measured.

Myotropic assay

Rings were washed with buffer and allowed to equilibrate for 20 min. Following this rings were exposed in a cumulative fashion to three concentrations of AII (1, 10 and 100 nM) over a period of 20 min. Control responses were obtained from rings left untreated for the 20 min equilibration period and during exposure to AII. Responses after treatment were obtained from matched rings (cut from the same sample of saphenous vein, from the same patient) incubated with either piroxicam (5 μM; in preliminary experiments this concentration abolished PGI_2 release) N^G-nitro-L-arginine methyl ester (L-NAME, 200 μM; this concentration totally inhibited the relaxation, in response to BK, of the precontracted saphenous vein) or both of these inhibitors simultaneously for the 20 min equilibration period and during AII administration. All contractile responses to AII were expressed as a percentage of the (maximum) KCl response for each ring.

Biochemical assays

Control rings were untreated for a period of 15 min prior to AII administration. Matched rings, obtained from the sample of vein were incubated with either piroxicam (5 μM), L-NAME (200 μM) or both of these inhibitors for 15 min prior to and during exposure to a single concentration of AII (100 nM) for 5 min. After this time, the organ bath buffer was analysed for either 6-oxo-$PGF_{1\alpha}$ (as indicator of PGI_2 release) or cyclic GMP (as an indicator of NO release).

For assessment of 6-oxo-$PGF_{1\alpha}$ content, the whole of the organ bath buffer (10 ml) was collected after the total incubation time of 20 min. 6-Oxo-$PGF_{1\alpha}$ was measured by radioimmunossay (RIA) as described by Nakamura & Bakhle (1991), the inter assay variation, by this method, was 4%. PGI_2 release was expressed as pmol of 6-oxo-$PGF_{1\alpha}$ measured in the organ bath. For each ring the amount of 6-oxo-$PGF_{1\alpha}$ released into the organ bath over 20 min without any stimulation (basal release) was measured separately. In pilot experiments the major cyclo-oxygenase product was found to be 6-oxo-$PGF_{1\alpha}$ with TXB_2 and PGE_2 being undetectable by RIA (less than 0.2 pmol ml^{-1}).

For assessment of cyclic GMP content, aliquots of organ bath buffer (100 μl) were removed immediately before (t = 0) and at 1, 3 and 5 min after AII administration. At the end of this procedure the rings of saphenous vein were removed, blotted dry with tissue paper and weighed. cyclic GMP content of the buffer was measured by scintillation proximity RIA kit (Amersham); the inter assay variation of this method was 2%. The cyclic GMP released was expressed as fmol per mg of tissue.

In another series of experiments, the stable PGI_2 analogue, iloprost (2.5 ng ml^{-1}) or the NO donor GTN (1 μM) (in a preliminary study these concentrations caused complete relaxation of contracted with either AII 100 nM or PE 10 μM) were added to the organ bath at the same time as the enzyme inhibitors, piroxicam and L-NAME, (15 min prior to AII administration). Organ bath buffer was assayed for its content of 6-oxo-$PGF_{1\alpha}$ or cyclic GMP.

Materials

KH buffer had the following composition (mM): NaCl 118, $NaHCO_3$ 25, KCl 4.7, KH_2PO_4 1.2, Mg_2SO_4 1.2, $CaCl_2$ 2.5, glucose 5.6 (all BDH, AnalaR grade). KCl (AnalaR grade) was obtained from BDH (Poole, U.K.) cyclic GMP assay kits were obtained from Amersham (U.K.), iloprost was a gift from Schering (Germany). The antisera to 6-oxo-$PGF_{1\alpha}$ was donated by Dr J.A. Salmon (Wellcome Foundation, Beckenham, Kent). All other substances were obtained from Sigma (Poole, U.K.).

Statistical analyses

Matched rings from the same patients were compared. In all cases n refers to the number of patients from whom vessels were obtained.

Normal distribution of results was confirmed by a non-significant result in an F-test on the data. For such data, Students t test for paired or unpaired data was then applied and significance of the differences between the means was assumed when $P < 0.05$. Graphs for normal data show values as the mean with standard error of the mean.

Non-parametric tests were applied whenever the data was not normally distributed (confirmed by a significant result in an F-test on the data). For such data the Wilcoxon test for paired data was applied. Significant differences between groups was assumed when $P < 0.05$. These results have been shown as box and whisker plots, which depict the median, the interquartile range and the 5th and 95th percentiles of the data. In the text they are quoted as medians and the 95% confidence interval (CI) of the median is given.

Results

Myotropic responses; effects of piroxicam and L-NAME (separately and simultaneously)

The addition of piroxicam (5 μM) to the KH buffer did not alter the basal tone of the saphenous vein and did not alter myotropic responses of saphenous vein when compared with control, unpretreated rings, taken from the sample of vein. The median changes in response to 1, 10 and 100 nM AII after

piroxicam were 1% (CI −5,53), 4% (CI −25,9) and −2% (CI −42,81), respectively).

As with piroxicam, addition of L-NAME (200 μM) to the KH buffer did not alter the basal tone of the saphenous vein. Pretreatment of the rings with L-NAME did not alter the contractile responses of saphenous vein to AII when compared to control, the median changes in response the three concentrations of AII were 1% (CI −17,42), −7% (CI −52,3) and 2% (CI −50,20), respectively.

A number of rings were treated with both L-NAME and piroxicam together prior to stimulation with AII. Such pretreatment of the rings significantly enhanced the myotropic responses of AII compared with control, unpretreated, rings. Median responses to 100 nM, expressed as a percentage of the responses to 90 mM KCl, were 51% (CI 32,93) for treated rings and 31% (CI 12,65) for control untreated rings. These results are summarized in Figure 1.

Biochemical assays

Release of 6-oxo-$PGF_{1\alpha}$ Following stimulation of saphenous vein with the standard concentration of AII (100 nM), the level of 6-oxo-$PGF_{1\alpha}$ measured in the organ bath buffer was low with a median value of 1.0 pmol (CI 0.7,3.1). This was not different from 6-oxo-$PGF_{1\alpha}$ levels measured basally (no stimulation for 20 min) from the same samples of vein when the median value was 1.1 pmol (CI 0.4,3.6). Therefore, the changes in 6-oxo-$PGF_{1\alpha}$ levels when comparing the basal and AII stimulated levels from the same ring of vein, were also not significant (median value 0.1 pmol (CI −0.8,0.5); these results are shown in Figure 2).

Effects of L-NAME on 6-oxo-$PGF_{1\alpha}$ release After pretreatment of the saphenous vein rings with L-NAME, levels of 6-oxo-$PGF_{1\alpha}$ measured in the organ bath had a median value of 1.6 pmol (range 1.5, 3.0). These amounts were not different from basal levels (without any treatment) from the same rings of saphenous vein which had a median value of 1.7 pmol (range 0.6,2.4). Addition of AII to the rings of saphenous vein treated with L-NAME, led to an increase in the levels of 6-oxo-$PGF_{1\alpha}$ to 3.2 pmol (CI 1.1, 4.8). This was significantly higher than the basal release from the same rings. Comparing the stimulated release with the basal levels from the same rings of vein, there was an increase of 1.4 pmol (CI −0.6,2.7) above the basal amounts. Further, these stimulated amounts of 6-oxo-$PGF_{1\alpha}$ (Figure 2) were significantly higher than the amounts released due to AII stimulation without pretreatment with L-NAME.

Pretreatment with both piroxicam and L-NAME Levels of 6-oxo-$PGF_{1\alpha}$ were reduced to a median value of 0.2 pmol (CI −0.4,0.6), these amounts were not different from basal amounts and significantly less than the enhanced amounts released following pretreatment with L-NAME alone (Figure 2). Under these conditions, i.e. pretreatment of rings with both L-NAME and piroxicam prior to AII stimulation, there was a significant enhancement in the myotropic responses to AII when compared with responses of untreated rings isolated from the same sample of saphenous vein (compare Figure 1).

Release of cyclic GMP The basal output of cyclic GMP, immediately before giving AII and in the absence of any inhibitor, was 2.1 ± 0.6 fmol mg^{-1} saphenous vein. After the addition of AII, the mean value of cyclic GMP rose to a maximum of 3.3 ± 0.5 fmol mg^{-1}, 1 min after the addition, but this value or the values at 3 and 5 min after AII addition were not significantly different from the basal values, as shown in Figure 3a.

Effects of piroxicam on cyclic GMP release Following pretreatment of the saphenous vein with piroxicam for 15 min, the basal amount of cyclic GMP rose to about double the control values. Now, the addition of AII gave a clear further increase reaching 6.4 ± 0.8 fmol mg^{-1} at 1 min after AII addition and this was maintained for at least 5 min. These levels were significantly higher than the basal levels for the same rings and than the levels of cyclic GMP generated by AII alone in the matched rings, from the same vein sample, measured at corresponding time points. The results are shown in Figure 3a. It is worth noting that under the conditions used for this assay, no enhancement was observed in the myotropic responses of the saphenous vein when compared with those from control rings (see above).

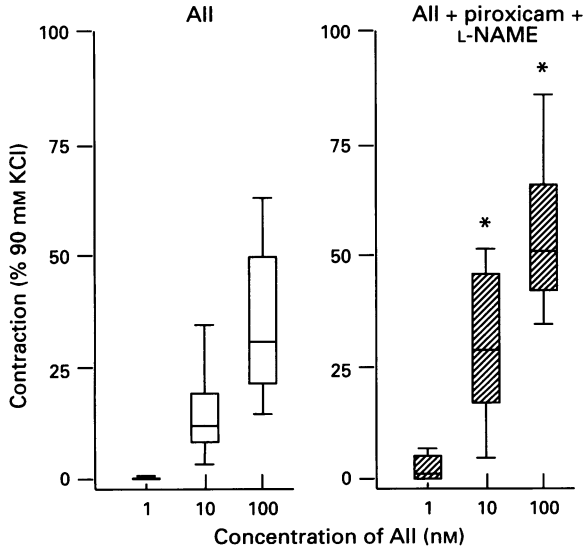

Figure 1 Effect of treatment with both piroxicam and L-NAME on contractions of rings of human saphenous vein stimulated by AII. The combined treatment augmented the response by nearly 70% at 100 nM, whereas treatment with either piroxicam or L-NAME alone was ineffective (data in text). The results are shown as a box and whisker plot, with median, interquartile range, 5th and 95th percentiles. *$P<0.05$ treated rings (hatched box) vs untreated control rings (open box), $n=12$.

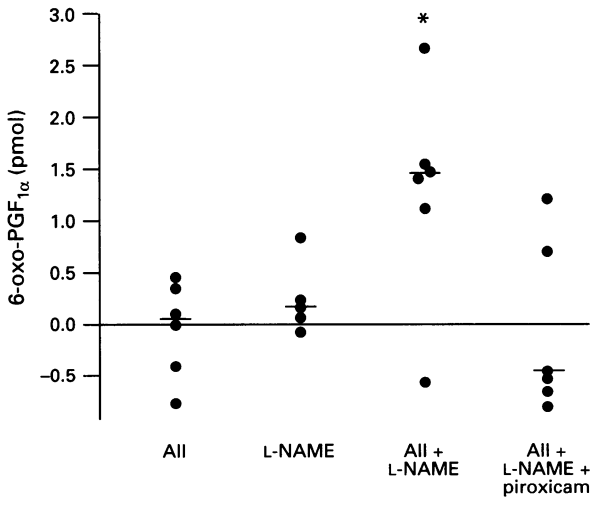

Figure 2 Release of PGI_2 (measured as 6-oxo-$PGF_{1\alpha}$) from saphenous vein. The individual values shown have been corrected for basal release of PGI_2 for each ring; the median for each group is shown by a bar. Neither stimulation by AII nor inhibition of NO synthesis (by L-NAME), singly, increased the release of PGI_2. However, stimulation by AII in the presence of L-NAME produced a three fold increase of PGI_2 output. The enhanced release due to L-NAME was inhibited by concomitant treatment with piroxicam. *$P<0.05$ AII stimulated with or without L-NAME.

Pretreatment with both piroxicam and L-NAME Pretreatment of the rings with both piroxicam and L-NAME abolished the release of cyclic GMP. Levels of cyclic GMP were reduced to 3.5 ± 1.0 fmol mg^{-1} (measured at 1 min) which was not different rom the basal release (at time 0) and significantly less than the enhanced amounts caused by pretreatment solely with piroxicam, as shown in Figure 3b. Under these conditions, i.e. pretreatment of rings with both L-NAME and piroxicam prior to AII stimulation, there was a significant enhancement in the myotropic responses to AII when compared with responses of untreated rings isolated from the same sample of saphenous vein (see Figure 1).

Effects of a NO donor and a PGI_2 analogue To examine the effects of exogenous NO on the increased release of PGI_2 seen when endogenous NO synthesis was inhibited with L-NAME, glyceryl trinitrate (GTN; a NO donor) was added to the incubation mixture for the rings of saphenous vein. The GTN attenuated the release of PGI_2, caused by AII in the presence of L-NAME. The median level of 6-oxo-$PGF_{1\alpha}$ measured was 0.6 pmol (CI 0.2,1.8) (corrected for basal release from the same rings) and was not different from the basal release of PGI_2 from these tissues or from release due to AII stimulation alone, shown in Figure 4a).

A similar set of experiments was designed to assess the effects of exogenous PGI_2 on increased release of NO seen in the absence of endogenous PGs. Here a stable analogue of PGI_2, iloprost, was added to the incubation mixture along with piroxicam. This addition caused a significant attenuation in cyclic GMP measured in the organ bath buffer, compared with the

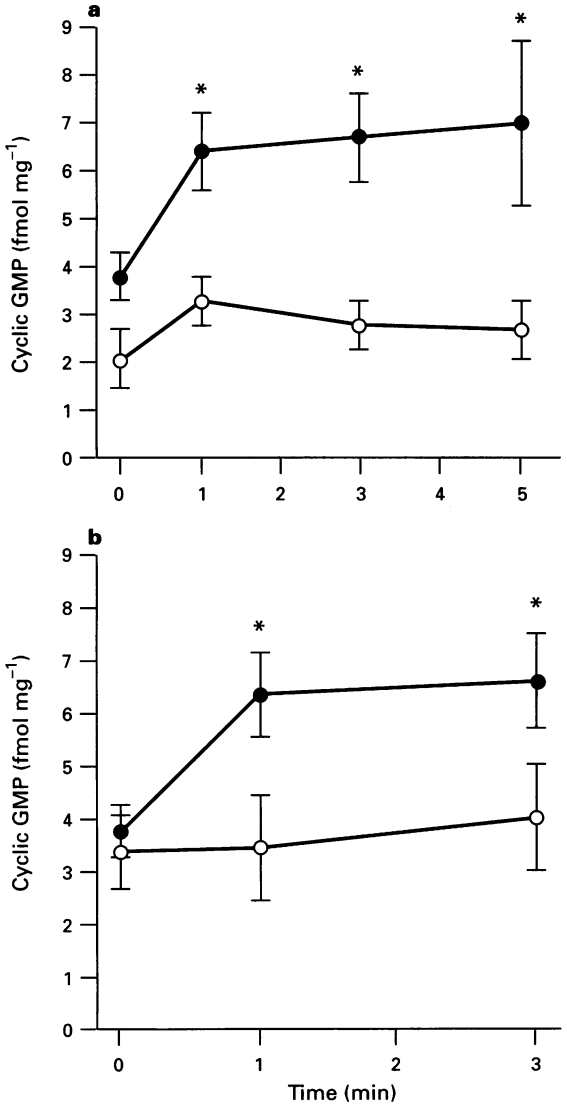

Figure 3 (a) No release, measured as cyclic GMP output from saphenous vein following stimulation with AII (100 nM). With AII alone, no increase in cyclic GMP was observed but after piroxicam, there was a rapid and sustained increase in cyclic GMP output from the vascular ring. AII alone (○; $n=5$) and AII after piroxicam treatment (●; $n=8$). *$P<0.05$, untreated vs piroxicam treated. (b) Effect of treatment with both L-NAME and piroxicam on release of cyclic GMP from saphenous vein stimulated by AII (100 nM). *$P<0.05$, piroxicam treated (●; $n=8$) vs piroxicam and L-NAME treated (○; $n=4$).

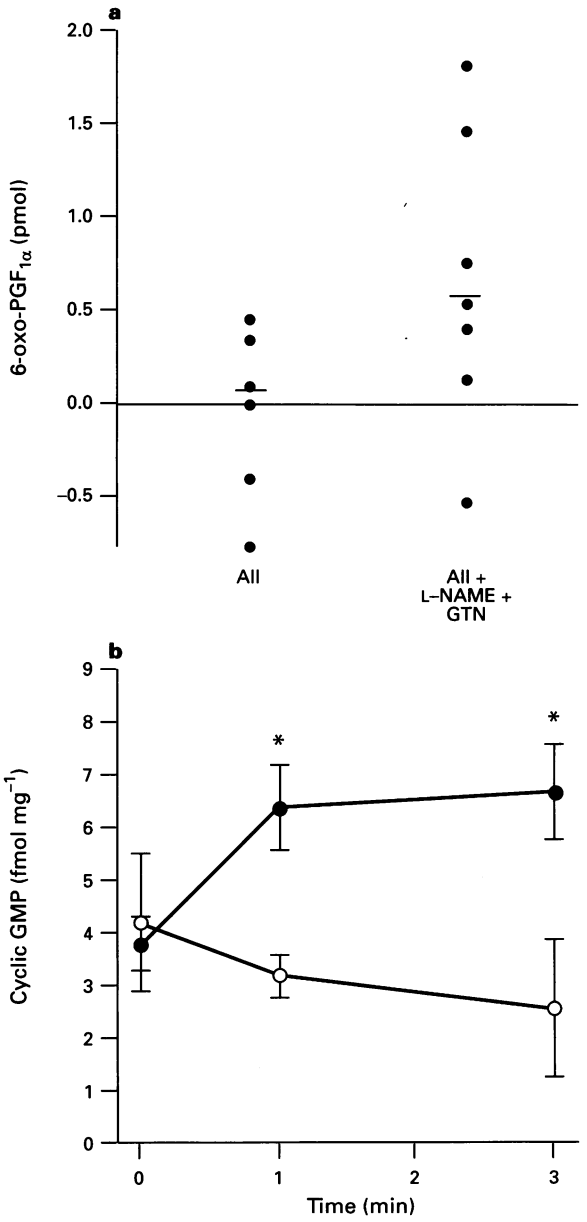

Figure 4 (a) Inhibition by GTN (1 μM) of 6-oxo-$PGF_{1\alpha}$ release from saphenous vein rings treated with L-NAME (200 μM) and stimulated by AII (100 nM). The increased release consequent on L-NAME treatment was reversed by the addition of GTN, an exogenous NO donor, and the output of 6-oxo-$PGF_{1\alpha}$ was not different from that of AII alone. (b) Effects of iloprost (2.5 ng ml^{-1}) on release of cyclic GMP stimulated by AII (100 nM) from piroxicam (5 μM) treated rings of saphenous vein. The increased release of cyclic GMP following piroxicam treatment was totally suppressed by the addition of iloprost. *$P<0.05$, piroxicam treated (●; $n=8$) vs piroxicam and iloprost treated (○; $n=3$).

enhanced release due to piroxicam at the same time points, levels being reduced to 2.6 ± 1.3 fmol mg^{-1} at 3 min. These results are shown in Figure 4b.

Discussion

In this study we have examined the role of NO and PGI_2, derived from endothelium, in modulating the myotropic responses of rings of the human saphenous vein to AII. The involvement of endogenous NO or PGI_2 was assessed by the use of inhibitors of NOS or COX and the myotropic responses (contractions of vascular rings) were correlated with biochemical responses (levels of PGI_2 and cyclic GMP). Two general comments must be made about our experimental conditions.

Firstly we used human tissues to approach as closely as possible the conditions in the coronary bypass graft *in vivo*. The samples of saphenous vein were therefore more variable (than from inbred animals) in their responses to AII or to KCl. Furthermore, the human samples were of necessity from patients with vascular disease, not from healthy subjects. We have attempted to minimize the effects of these sources of variation by selecting only those samples with a good response to KCl, a good relaxation to BK after PE (a sign of endothelial integrity) and by expressing the myotropic responses to AII relative to each ring's contractile response to KCl.

Secondly we correlated the contractile responses to AII with biochemical events, release of NO or PGI_2. Release of PGI_2 was measured directly by RIA for 6-oxo-$PGF_{1\alpha}$ but output of NO was measured indirectly by its action in increasing cyclic GMP. The level of cyclic GMP nevertheless is a highly relevant factor in the present work as the relaxant effects of NO on smooth muscle are mediated by increases in cyclic GMP and in vascular preparations such as rat isolated aorta, extracellular levels of cyclic GMP correlate well with intracellular levels (Rapoport & Murad, 1983; Schini *et al.*, 1989). A single concentration of NOS or COX inhibitor was used, giving a marked decrease of the corresponding variable (cyclic GMP or PGI_2). Although both biochemical variables could have been assayed under different and perhaps more ideal conditions, the conditions for measuring the biochemical changes were deliberately limited to those used for the myotropic assays to strengthen the causal link between contractile and biochemical responses.

That piroxicam or L-NAME used separately had no effect on the contractions induced by AII has two possible explanations; either the concentration of inhibitors used was inadequate, or AII failed to stimulate either NOS or COX activity in the endothelium of this tissue. The first explanation seems unlikely; the biochemical assays showed that the enzyme inhibitors used were effective in inhibiting NOS and COX activity. In view of the observation that the simultaneous inhibition of both NO and PGI_2 synthesis (treatment with L-NAME and piroxicam together) augmented the contractions to AII, the second explanation also seems unlikely. Indeed, this observation implies, somewhat paradoxically, that both NO and PGI_2 are involved in modulating the response to AII. While the involvement of NO and PGI_2 in endothelium-dependent responses to AII has been described in a number of vascular tissues, usually only one mediator appears to be the controlling factor. The identity of the relevant modulator is often determined by tissue type and species. For example in the canine renal arteries, basilar arteries and mesenteric arteries and veins, AII stimulates PGI_2 release, and in the rat and bovine aorta it is NO that is released by AII (Toda & Miyazaki, 1981; Greutter *et al.*, 1987).

The increase in PGI_2 production from saphenous vein in response to AII, found in these experiments, is in keeping with several reports where endothelium-dependent relaxation or inhibition of contraction in response to AII has been attributed to the stimulated release of PGI_2 by AII (Toda & Miyazaki, 1981). However, in the experiments described here AII increased release of PGI_2 in vascular rings only when NO synthesis had been inhibited. These observations generate two comments. Firstly, PGI_2 release in response to AII may not, initially, be responsible for the observed, endothelium-dependent attenuation of contraction. Secondly, the observations suggest that NO derived from endothelium can inhibit the release of PGI_2 from the saphenous vein. This suggestion is supported by our previous report that PGI_2 release, stimulated by AII, was increased in samples of saphenous vein without endothelium (Barker *et al.*, 1994a).

We also observed increased output of cyclic GMP but only in the presence of piroxicam, i.e. when release of endogenous prostaglandins was inhibited. Since the amounts of cyclic GMP in the extracellular solution are usually less than those reported intracellularly (Schini *et al.*, 1989) it may require a large increase in intracellular cyclic GMP before changes are seen extracellularly. Only after the addition of piroxicam was the increase in intracellular cyclic GMP sufficient to cause a rise in extracellular cyclic GMP. Nevertheless, that piroxicam did increase extracellular cyclic GMP discloses an inhibitory effect of PGs on the levels of cyclic GMP. The simplest inference from the effects of piroxicam is that release of NO (and cyclic GMP) was attenuated by a PG, possibly PGI_2.

From the results of the experiments with NOS or COX inhibitors therefore, there appears to be for each agonist (NO or PGI_2), endogenous inhibition of each other's synthesis. This postulate was supported by the effects of exogenous agonists. Since both PGI_2 and NO are chemically labile compounds, we used more stable alternatives, iloprost, a stable analogue of PGI_2 and GTN, which acts as an NO donor compound. Either of these two exogenous agonists was able to reverse the increased output of cyclic GMP or PGI_2 due to inhibition of the synthesis of endogenous agonist, as predicted. It therefore seems most likely that, in our system, the human saphenous vein stimulated by AII, the myotropic responses are indeed modulated by both and either NO and PGI_2. Further confirmation of the role of NO could be derived from the use of D-NAME and L-arginine in suitable experiments.

The finding that NO inhibits COX activity is both in keeping and in contrast to other recent reports. In bovine aortic endothelial cells, NO acted to inhibit the release of PGI_2 following BK stimulation and in porcine splenic artery L-NAME enhanced the release of contractile PGs (Doni *et al.*, 1988; Lot *et al.*, 1993). In contrast, in macrophages or smooth muscle cells, NO enhanced COX activity (Salvemini *et al.*, 1993; Inoue *et al.*, 1993) and another NO donor, 3-morpholinosydnonimine (SIN-1), potentiated the oedema induced by arachidonic acid (Sautebin *et al.*, 1995). The situation is further confused by the existence of COX-1 and COX-2 isoforms and the possibility that induction of COX-2 may be affected by NO as well as the activity of COX-2 itself (Salvemini *et al.*, 1993; Swierkosz *et al.*, 1995). However in none of these reports of NO-COX interactions has there been experimental indications of a PG-NOS interaction. For instance, indomethacin did not change the output of, nor the responses to, NO in different experimental systems (Salvemini *et al.*, 1993; Inoue *et al.*, 1993). Furthermore, in our own system the relaxation of saphenous vein induced by BK and the accompanying increase in cyclic GMP, were both unaffected by piroxicam (unpublished data), suggesting that during stimulation with BK there was no cross-talk between endogenous PGs and NO. Nevertheless, there are reports of inhibition of the NO system by PGs. Marotta *et al.* (1992) found that PGE_2 and iloprost inhibited the induction of NOS in macrophages activated by LPS, but the PGs had no effect on NOS acivity once it had been induced. Our experiments were generally short-term and, although we did not specifically look for evidence of induction of either COX-2 or iNOS, such induction would probably not contribute significantly to the output over the 5–15 min exposure times we used. By contrast, in vascular smooth muscle cells, iloprost increased the output of NO stimulated by LPS (Gross *et al.*, 1994). Further examples of the heterogeneity of results from NOS-COX interactions have been given in a short

review (Battistini *et al.*, 1994). Thus, there is no clear common pattern of interaction between NO and COX activity. The most generally applicable rule may be that the interaction between NOS and COX will depend on the exact experimental conditions such as cell type, species and stimulus. However, our findings may be the first to demonstrate an acute and thus direct effect of endogenous and exogenous PGs on NOS activity and the first to suggest a reciprocal relationship between the two vasorelaxant systems.

Overall this study has demonstrated the existence in human vascular tissue of a reciprocally inhibitory interaction between the two endogenous vasorelaxant mediators. NO and PGI_2; in the absence of either NO or PGI_2, levels of the other relaxant mediator can be raised sufficiently to maintain the attenuation of the contractile effects of AII. Although this interaction may be restricted to this particular set of tissue, species and stimulus, it would have practical relevance in ensuring that, in the coronary artery bypass graft, levels of vasorelaxation (and anti-aggregatory activity) are maintained in the event of dysfunction of one enzyme pathway. This would provide an important mechanism for the maintenance of patency and low thrombogenicity of the graft and thus for the long term success of the coronary bypass procedure.

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