Determinants of the Response of Human Blood Vessels to Nitric Oxide Donors In Vivo

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ABSTRACT

The potency of the nitric oxide (NO) donors glyceryltrinitrate (GTN) and 3-morpholinosydnonimine was compared in human dorsal hand veins, the radial artery, and the forearm resistance vessels. NO donors were more potent in veins and the radial artery (vessels with minimal basal NO-mediated dilatation) than in the resistance vascular bed (where basal NO is a major determinant of vascular tone). In contrast, 8-bromoguanosine 3',5'-cyclic monophosphate (a cGMP mimetic) was approximately equipotent in resistance arteries and veins and was less potent in the radial artery. Inhibition of phosphodiesterase V with dipyridamole did not alter the arteriovenous profile of GTN. Increasing the local concentration of NO in veins (by infusing sodium nitroprusside) reduced their sensitivity to GTN but not to 8-bromoguanosine 3',5'-cyclic monophosphate. Conversely, reducing endogenous NO production in the resistance vasculature led to time-dependent increases in the response to GTN. These data suggest that soluble guanylate cyclase rather than cGMP-dependent protein kinase or phosphodiesterase V is the site in the second messenger pathway that determines the arteriovenous profile of NO donors. Moreover, the sensitivity of soluble guanylate cyclase to NO donors might be regulated by the ambient concentration of NO, with increased local NO down-regulating the dilator response to NO donors.

Nitric oxide (NO) is an endogenous dilator synthesized by the vascular endothelium. Under physiological conditions in vivo, it provides background dilatation of the arterial resistance bed and is an important determinant of blood pressure in humans (Vallance et al., 1989a). In addition, NO has effects on smooth muscle cell growth, platelet activation, and leukocyte adhesion that might be antiatherogenic (Cooke and Dzau, 1997). In human cardiovascular disease, reduced NO-mediated dilatation has been demonstrated in resistance and conduit arteries (Calver et al., 1993; Mullen et al., 1997) that might have a pathogenic role in the initiation and progression of atherosclerosis. Therefore, there might be advantages in using exogenous NO to reverse vasoconstriction and possibly retard atherogenesis in the arterial vascular bed. Currently available NO donors are more potent dilators of veins than the resistance vasculature when used clinically, and this vascular profile contributes to their efficacy in the treatment of angina and heart failure (Collier et al., 1978). However, it is possible that venulectivity might limit the effectiveness of NO donors in the treatment of arterial NO deficiency (MacAllister et al., 1995b). Therefore, understanding the vascular profile of NO donors might suggest ways in which NO-replacement therapy could be targeted to the arterial vascular bed.

It has been suggested that the increased sensitivity of veins to NO donors compared with resistance arteries is related to the degree of basal endogenous NO-mediated dilatation in these vessels (Vallance et al., 1989b). Veins exhibit minimal basal NO in contrast to resistance vessels (Vallance et al., 1989a), and this might lead to increased sensitivity of venous vascular smooth muscle to NO donors (Moncada et al., 1991) secondary to up-regulation of the transduction pathway for NO in the vascular smooth muscle. NO stimulates soluble guanylate cyclase (sGC) in vascular smooth muscle (Hobbs and Ignarroz, 1996) to synthesize cGMP. In the presence of endogenously synthesized NO, there is evidence that sGC is down-regulated (Papapetropoulos et al., 1996b; Fillipov et al., 1997), resulting in reduced responsiveness to NO donors. In addition, there might also be down-regulation of the pathway distal to sGC. cGMP relaxes smooth muscle (at least in part) by activating a specific cGMP-dependent protein kinase (G kinase) (Vaandrerger and de Jonge, 1996), and G kinase expression is reduced by exposure to NO (Soff et al., 1997). The action of cGMP is terminated by phospho-

ABBREVIATIONS: PDE V, phosphodiesterase type V; G kinase, cGMP-dependent protein kinase; SNP, sodium nitroprusside; AUC, area under the dilatation/time curve; GTN, glyceryltrinitrate; SIN-1, 3-morpholinosydnonimine; sGC, soluble guanylate cyclase; 8-Br-GMP, 8-bromoguanosine 3',5'-cyclic monophosphate; NE, norepinephrine; L-NMMA, Nω-monomethyl-L-arginine.
diesterase type V (PDE V) (Beavo 1995), and increased activity of PDE V has been reported in blood vessels with high NO concentrations (Holzmann et al., 1996). It is therefore possible that differential sGC, G kinase, or PDE V activity in arteries and veins is related to the basal NO concentration in these vessels and could determine the arteriovenous profile of NO donors.

The aim of this study was to assess the relative potency of NO donors in resistance arteries, conduit arteries, and veins and determine the contribution of sGC, G kinase, and PDE V to the dilator profile of NO donors. The responsiveness of sGC was determined by comparing the potency of NO donors in dorsal hand veins, the forearm resistance vasculature, and the radial artery (a conduit artery that also has minimal basal NO-mediated dilatation in vivo (Jonnides et al., 1995; Lieberman et al., 1996). The responsiveness of G kinase was assessed by comparing the potency of 8-bromoguanosine 3',5'-cyclic monophosphate (8-Br-GMP; a cell permeable analog of cGMP) in these vessels. The activity of PDE V was examined by comparing the arteriovenous profile of NO donors before and after inhibition of PDE V with dipyridamole. To determine whether there was functional evidence for up- or down-regulation of the sGC-G kinase pathway in vivo, the potency of GTN was determined in the presence and absence of basal NO.

**Experimental Procedures**

Sixty-six studies were performed on 30 healthy volunteers (age, 25 ± 1 years) in a temperature-controlled laboratory (24–28°C). All studies had been approved by the local committee on the ethics of research in humans.

**Methodology of Venous and Arterial Studies**

The dilator effect of infused drugs on vein and conduit arteries was assessed by direct measurement of vessel diameter. The dilator effect on the resistance vasculature of the forearm was determined by measuring changes in blood flow at constant perfusion pressure. Doses of drugs were chosen to give approximately equivalent dilatation in each vessel type and were based on the results of pilot studies (data not shown).

**Venous Studies.** The dorsal hand vein technique was used to investigate venous reactivity in response to locally infused drugs (Vallance et al., 1989b). Single dorsal hand veins were cannulated using a butterfly needle and vehicle (0.9% saline (w/v)) or drugs dissolved in vehicle were infused at 0.25 ml/min. Veins were dissected by inflating an upper arm cuff to 40 mm Hg, and the internal diameter 5 to 10 mm downstream from the tip of the cannula was continuously recorded by measuring the linear displacement of a probe resting on the vein summit when the cuff was deflated. Under these experimental conditions, dorsal hand veins have no active tone; to observe a dilator response, veins were precontracted using norepinephrine (NE) (10–640 pmol/min; each dose for 5 min) was compared with that for 8-Br-GMP (50, 100, 200, and 400 nmol/min; each dose for 6 min; n = 6). In five subjects, the response of veins to 3-morpholinosydnonimine (SIN-1) (50, 100, and 200 pmol/min; each dose for 5 min) was compared (after a 15-min washout period) with that for 8-Br-GMP (500, 1000, and 2000 nmol/min; each dose for 6 min). In four subjects, the dilator response of the radial artery to GTN (25, 50, and 100 pmol/min; each dose for 5 min) was compared with that for 8-Br-GMP (500, 1000, and 2000 nmol/min; each dose for 6 min). In further experiments, the response of veins to 3-morpholinosydnonimine (SIN-1) (50, 100, and 200 pmol/min; each dose for 5 min; n = 6) was compared with the dilator effect of SIN-1 in the forearm (50, 100, and 200 nmol/min; each dose for 5 min; n = 4).

**Protocol 1: Comparison of Arteriovenous Profile of NO Donors with 8-Br-GMP**

The venodilator effect of GTN (0.5, 1, 2, and 4 pmol/min; each dose for 5 min; n = 6) was compared (after a 15-min washout period) with that of 8-Br-GMP (50, 100, 200, and 400 nmol/min; each dose for 6 min; n = 6). In five subjects, the response of the resistance vasculature to GTN (0.5, 1, and 2 nmol/min; each dose for 5 min) was compared (after a 15-min washout period) with that for 8-Br-GMP (500, 1000, and 2000 nmol/min; each dose for 6 min). In four subjects, the dilator response of the radial artery to GTN (25, 50, and 100 pmol/min; each dose for 5 min) was compared with that for 8-Br-GMP (500, 1000, and 2000 nmol/min; each dose for 6 min). In further experiments, the response of veins to SIN-1 in the forearm (50, 100, and 200 nmol/min; each dose for 5 min; n = 4).

**Protocol 2: Effects of Dipyridamole on Dilator Profile of GTN**

The venodilator response to GTN (0.25, 0.5, and 1 pmol/min; each dose for 5 min; n = 6) was determined; after a washout period of 15 min, dipyridamole (1 nmol/min) was infused for an additional 15 min and then coinfused with GTN as above. In the forearm resistance vasculature, the dilator response to GTN (0.25, 0.5, and 1 nmol/min; n = 6) was determined; after a washout period of 15 min, dipyridamole alone was infused (25 nmol/min) for an additional 15 min and then coinfused with GTN. The doses of dipyridamole were chosen to give an approximate plasma concentration of 1 to 2 μM (assuming blood flow in hand veins of 0.5–1 ml/min and in the forearm of 15–40 ml/min; Collier et al., 1978).

**Protocol 3: Effects of Sodium Nitroprusside (SNP) on Sensitivity of Veins to Dilators**

The venodilator response to GTN (0.5, 1, and 2 pmol/min; each dose for 5 min; n = 6) or bradykinin (1, 2 and 4 pmol/min; n = 7) was determined in the presence of NE alone and subsequently in the presence of NE and SNP (5 pmol/min). In these studies, after the first dose-response curve to the dilator was constructed, NE alone was infused until vessels were precontracted to baseline diameter. SNP was coinfused until a stable venodilatation had occurred (10 min) and continued for the duration of the experiment. The vein was precontracted by increasing the dose of NE, and the dose-response to GTN (0.5–2 pmol/min) or bradykinin (1–4 pmol/min) was repeated. The prolonged duration of action of 8-Br-GMP (dilatation persisting
for more than 60 min after cessation of infusion; data not shown) precluded repetition of the dose-response curves in the same experiment. Therefore, the dilator responses to 8-Br-GMP (100, 200, and 400 nmol/min; \( n = 5 \)) were assessed in veins precontracted with NE in the presence of SNP (5 pmol/min) and compared with the response observed when 8-Br-GMP alone was used.

Protocol 4: Effects of Inhibition of Basal NO-Mediated Dilatation on Sensitivity of Resistance Arteries to GTN

In the forearm resistance bed, the effect of GTN (0.25, 0.5, and 1 nmol/min) was determined alone and after local inhibition of NO synthase for 15 and 60 min by coinfusion of the NO synthase inhibitor \( \text{N}^\cdot \text{G} \)-monomethyl-L-arginine (\( \text{l-NMMA} \); 4 \( \mu \)mol/min; \( n = 7 \)). In this vascular bed, \( \text{l-NMMA} \) increases basal vascular tone and reduces blood flow, effects that might nonspecifically alter the response to infused dilators (O’Kane et al., 1994). To allow for nonspecific effects, in separate experiments the dilator response to GTN (0.25, 0.5, and 1 nmol/min) was determined alone and after 15 and 60 min of a local infusion of NE (240 pmol/min; \( n = 4 \)) to cause basal constriction equivalent to that caused by \( \text{l-NMMA} \).

Materials

Ascorbic acid was from Evan’s Medical Ltd. (Horsham, UK). Bradykinin was from Clinalpha (Laufelfingen, Switzerland). 8-Br-GMP was from Sigma Chemical Co. Ltd. (Dorset, UK). Dipyridamole was from Boehringer Ingelheim Ltd. (Berkshire, UK). GTN was from Du Pont Pharmaceuticals (Hertfordshire, UK). Lignocaine was from Antigen Pharmaceuticals Ltd. (Roscrea, Ireland). SIN-1 was from Alexix Corporation Ltd. (Nottingham, UK). \( \text{l-NMMA} \) was from Clinalpha. NE was from Winthrop Laboratories (Guildford, UK). We obtained 0.9% saline from Baxter Healthcare Ltd. (Norfolk, UK). SNP was from David Bull Laboratories (Warwick, UK). Drugs were dissolved in sterile saline and filtered (Acrodisc; Gelman Sciences, Ann Arbor, MI) before use.

Calculations and Statistics

Hand vein size was measured in millimeters, and the dilatation of precontracted veins was expressed as a percentage reversal of the precontraction (MacAllister et al., 1995b). Radial artery diameter was measured in millimeters, and the dilatation was expressed as a percentage of the dilatation caused by sublingual GTN. Forearm blood flow, expressed as ml/100 ml forearm/min, was calculated according to the method of Whitney (1953). The ratio of the blood flow in the infused arm to that in the control arm was calculated for each measurement period. The effect of drugs on blood flow was expressed as a percentage change in this ratio from the preceding baseline period.

The dilator potency of the infused drugs was determined by calculating the doses that caused a 25% dilatation (ED\(_{25}\)) in veins and conduit arteries or a 25% increase in blood in resistance vessels. The conduit- and resistance arteriovenous profiles of GTN, 8-Br-GMP, and SIN-1 were derived from the ratio of the ED\(_{25}\) values in conduit or resistance arteries to the mean ED\(_{25}\) values in veins. The effect of coinfused drugs on the dilatation caused by GTN or 8-Br-cGMP was analyzed by comparing the area under the dilatation/time curves (AUCs) (MacAllister et al., 1995a). Results are expressed as mean ± S.E.M. and compared by paired and unpaired \( t \) test or ANOVA as appropriate where \( P < .05 \) is considered significant.

Results

Arteriovenous Profile of Dilators. The relative resistance-arteriovenous potencies of GTN and SIN-1 in vivo were similar but differed significantly from the profile of 8-Br-GMP (Fig. 1). The ED\(_{25}\) values for GTN and SIN-1 were 0.9 ± 0.2 and 75 ± 10 pmol/min, respectively, in veins (\( n = 6 \)) and 0.6 ± 0.2 (\( n = 5 \)) and 61 ± 7 nmol/min (\( n = 4 \)), respectively.

![Arteriovenous profiles of dilators. Dilator responses of dorsal hand veins (\( \triangle \); \( n = 6 \); left axis), the radial artery (\( \bullet \); \( n = 4 \); left axis), and the forearm resistance bed (\( \square \); \( n = 4–5 \); right axis) to local infusions of GTN (A), 8-Br-GMP (B), and SIN-1 (C). Allowing for the approximately 50-fold differences in blood flow between dorsal hand veins and the forearm arterial bed, these results suggest that NO donors are approximately equipotent in dorsal hand veins and the radial artery and 10-fold less potent in the resistance vasculature. 8-Br-GMP was approximately equipotent in veins and the resistance vasculature but was a less potent dilator of the radial artery.](image-url)
in the forearm resistance bed. The mean ratios of the ED<sub>25</sub> values in resistance arteries and veins were 705 ± 200 for GTN (n = 5) and 816 ± 87 for SIN-1 (n = 4; P > .5 by ANOVA). 8-Br-GMP was less potent in veins and the resistance vasculature (ED<sub>25</sub> = 138 ± 53, n = 6, and 758 ± 159 nmol/min, n = 5, in veins and resistance vessels, respectively) and had an ED<sub>25</sub> ratio of 5.4 ± 1.1 (n = 5; P < .01 compared with GTN and SIN-1 by ANOVA). GTN was a more potent dilator of the radial artery than the forearm resistance vasculature (Fig. 1A; ED<sub>25</sub> in the radial artery was 32 ± 4.4, with a conduit-arteriovenous ratio of 36.2 ± 6.3, n = 4, P < .01, compared with the resistance-arteriovenous ratio). In contrast, 8-Br-GMP was less potent in the radial artery compared with the resistance vasculature (Fig. 1B) and at the doses infused did not consistently cause a 25% dilatation of this vessel.

**Effect of Dipyridamole on Dilator Effect of GTN.** Dipyridamole alone had no significant effect on precontracted vein size (45 ± 7% and 41 ± 10% of control size in the absence and presence of dipyridamole, respectively; n = 6; P > .05) or basal forearm blood flow (8.7 ± 7.8% dilatation in the presence of dipyridamole alone; n = 6; P > .05). However, the dilator effect of GTN (0.25, 0.5, and 1 nmol/min) in veins was increased by coinfusion with dipyridamole (1 nmol/min; Fig. 2A). The AUC values were 420 ± 129 for GTN alone and 828 ± 96 in the presence of dipyridamole (P < .03; n = 6). In the forearm, the dilator effect of GTN (0.25, 0.5, and 1 nmol/min) was also increased by coinfusion with dipyridamole (AUC = 338 ± 128 for GTN alone and 607 ± 90 in the presence of dipyridamole 25 nmol/min; P < .05; n = 6; Fig. 2B). The mean ratio of the AUC values for GTN in the presence of dipyridamole compared with GTN alone was 2.0 ± 0.2 (n = 6) in veins and 1.8 ± 0.3 (n = 6) in the resistance vasculature (P > .5).

**Effects of Coinfusion of SNP on Sensitivity of Veins to Dilators.** The effects of coinfusion of SNP on the response to dilators are shown in Fig. 3. SNP reduced the dilator response to GTN (AUC = 746 ± 212 before and 305 ± 191 in the presence of SNP; P < .05; n = 6; Fig. 3A) and bradykinin (AUC = 329 ± 56 before and 90.5 ± 83 in the presence of SNP; P < .05; n = 7; Fig. 3B). The response to 8-Br-GMP alone (data taken from Fig. 1B) was unchanged in the presence of SNP [AUC values in the absence and presence of SNP = 787 ± 150 (n = 6) and 624 ± 164 (n = 5), respectively; P > .2; Fig. 3C].

**Effects of Inhibition of Basal NO-Mediated Dilatation to GTN.** In the forearm, the dilator effects of GTN (0.25, 0.5, and 1 nmol/min) were reduced in the presence of L-NMMA (4 μmol/min; preinfused for 10 min; AUC = 569 ± 177 before and 172 ± 93 in the presence of L-NMMA; n = 7; P < .05 by ANOVA). However, when L-NMMA was preinfused for 60 min, the response to GTN had returned to normal (AUC = 633 ± 207; P > .2 compared with control; Fig. 4A). To account for the effect of increased arteriolar constriction caused by L-NMMA on the response to dilators, the response to GTN was also assessed in the presence of an equieffective constritor dose of NE (240 pmol/min; 35 ± 14.6% reduction in flow; n = 4). The arteriolar dilator effects of GTN (0.25, 0.5, and 1 nmol/min) were reduced in the presence of NE preinfused for 10 and 60 min (AUC = 620 ± 200 before NE and 236 ± 135

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**Fig. 2.** Effect of dipyridamole on the dilatation to GTN. GTN was infused alone and subsequently in the presence of dipyridamole in veins (1 nmol/min) (A) and in the forearm (25 nmol/min) (B). Dipyridamole increased the dilatation to GTN in dorsal hand veins and the forearm (*P < .05 for AUC values before and during dipyridamole); ▲, dipyridamole; Δ, control.

**Discussion**

The results of this study indicate that human blood vessels in vivo differ in their sensitivity to NO donors. Dorsal hand veins and the radial artery were more sensitive to NO donors than the forearm resistance vasculature. In contrast, 8-Br-GMP was less venoselective than NO donors and more potent in the resistance vasculature than in conduit arteries, suggesting that the activity of G kinase was not the major determinant of the dilator profile of NO donors. Furthermore, dipyridamole did not alter the arteriovenous profile of GTN, indicating that arteriovenous profile of exogenous NO is not determined by PDE V activity. These results are consistent with increased sensitivity of sGC to NO donors in veins and conduit arteries and might be a consequence of the lack of basal endogenous NO in these vessels. To determine whether basal NO-mediated dilatation altered the sensitivity of these vessels to NO donors, the potency of GTN was determined in veins and the resistance vasculature in the presence or absence, respectively, of basal NO. SNP (infused locally to mimic basal NO in dorsal hand veins) reduced the
sensitivity of these vessels to endogenous and exogenous NO, but not to 8-Br-GMP, which is consistent with SNP-mediated reduction in the sensitivity of sGC to GTN. In the arterial resistance bed, L-NMMA (to inhibit basal NO synthesis) resulted in time-dependent changes in the sensitivity to GTN that were consistent with increased sensitivity of sGC to NO. Taken together, these findings suggest that NO down-regulates sGC in human vessels in vivo and might contribute to the arteriovenous profile of NO donors.

We have shown previously that NO donors are more potent dilators of human veins than resistance arteries in vivo and that this profile of activity is independent of the mechanism of biotransformation of NO donors to NO (MacAllister et al., 1995b). In the present study, GTN was 500- to 1000-fold more potent in the venous than in the arterial resistance bed. Allowing for the approximately 50-fold difference in blood flow between the two vascular beds (Collier et al., 1978), these results suggest that dorsal hand veins are 10- to 20-fold more sensitive to GTN. Preferential metabolism of GTN to NO in veins and arteries is unlikely to be the sole explanation because SIN-1 (which is less dependent on metabolism for NO release than GTN) had a similar dilator profile to GTN. A further explanation for the differences between arteries and veins might be the increased rate of inactivation of NO in the arterial circulation (perhaps due to differences in oxyhemoglobin concentration; Wennmalm et al., 1992). However, this mechanism cannot explain why conduit arteries were more sensitive to GTN than the resistance vasculature. It is therefore more likely that the profile of the NO donors studied reflects the sensitivity of vascular smooth muscle to NO.

The difference in the sensitivity of these vessels to NO might be related to their ambient NO concentration. NO synthase inhibitors, such as L-NMMA, have been used to assess NO-mediated dilatation of different types of vessels in humans in vivo. L-NMMA constricts human forearm resistance vessels (resistance arteries and arterioles) after local infusion in vivo (Vallance et al., 1989a), and when given systemically, increases blood pressure (Stamler et al., 1994). In contrast, L-NMMA does not increase the tone of NE-constricted human dorsal hand veins (Vallance et al., 1989b). Similarly, L-NMMA does not constrict peripheral conduit arteries (Jonnides et al., 1995; Lieberman et al., 1996) in vivo. It appears from our results that vessels with basal NO-mediated dilatation (resistance arteries) are less sensitive to NO than those with minimal basal NO (peripheral conduit arteries or veins). One possible mechanism for this might be down-regulation of the transduction pathway for NO by endogenous NO. NO reduces the sensitivity of sGC to NO, reduces the expression of sGC (Moncada et al., 1991; Papapetropoulos et al., 1996a; Papapetropoulos et al., 1996b) and G kinase (Soff et al., 1997), and increases the activity of PDE V (Holzmann et al., 1996). Each of these effects of NO could desensitize vascular smooth muscle to NO donors.

To determine whether there was differential activity of G kinase in these vessels, 8-Br-GMP (a cell wall-permeable analog of cGMP) was used to stimulate G kinase in veins and arteries. The arteriovenous profile of 8-Br-GMP differed from that of the NO donors; resistance vessel dilatation occurred at doses that were 10-fold greater than those that caused venodilatation, consistent with equipotency of cGMP in these vessels. Moreover, 8-Br-GMP was a less potent dilator of conduit arteries than resistance vessels. Assuming that 8-Br-GMP is equally permeable in these vessels, these observations suggest that the increased sensitivity of veins and con-
Fig. 4. Effect of NO synthase inhibition on the arterial response to GTN. Effect of infusion of L-NMMA (A; n = 7) or NE (B; n = 4) on the response of the forearm resistance bed to GTN. The dilator responses are shown to GTN alone and during coinfusion with L-NMMA or NE (preinfused for 10 and 60 min). A, \( *P < .05 \) for the AUC values at 10 min compared with control and after 60 min of infusion of L-NMMA; \( \triangle \), control; \( \bullet \), L-NMMA 10 min; \( \Delta \), L-NMMA 60 min. B, \( *P < .05 \) for the AUC values at 60 min compared with control; \( \triangle \), control; \( \Delta \), NE 10 min; \( \bullet \), NE 60 min.

duart arteries to NO donors is not accounted for by increased sensitivity of venous or conduit artery G kinase to cGMP.

To investigate whether PDE activity determined the smooth muscle response to NO donors, dipyridamole was used to inhibit PDE V in veins and the resistance vasculature. Dipyridamole was infused in veins and arteries at doses to achieve predicted concentrations in the micromolar range, which would be expected to inhibit PDE V (Ziegler et al., 1995). Dipyridamole alone had no significant dilator effect on dorsal hand veins or in the forearm. In contrast, dipyridamole augmented dilatation to GTN in both vascular beds, suggesting that PDE activity modulates cGMP-mediated dilatation when sGC is stimulated by NO donors. Augmentation of the dilatation to GTN in veins and the resistance bed was similar, and the arteriovenous profile of GTN was unchanged. These results suggest that PDE V activity is not a major determinant of the arteriovenous profile of GTN. However, there are limitations to the use of dipyridamole in these studies. Dipyridamole inhibits adenosine uptake, which might contribute to the dilatation observed. Moreover, it is impossible to be certain whether the doses of dipyridamole used in veins and arteries caused equivalent inhibition of PDE V. Additionally, it is unclear why dipyridamole alone did not dilate the resistance vasculature. It is possible that the dose infused was subthreshold to augment basal NO-mediated effects because similar observations have been made in the human pulmonary circulation, where low doses of dipyridamole that did not alter pulmonary vascular resistance increased the dilator response to inhaled NO (Fullerton et al., 1997). These limitations notwithstanding, the results of the experiments with 8-Br-GMP and dipyridamole are consistent with smooth muscle sensitivity to NO donors being determined at a site proximal to G kinase and PDE V.

To determine whether the sensitivity of sGC to NO was modulated by basal NO, the response of veins to GTN was compared in the absence or presence of SNP to provide background NO and sGC stimulation. Coinfusion of SNP reduced the dilator effect of GTN and bradykinin (an NO-dependent dilator in these vessels; Vallance et al., 1989b). SNP did not alter the response to 8-Br-GMP, excluding a direct effect of SNP on the sensitivity of G kinase. These observations are consistent with an effect of NO derived from SNP to reduce sGC-mediated effects of NO.

In the arterial resistance bed, the effects of NO synthase inhibition on the sensitivity of these vessels to GTN was determined. In the presence of L-NMMA infused for 10 min, the dilator effect of threshold doses of GTN was reduced. A similar reduction was seen when NE was infused at an equieffective constrictor dose, suggesting that inhibition of the effect of GTN by L-NMMA and NE was a consequence of vasoconstriction per se (a reduction in sensitivity of blood vessels to dilators due to increased constrictor tone; O’Kane et al., 1994). However, after 60 min of treatment with L-NMMA, the response to GTN was restored, yet there was no change in the response to GTN in the presence of NE at this time point. Restoration of the response to GTN after 60 min of L-NMMA is consistent with increased sensitivity of sGC to overcome the effects of functional antagonism.

These data suggest that basal NO acutely modulates the sensitivity of arteries and veins to NO donors but not to 8-Br-GMP and are consistent with an effect of NO to reduce the responsiveness of sGC. However, the molecular mechanisms responsible for desensitization of sGC to NO donors remain unclear. From the time course of the effects of SNP and L-NMMA, it is likely that there was a change in the sensitivity of sGC rather than an effect on protein synthesis. NO or cGMP might cause a conformational change in sGC to reduce its sensitivity to NO. Alternatively, basal NO might cause near-maximal activation of sGC, so reducing its responsiveness to further stimulation by NO donors. At present, it is not possible to differentiate between these possibilities.

In conclusion, the results of this investigation suggest that in the human vasculature, the sensitivity of arteries and veins to exogenous NO is reduced by an effect of endogenous NO to desensitize sGC. Physiologically, this mechanism might provide feedback control over the NO pathway to dampen the responses to NO. Our observations might also explain in part the arteriovenous profile of NO donors because vessels with significant basal NO-mediated dilatation exhibit reduced sensitivity to NO donors. Whether arteriovenous differences in the transformation of NO donors to NO or the expression of the components of the sGC/G kinase system also contributes to the arteriovenous profile of NO donors in human vessels remains to be determined. If our
results are representative of human vessels in general, then drugs such as the organic nitrates will target NO replacement to conduit arteries and vein at doses that will have little effect on the resistance vasculature. Whether down-regulation of sGC might contribute to NO tolerance remains to be determined.

References

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