Reciprocal regulation of cGMP-mediated vasorelaxation by soluble and particulate guanylate cyclases

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Hussain, Monira B., Raymond J. MacAllister, and Adrian J. Hobbs. Reciprocal regulation of cGMP-mediated vasorelaxation by soluble and particulate guanylate cyclases. Am J Physiol Heart Circ Physiol 280: H1151–H1159, 2001.—Nitric oxide (NO) and atrial natriuretic peptides (ANP) activate soluble (sGC) and particulate guanylate cyclase (pGC), respectively, and play important roles in the maintenance of cardiovascular homeostasis. However, little is known about potential interactions between these two cGMP-generating pathways. Here we demonstrate that sGC and pGC cooperatively regulate cGMP-mediated relaxation in human and murine vascular tissue. In human vessels, the potency of spermine-NONOate (SPER-NO) and ANP was increased after inhibition of endogenous NO synthesis and decreased by prior exposure to glyceryl trinitrate (GTN). Aortas from endothelial NO synthase (eNOS) knockout (KO) mice were more sensitive to ANP than tissues from wild-type (WT) animals. However, in aortas from WT mice, the potency of ANP was increased after pretreatment with NO synthase (sGC) inhibitor. Vessels from eNOS KO animals were less sensitive to ANP after GTN pretreatment, an effect that was reversed in the presence of an sGC inhibitor. cGMP production in response to SPER-NO and ANP was significantly greater in vessels from eNOS KO animals compared with WT animals. This cooperative interaction between NO and ANP may have important implications for human pathophysiology involving deficiency in either mediator and the clinical use of nitrovasodilators.

soluble guanylate cyclase; particulate guanylate cyclase; nitric oxide; atrial natriuretic peptide; guanosine cyclic 3′,5′-monophosphate

THE CARDIOVASCULAR ACTIONS of nitric oxide (NO) and atrial natriuretic peptide (ANP) are fundamental to the regulation of blood pressure (15, 16, 19, 29, 35). NO is released from vascular endothelial cells in response to chemical mediators (e.g., bradykinin) and shear stress, whereas ANP is a hormone released from the cardiac atria in response to hypervolemic states (i.e., atrial stretch). Both mediators possess multifaceted actions that coordinate to maintain cardiovascular homeostasis, including reducing vascular tone, inhibiting platelet aggregation, and promoting natriuresis and diuresis. These actions of NO and ANP are brought about via activation of specific receptor proteins, the guanylate cyclases. NO stimulates the cytoplasmic heterodimeric hemoprotein, soluble guanylate cyclase (sGC) (6); ANP activates the membrane-bound protein, particulate guanylate cyclase (pGC) (30, 33, 36), which possesses an ANP-binding motif at its extracellular NH2 terminus and guanylate cyclase activity at its COOH-terminal intracellular domain. Stimulation of either cyclase results in the conversion of GTP to the intracellular second messenger cGMP, which is responsible for mediating the majority of cardiovascular effects of these mediators. Because of the profound hypotensive actions of both NO and ANP, precise regulation of the sGC and pGC signaling pathways is a prerequisite for maintaining cardiovascular homeostasis.

Synergistic with the feedback regulation of many classical neurotransmitter and hormone systems, we (7) recently demonstrated that the NO-sGC-cGMP pathway is regulated by the ambient concentration of NO, possibly through cGMP. A decrease in the basal NO level, as occurs in mice deficient in endothelial NO synthase (eNOS), causes an enhanced sensitivity of the system to NO/NO donors. In contrast, after chronic exposure to an NO excess, the system downregulates, and a decrease in response to NO/NO-donors is observed. The ANP-pGC-cGMP system also incorporates a self-regulating mechanism such that after exposure to its endogenous ligand, ANP, the receptor becomes dephosphorylated and desensitizes to subsequent activation (22, 25, 26). Because both the sGC- and pGC-cGMP systems have complementary roles to play in cardiovascular homeostasis, an interaction between the two pathways to regulate cGMP levels might represent an important physiological control mechanism. In this way, an excess or deficiency in one mediator could be compensated by the other, or, conversely, the interaction may constitute a negative-feedback system that prevents overactivation of cGMP signaling in one cell type by NO and/or ANP. This thesis is consistent with the observation that prepro-ANP mRNA expres-
sion is upregulated in the atria of eNOS knockout (KO) mice (3); moreover, many cell types possess both sGC and pGC (4, 33).

It is not clear whether changes in the ambient NO/cGMP concentration can affect the sensitivity of the pGC system or vice versa. Previous reports have hinted that an interaction between the two pathways may exist, but the observed effects are inconsistent and appear to be species, and often tissue, specific. Thus NO donors have been reported to increase (9, 38), decrease (18), or have no effect (21, 23, 34) on the response of blood vessels to ANP; a lack of effect of NO donors on ANP responses is also observed in airway smooth muscle cells in culture (5). Similarly, ANP has been reported to both increase (37) and decrease (17, 32) the activity of the NO pathway in cultured cells.

Thus a definitive interaction between NO- and ANP-mediated responses remains to be demonstrated, yet such an interplay may be fundamental to the control of cardiovascular homeostasis. In the present study, we have investigated the interactions of NO and ANP involved in regulating vascular smooth muscle reactivity in human and mouse vessels in vitro. The dissection of the mechanisms involved was aided by the use of eNOS KO animals, which can be used as a model of chronic vascular NO deficiency.

METHODS

All studies described in this manuscript were conducted in accordance with the University College London guidelines on the use of human and animal tissue for research purposes. The use of human tissue was approved by the local Ethics Committee.

Human Studies

Internal mammary artery (IMA) was obtained from patients undergoing coronary artery bypass surgery and set up for pharmacological characterization following an essentially identical protocol to that described for murine tissue (7). Briefly, IMA rings (3–5 mm in width) were suspended in 25-ml organ baths for isometric tension measurements. Tissues were subjected to 1 g of resting tension and equilibrated for 120–180 min before experimentation. Tissues were primed with KCl (4.8 × 10\(^{-2}\) mol/l) before a supramaximal concentration of phenylephrine (PE; 10\(^{-4}\) mol/l) was added. After washout, a concentration of PE producing ~60% of the maximum contraction was administered. Once this response had stabilized, acetylcholine (ACh; 10\(^{-6}\) mol/l) was added to assess endothelial integrity. If the contractions to PE were not maintained or relaxations >50% of the PE-induced tone to ACh were not observed, the tissues were discarded.

To study the effects of acute NO/cGMP deficiency on responses to the NO donor N-(2-aminoethyl)-N-(2-hydroxy-2-nitrosohydrazino)-1,2-ethylenediamine (SPER-NO) and ANP, concentration-response curves to each agent were constructed in the presence of the NO synthase (NOS) inhibitor N\(^{\text{g}}\)-methyl-l-arginine (l-NMMA; 3 × 10\(^{-4}\) mol/l; 30-min incubation). The effect of NO/cGMP excess was investigated by preincubation of vessels with the NO donor glyceryl trinitrate (GTN; 3 × 10\(^{-5}\) mol/l; 30-min incubation followed by washout).

Marine Studies

To more fully characterize the interactions between the NO/sGC and ANP/pGC systems, murine aortas originating from wild-type (WT) and specific NOS KO animals were employed as models of chronic NO deficiency, as described previously (7).

To examine the effects of acute NO/cGMP deficiency on ANP responses, concentration-response curves to ANP were obtained in tissues from WT mice in the presence of the NOS inhibitor \(N^2\)-nitro-l-arginine methyl ester (l-NNAME; 3 × 10\(^{-4}\) mol/l; 30-min incubation) or the sGC inhibitor 1H-[1,2,4]-oxadiazolol-[4,3-a]quinoxalin-1-one (ODQ; 5 × 10\(^{-6}\) mol/l; 30-min incubation). To investigate the effect of chronic NO/cGMP deficiency on ANP responses, concentration-response curves were constructed for ANP in aortas from eNOS KO mice.

The effect of NO/cGMP excess was investigated by preincubation of vessels from eNOS KO animals with GTN (3 × 10\(^{-5}\) mol/l; 30-min incubation followed by washout). To examine whether cGMP-dependent or -independent actions of NO were responsible for the decreased response to ANP in the presence of GTN, identical experiments were conducted in the presence of ODQ (5 × 10\(^{-6}\) mol/l). The effect of cGMP excess on SPER-NO responses was also tested by incubating vessels from eNOS KO animals with ANP (10\(^{-7}\) mol/l; 30-min incubation followed by washout).

Possible differences in the starting PE-induced tone between control and treated tissues were eliminated by adjusting the PE concentration such that the starting tone was not significantly different after any intervention.

Measurement of Tissue cGMP

Aortas were dissected from WT and eNOS KO mice, divided into two equivalent rings, and mounted in organ baths as described above. Tissues were allowed to equilibrate for 60 min, after which baseline tension was adjusted to 0.3 g. A supramaximal concentration of PE (10\(^{-5}\) mol/l) was added, and tissues were washed repeatedly over a period of 45 min to regain baseline tension. After washout, a concentration of PE producing ~80% of the maximum contraction was administered. Once a stable level of contraction was maintained, SPER-NO (3 × 10\(^{-7}\) mol/l) or ANP (4 × 10\(^{-7}\) mol/l) was added to the bath. At the point of maximal relaxation, the tissues were snap-frozen in liquid nitrogen. Some tissues were allowed to maintain contraction and frozen without the addition of vasorelaxants to obtain baseline readings for cGMP in tissues from both WT and eNOS KO animals. The concentrations of SPER-NO (3 × 10\(^{-7}\) mol/l) and ANP (4 × 10\(^{-7}\) mol/l) used in these studies corresponded to an approximate EC\(_{50}\) concentration established in WT vessels.

Tissues were homogenized in 0.7 ml acidified ethanol (HCl; pH 3.4) and sonicated for 15 min before incubating at 4°C for 15–18 h. Samples were then centrifuged at 10,000 g for 15 min at 4°C. The supernatent was assayed for cGMP by a commercially available kit according to the manufacturer’s instructions (cGMP enzyme immunoassay system; Amersham Pharmacia Biotech).

Data Analysis

Relaxations are expressed as a percent reversal of the PE-induced tone (means ± SE). Curves were fitted to all data
by nonlinear regression. The $-\log [M]$ of a given drug giving a half-maximal response (pEC$_{50}$) values were used to compare the relaxant effects of the drugs. Data were analyzed using Student’s t-test; $P < 0.05$ was taken as statistically significant.

RESULTS

Human Studies

Interactions of sGC and pGC in human IMA. PE (10^{-4} mol/l) produced a maximal contraction of the human IMA (3.78 ± 0.14 g, $n = 14$). For experimentation, a concentration of PE that produced ~60% of this response was employed (2.42 ± 0.17 g, $n = 14$). Tissues precontracted in this manner relaxed to ACh (10^{-6} mol/l) by 84.95 ± 1.93% ($n = 14$).

Preincubation of tissues with the NOS inhibitor L-NMMA increased the potency of both SPER-NO (pEC$_{50}$ 6.91 ± 0.10 and 7.21 ± 0.09 in the absence and presence of L-NMMA, respectively, $n = 4$, $P < 0.05$; Fig. 1A) and ANP (pEC$_{50}$ 8.51 ± 0.07 and 8.80 ± 0.06 in the absence and presence of L-NMMA, respectively, $n = 4$, $P < 0.05$; Fig. 1B). In contrast, prior exposure of tissues to the NO donor GTN significantly decreased the potency of both SPER-NO (pEC$_{50}$ 7.00 ± 0.05 and 6.66 ± 0.06 before and after GTN, respectively, $n = 5$, $P < 0.05$; Fig. 2A) and ANP (pEC$_{50}$ 8.74 ± 0.05 and 8.40 ± 0.13 before and after GTN, respectively, $n = 4$, $P < 0.05$; Fig. 2B). GTN pretreatment also decreased the maximum response to ANP (99.61 ± 3.62 and 66.74 ± 5.68% before and after treatment, respectively, $n = 5$, $P < 0.05$). GTN pretreatment had no effect on the potency of forskolin (pEC$_{50}$ 7.03 ± 0.13 and 7.31 ± 0.15 before and after GTN, respectively, $n = 5$, $P > 0.05$).

Mouse Studies

Effect of NOS gene disruption on the contractile response to phenylephrine. PE produced concentration-dependent contractions of the mouse thoracic aorta with similar pEC$_{50}$ values (7.16 ± 0.05 and 7.20 ± 0.04, $P > 0.05$) and maximal response (0.83 ± 0.04 and
0.91 ± 0.03 g; P > 0.05), respectively, in WT (n = 20) and eNOS KO (n = 40) animals. ACh relaxed preconstricted vessels from WT (66.05 ± 1.94%, n = 36 from 20 animals) but not eNOS KO mice, as previously described (Hussain et al., Ref. 7).

Effect of NOS gene disruption on sensitivity of the pGC-cGMP pathway. ANP was more potent on aortas from eNOS KO animals compared with vessels from WT mice (pEC$_{50}$ 8.85 ± 0.01 and 8.41 ± 0.02, respectively, n = 6, P < 0.05; Fig. 3A) but had similar potency on vessels from neuronal NOS WT and KO mice (pEC$_{50}$ 8.43 ± 0.02 and 8.39 ± 0.03, respectively, n = 8, P > 0.05; Fig. 3B) and inducible NOS (iNOS) WT and KO mice (pEC$_{50}$ 8.47 ± 0.03 and 8.45 ± 0.04, respectively, n = 6, P < 0.05; Fig. 3C).

Effect of NOS and sGC inhibition on ANP responses. In WT aortas, the potency of ANP was increased after pretreatment with either the NOS inhibitor L-NAME (pEC$_{50}$ 8.74 ± 0.09 and 9.15 ± 0.09 in the absence and presence of L-NAME, respectively, n = 4, P < 0.05; Fig. 4A) or the sGC inhibitor ODQ (pEC$_{50}$ 8.56 ± 0.05 and 9.26 ± 0.03 in the absence and presence of ODQ, respectively, n = 4, P < 0.05; Fig. 4B).

Effect of NO excess on ANP responses. After incubation with GTN, vessels from eNOS KO animals were less responsive to SPER-NO (pEC$_{50}$ 7.39 ± 0.04 and 6.40 ± 0.05 before and after GTN, respectively, n = 4, P < 0.05; Fig. 5A) and ANP (pEC$_{50}$ 9.24 ± 0.05 and 8.43 ± 0.04 before and after GTN, respectively, n = 4, P < 0.05; Fig. 5B). GTN pretreatment had no effect on the potency of forskolin (pEC$_{50}$ 7.16 ± 0.04 and 7.13 ± 0.04 before and after GTN, respectively, n = 5, P > 0.05). To examine whether cGMP-dependent or -independent actions of NO were responsible for the decreased response to ANP in the presence of GTN,
similar experiments were conducted in the presence of ODQ and GTN. In tissues from eNOS KO animals, ODQ alone did not alter responses to ANP (Fig. 6). In the presence of ODQ, the hyporesponsiveness to ANP after pretreatment with GTN was partially reversed (pEC50 8.81 ± 0.02 for control responses, 8.77 ± 0.04 in the presence of ODQ, 8.28 ± 0.06 after GTN pretreatment, and 8.58 ± 0.06 after GTN pretreatment in the presence of ODQ; all n ≥ 5, P < 0.05 comparing the latter two conditions; Fig. 6).

**Effect of ANP excess on SPER-NO and ANP responses.** Prior exposure of tissues with ANP produced a rightward shift of the SPER-NO (pEC 50 7.33 ± 0.06 and 6.17 ± 0.05 before and after ANP, respectively, P < 0.05; Fig. 7A) and ANP (pEC 50 8.93 ± 0.03 and 8.01 ± 0.17 before and after ANP, respectively, n = 4, P < 0.05; Fig. 7B) concentration-response curves. Forskolin responses were not significantly affected by ANP pretreatment (pEC 50 7.26 ± 0.03 and 6.91 ± 0.05 before and after ANP, respectively, n = 4, P > 0.05).

cGMP Measurements

The basal cGMP content of aortas from WT animals was significantly greater than that detected in vessels from eNOS KO mice (437.6 ± 69.3 and 302.2 ± 54.5 fmol cGMP/mg protein in WT and KO vessels, respectively, n = 4–6, P < 0.05). In tissues treated with SPER-NO or ANP, the increase in cGMP levels was significantly greater in aortas from KO animals compared with WT animals (Fig. 8).

**DISCUSSION**

The present study demonstrates that there is reciprocal interaction between the sGC and pGC systems in human and murine blood vessels. NO-deficient vessels (human IMA or WT aortas treated with a NOS inhibitor or eNOS KO aortas) exhibit increased sensitivity to ANP. Conversely, pretreatment of these vessels with a NO excess (supramaximal concentrations of GTN) reduced their responsiveness to ANP. The effects observed are specific for cGMP-mediated relaxation, because the relaxation of vessels in response to increases in cAMP (elicited by the adenylate cyclase activator forskolin) was unaffected by pretreatment with ANP or NO donors. The changes in sensitivity to ANP, induced by manipulation of the ambient NO concentration, were, in part, dependent on the generation of cGMP because the sGC inhibitor ODQ also increased the potency of ANP and ameliorated the reduction in potency of ANP as a result of GTN pretreatment. Consistent with modulation of guanylate cyclase activity by cGMP, ANP pretreatment of vessels reduced subsequent sensitivity to the NO donor SPER-NO. Moreover, increases in tissue cGMP levels in response to both SPER-NO and ANP were larger in vessels from KO animals compared with WT animals.

We (7) recently demonstrated that the NO-sGC-cGMP axis is exquisitely sensitive to the ambient concentration of NO, allowing rapid up- and downregulation of this signaling cascade in response to the degree of stimulation. Previous reports (22, 25, 26) have sug-
gested that, in an analogous fashion, the pGC-cGMP system can also be downregulated by its endogenous ligand, ANP; this process involves dephosphorylation and desensitization of the receptor. Despite these findings, the possibility of interaction between sGC and pGC has only been examined sporadically and with contradictory results. Pretreatment of vessels with GTN has been reported to decrease responses to NO donors or endothelium-dependent agonists (e.g., bradykinin) but increase the sensitivity of the pGC system to ANP (9, 27, 38). In direct contrast, several studies (14, 21, 28) report that prior exposure to GTN is without effect on ANP responses.

Biochemical analysis of crude guanylate cyclase preparations from the rat aorta and human coronary arteries reveals that pretreatment with GTN causes homologous desensitization to GTN, SNP, and NO but does not induce heterologous desensitization to ANP (34); the same pattern of tolerance is seen in vascular smooth muscle cells of several other species (23) and airway smooth muscle cells (5) in culture. Importantly, little or no functional information is available with respect to a putative sGC-pGC interaction in the human vasculature, despite the extensive investigation of the clinical phenomenon of nitrovasodilator-induced tolerance (20, 24).

This study represents the first investigation looking at a functional interaction between the two cGMP-generating systems in human vascular tissue and has identified a specific cross-talk between the sGC and pGC systems. This observation suggests that cGMP-mediated responses are regulated in a reciprocal fashion by NO and ANP and that this self-regulating system is important in human physiology/pathophysiology. In the presence of L-NMMA, the responses to both SPER-NO and ANP in the human IMA were significantly more potent. Conversely, in the presence of NO excess, the responses to both ANP and SPER-NO were decreased; moreover, the potency of forskolin was unchanged. These observations are analogous with those in murine aortas (see below), indicating that parallel mechanisms for the cooperative regulation of cGMP levels by NO and ANP exist in humans and mice. The present study therefore demonstrates that murine tissue represents a good model in which to study cyclic nucleotide signaling with relevance to human physiology; this is particularly important in light of the inconsistent literature regarding sGC/pGC signaling in other species.

After identification of cross-talk between the sGC and pGC systems in the human vasculature, we turned to murine tissue as a model for further, more-detailed mechanistic studies. We and others (1, 2, 7, 12) have previously reported that in eNOS KO mice, the responses to NO donors are enhanced. In this study, the eNOS KO mouse was used to assess the effects of chronic NO deficiency on the activation of pGC by ANP. Under these conditions, responses to ANP were mark-

![Fig. 7. Concentration-response curves to SPER-NO (A) and ANP (B) in eNOS KO mouse aortic rings in the absence and presence of ANP pretreatment (1 × 10^{-7} mol/l; 30-min incubation followed by wash-out, n = 4 for both). Relaxation is expressed as the means ± SE percent reversal of the PE-induced tone. After incubation with ANP, there was a significant decrease in the potency of both ANP and SPER-NO.](http://ajpheart.physiology.org/)

![Fig. 8. cGMP content of aortas from WT (open bars) and eNOS KO (solid bars) animals exposed to SPER-NO (3 × 10^{-7} mol/l) and ANP (4 × 10^{-9} mol/l, n = 4–6). (cGMP levels are expressed as means ± SE fmol/mg protein.) After incubation with SPER-NO or ANP, increases in cGMP content were significantly greater in vessels from eNOS KO animals. *P < 0.05, significantly different to corresponding WT tissue.](http://ajpheart.physiology.org/)
edly enhanced, mirroring the increased sensitivity of the NO-sGC-cGMP system, indicating that both pathways are upregulated during NO deficiency. This effect was specific to a deficiency in endothelial-derived NO because ANP was equipotent in aortas from neuronal NOS and iNOS WT and KO animals.

The effect of acute NO deficiency was studied using the NOS inhibitor l-NAME. In the presence of this inhibitor, the responses to ANP were enhanced in an analogous fashion to NO donors (7). The magnitude of the change in the ANP responses in the presence of l-NAME was similar to that observed in the eNOS KO animals. This suggests that the change in the sensitivity of the pGC-system occurs within minutes and is most likely to be the result of a conformational modification of preformed enzyme. However, a change in pGC expression after chronic NO deficiency may also contribute to the altered sensitivity of the ANP-pGC system, particularly in light of the fact that chronic ANP exposure causes a decrease in receptor expression (9). These observations are consistent with the finding that, in the rat renal glomeruli, treatment with l-NAME leads to an increase in the activity/sensitivity of the ANP-dependent guanylate cyclase system (13).

The results with l-NAME suggest that either NO or mediator distal to NO (e.g., cGMP, cyclic nucleotide phosphodiesterase, or G kinase) is responsible for the regulation of the sensitivity of the pGC-system. The sGC inhibitor ODQ was used to assess the involvement of cGMP (and therefore downstream mediators) in this desensitization by reducing the production of cGMP from basal NO in the WT tissues. The increased sensitivity of ANP in ODQ-pretreated tissues suggests that cGMP is involved, at least in part, in modifying the sensitivity of the pGC-system to ANP.

Having established the ability of NO-sGC and ANP-pGC signaling to upregulate in response to chronic NO deficiency, subsequent experimentation focused on the possibility of heterologous desensitization after NO excess. The effects of NO excess on ANP responses were investigated in the eNOS KO mice aorta, because this preparation lacks endogenous endothelial-derived NO production and is more sensitive to the effects of NO donors. In this system, after GTN treatment, both the responses to SPER-NO and ANP were reduced. This demonstrates that prior exposure of vessels to GTN decreases not only the sensitivity of the NO-sGC-cGMP system but also that of the ANP-pGC system. These data are in agreement with a study (18) in canine coronary arteries in which chronic exposure to NO donors shifts ANP concentration-response curves to the right.

In the present study, experiments were carried out in the presence of both ODQ and GTN to assess whether cGMP-dependent or -independent mechanisms are responsible for this downregulation of ANP responses. The inhibitory effect of GTN on ANP responses was partially reversed by ODQ, suggesting that cGMP is involved, at least in part, in modulating the sensitivity of the ANP-pGC system. Previous studies (22, 25, 26) have demonstrated that ANP can desensitize its own receptor, but it is unclear whether this is a direct effect of ANP binding itself or dependent on cGMP synthesis. However, 8-bromo-cGMP, a cell-permeable analog of cGMP, has been shown to significantly suppress the long-term recovery of ANP receptors after desensitization (8) and may also have an inhibitory effect on new ANP receptor synthesis (9).

In a reciprocal fashion, ANP has been reported to play a role in modulating NO production by iNOS during inflammatory episodes. ANP reduces NO2/NO3 production in murine macrophages (32) and rat aortic smooth muscle cells (17) and inhibits iNOS protein expression in murine macrophages by accelerating iNOS mRNA decay and preventing nuclear factor-kB binding (11). Furthermore, iNOS induction in rabbit aortic smooth muscle cells in culture increases intracellular cGMP levels, which results in decreased expression of the ANP-C receptor and 125I-labeled ANP binding (10). In contrast, however, in rat cardiac myocytes exposed to interleukin-1β, ANP causes an increase in iNOS mRNA and protein levels via a G-kinase-dependent mechanism (37). In the present study, we have demonstrated that ANP can downregulate the NO-sGC-cGMP pathway after excessive stimulation of pGC; this defines a novel role for ANP/pGC in modulating the signal conveyed by eNOS-derived NO. This heterologous desensitization therefore operates in a reciprocal fashion to regulate cGMP levels in the same cell type. Moreover, because ANP is unlikely to have a direct effect on NO or sGC, it is probable that the desensitization of NO signaling after ANP excess is mediated by cGMP (or a mediator distal to this point in the pathway). However, it is not known whether deficiencies in ANP-pGC signaling can be compensated for by enhanced NO-sGC activation.

The role of cGMP in the autoregulation of sGC and pGC signaling was confirmed by biochemical studies investigating the cGMP levels in aortas after exposure to SPER-NO and ANP. The resting cGMP content in vessels from WT animals was significantly greater than that in tissues from eNOS KO mice. This finding is consistent with a basal release of NO from the eNOS present in the endothelium of WT vessels. After SPER-NO or ANP was administered, the cGMP levels in the aortas from both WT and eNOS KO animals were significantly increased. However, the increase in cGMP concentration in the aortas from eNOS KO animals was greater in response to both vasodilators. These observations are in accord with the greater potency of SPER-NO and ANP in NO-deficient vessels and indicates that increased cGMP production plays a fundamental role in the altered sensitivity of these vessels to guanylate cyclase activation.

A number of putative mechanisms may explain our observations of heterologous (de)sensitization to guanylate cyclase activation in vascular smooth muscle. However, our finding that increased production of cGMP is involved in the altered sensitivity of NO-deficient vessels to SPER-NO and ANP indicates that increased expression or activity of guanylate cyclases and/or phosphodiesterases is likely to underlie the au-
toregulation of cGMP-mediated responses. In terms of ANP-pGC signaling, this may involve 1) an altered sensitivity of pGC to ANP, perhaps the result of phosphorylation and (de)sensitization of the ANP receptor (22, 25, 26); or 2) a change in the expression of pGC protein (analogous to the decreased receptor expression after iNOS induction; see Ref. 10). Alternatively, a direct action of NO on pGC (e.g., S-nitrosation) might underlie the altered sensitivity to ANP. The mechanisms underlying cross-tolerance to the NO-sGC pathway after excess ANP are more difficult to hypothesize, although a G kinase-dependent phosphorylation of sGC represents a plausible process (particularly because G kinase has been shown to be an important determinant of smooth muscle sensitivity to nitrovasodilators; see Ref. 31). Altered activity and/or expression of phosphodiesterases represents an appealing mechanism with which to bring about heterologous (de)sensitization, because this common regulator of cGMP levels could account for the altered sensitivity to activation of either guanylate cyclase. However, we (7) have previously shown that the phosphodiesterase type 5 inhibitor zaprinast has little or no influence on the vasorelaxant activity of SPER-NO in the mouse aorta. This observation suggests that alterations in phosphodiesterase type 5 expression and/or activity are unlikely to account for the heterologous (de)sensitization, although changes in the expression and/or activity of other phosphodiesterase isozymes may be involved. We are currently investigating the mechanisms underlying the heterologous (de)sensitization.

In summary, the results of the present study suggest that the ambient concentration of NO and/or ANP can modulate the sensitivity of the sGC and pGC signaling cascades, indicating that sGC and pGC reciprocally regulate cGMP-mediated vasorelaxation in the human and murine vasculature. Consequently, we have demonstrated for the first time that a cooperative interaction between NO and ANP, via sGC and pGC, might regulate vascular tone in human vessels and might have important implications for human physiology and pathophysiology. The existence of such a cooperative system is easy to reconcile with mammalian physiology. This mechanism would allow for feedback regulation of complementary responses mediated by NO and ANP (e.g., smooth muscle relaxation). Moreover, this mechanism would provide a compensatory signaling pathway should a deficiency arise in either mediator. In particular, the endocrine ANP/cGMP system could supplement the activity of the paracrine NO/cGMP pathway in cardiovascular diseases associated with generalized endothelial dysfunction, where other endothelial mediators might be unable to compensate for reduced NO bioactivity. This is supported by the recent observation that prepro-ANP mRNA expression is up-regulated in the atria of eNOS KO mice. Conversely, in disease states associated with excessive activation of either cGMP-generating system (e.g., sepsis and heart failure), there might reciprocal downregulation of responses elicited by the parallel pathway. Furthermore, these data may have clinical implication for nitrovasodilator therapy. The tolerance observed with the prolonged use of NO donors, such as GTN, may also blunt the effect of endogenous ANP activity; this may be responsible, at least in part, for the sodium retention, hypervolemia, and reduced vasodilation that is characteristic of nitrate tolerance.

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