Impaired nitric oxide-mediated vasodilation in transgenic sickle mouse

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Kaul, Dhananjaya K., Xiao-Du Liu, Mary E. Fabry, and Ronald L. Nagel. Impaired nitric oxide-mediated vasodilation in transgenic sickle mouse. Am J Physiol Heart Circ Physiol 278: H1799–H1806, 2000.—In transgenic sickle mice expressing human β6Glu and βS-Antilles globins, show intravascular sickling, red blood cell adhesion, and attenuated arteriolar constriction in response to oxygen. We hypothesize that these abnormalities and the likely endothelial damage, also reported in sickle cell anemia, alter nitric oxide (NO)-mediated microvascular responses and hemodynamics in this mouse model. Transgenic mice showed a lower mean arterial pressure (MAP) compared with control groups (90 ± 7 vs. 113 ± 8 mmHg, P < 0.00001), accompanied by increased endothelial nitric oxide synthase (eNOS) expression. NO-dependent arteriolar responses to NO-mediated effects (ACh and sodium nitroprusside [SNP]). Indomethacin did not alter the responses to ACh and SNP. Forskolin, a cyclic AMP-activating agent, caused a comparable dilation of A2 and A3 vessels (44 and 70%) in both groups of mice. Thus in transgenic mice, an increased eNOS/NO activity results in lower blood pressure and diminished arteriolar responses to NO-mediated vasodilators. Although the increased eNOS/NO activity may be compensated for with remodeling abnormalities, it may also cause pathophysiological alterations in vascular tone.

Sickle cell anemia (SS) is due to a single amino acid substitution in the hemoglobin S (HbS) molecule (β6Glu→Val) that results in the polymerization of HbS and sickling of red blood cells under deoxygenated conditions (17). This disease is characterized, among other features, by recurring painful vasoocclusive episodes. Sickle cell anemia presents a spectrum of abnormalities (pleiotropism) involving red blood cells and multiple organs.

Abnormal rheological properties of heterogeneous SS red blood cells may result in episodes of transient occlusion at the microvascular level (16). Repeated occurrence of such episodes may cause local ischemic-hypoxic injury and result in impaired endothelium-dependent vascular reactivity as reported for other ischemic diseases (12). In addition, the increased red blood cell rigidity and red blood cell-endothelium interactions in sickle cell anemia (14, 17) may inflict mechanical injury to the vessel endothelial lining. Evidence for endothelial damage in sickle cell anemia comes from electron microscopic studies (19), as well as from the presence of sloughed-off endothelial cells in the circulation of these patients (33, 35). Among the reported consequences of the endothelial injury are an increase in circulating levels of prostacyclin and von Willebrand factor (24, 34). In addition, peripheral vasodilation and cardiovascular adjustments have been reported in SS patients (13, 22, 23, 29), but the underlying mechanisms remain undefined.

From the reported endothelial abnormalities, the role of nitric oxide (NO) in sickle cell disease is of particular interest. NO is constitutively generated and released by the vascular endothelial cells and acts on the adjacent smooth muscle cells to produce vasorelaxation. NO is generated from L-arginine and oxygen by nitric oxide synthases (NOS), i.e., endothelial NOS (eNOS), neuronal NOS (nNOS), and inducible NOS (iNOS). NO has diverse effects, including important roles in the regulation of blood pressure, dynamic modulations of the vascular tone (vasomotion), blood flow, and leukocyte adhesion (21, 26, 27). Several pathological conditions such as hypertension, diabetes, and ischemia are associated with impaired endothelial NO production (12).

Transgenic sickle mice expressing human α-, β6Glu-, and βS-Antilles-globins on a background that is homozygous for deletion of mouse βmajor (S+S-Antilles mice) (10) have more severe pathology than the earliest mice described by our group (9), which express human α- and βS-globins on a background homozygous for deletion of mouse βmajor. The more severe pathology of S+S-Antilles mice is characterized by elevated reticulocytes and presence of dense red blood cells, spontaneous urine concentrating defect, shortened delay times for hemoglobin polymerization, multiple organ damage, and reduced life expectancy; all of these features are characteristics of sickle cell anemia. The greater severity is due to the presence of HbS-Antilles, which is a...
mutant hemoglobin containing β^S mutation at β6 (Glu→Val) and a second mutation at β23 (Val→Ile). The second mutation results in a lower oxygen affinity and lower solubility of HbS-Antilles under deoxygenated conditions than HbS (25). The solubility (Csat) is the concentration of deoxygenated hemoglobin in equilibrium with polymer; in other words, the minimum concentration of deoxygenated HbS-Antilles required for polymer formation is lower than that required for HbS. In contrast to patients heterozygous for HbS who have no clinical symptoms, patients heterozygous for HbS-Antilles have significant pathology.

We have previously reported that S+S-Antilles mice have intravascular sickling and red blood cell-endothelial interaction in the cremaster muscle microcirculation (16). Furthermore, these mice show an attenuated arteriolar constriction in response to hyperoxia that may involve contributions from both abnormal red blood cells and endothelial factors. In the less severe transgenic mouse line expressing human α- and β^S-globins (9), both eNOS and iNOS show a marked increase in the kidneys (3). These observations indicate that in transgenic sickle mice, NO production may be altered. However, no in vivo study has been carried out to investigate whether altered NO production would affect microvascular responses and systemic hemodynamics in the mouse model of sickle cell anemia.

In the present studies, we have used the S+S-Antilles transgenic mouse to test the hypothesis that microvascular flow abnormalities, likely endothelial damage, and altered NO production will result in significant vascular tone adjustments, affecting systemic hemodynamics, as well as responses of the resistance vessels (i.e., arterioles). The work presented here demonstrates compromised arteriolar diameter and flow responses in S+S-Antilles mice to NO-mediated vasodilators, i.e., ACh, an endothelium-dependent vasodilator (12), and sodium nitroprusside (SNP), an NO donor. We also present evidence indicating that lower blood pressure and the impaired microvascular responses in the S+S-Antilles mouse are mainly a consequence of elevated NOS expression.

**MATERIALS AND METHODS**

Transgenic mice Transgenic mice expressing human α-, β^S-, and β^S-Antilles-globins on a homozgyous mouse β^major deletion background (β^MDD) and symbolized as α^Hβ^Sβ^S-Antilles[β^MDD] were produced by breeding α^Hβ^S[β^MDD] mice (9) into a line expressing α^H and β^Antilles[β^MDD] (31) on a homozgyous mouse β^major deletion background, as previously described (10). The globin composition in mature mice was determined by HPLC and revealed the following: human α, 58.2% of all α-globin; β^S, 42.2% and β^S-Antilles, 35.9% of all β-globins (10). Transgenic sickle mice expressing human α- and β^S-globins (~75% β^S of all globins, mouse β^major deletion background, or α^HIβ^S[β^MDD]) and mice expressing human α- and β^S-globins (30% of all β-globins or α^HIβ^S with no deletions) were used for blood pressure measurements.

Intravital microscopy. Male C57BL/6j control (n = 44) and S+S-Antilles (n = 35) mice weighing ~25–30 g (4–6 mo old) were used. The mice were maintained on a standard diet and water ad libitum. Mice were anesthetized intraperitoneally with 10% urethane and 2% α-chloralose in saline (5 ml/kg). The animals were tracheostomized. The right jugular vein was cannulated for infusion of vasoactive substances. To monitor arterial pressure, the left carotid artery was cannulated using PE-10 polyethylene tubing. In vivo microcirculatory observations were made in the open cremaster muscle preparation and prepared according to the method of Baez (1). The suffusion and maintenance of the mouse cremaster preparation was done as described (16). Briefly, the preparation was suffused with a bicarbonate Ringer solution (mmol/l: 135.0 NaCl, 5.0 KCl, 27.0 NaHCO3, 0.64 MgCl2, and 11.6 glucose); pH of the solution was adjusted to 7.35–7.4 by continuous bubbling with 94.4% N2.5% CO2. The osmolarity of the solution, as measured by a Microsmette (Precision systems, Sudsbury, MA) was 330 mosM, as described for the mouse plasma (4). The temperature of the suffusion solution (flow rate, 5–6 ml/min) was maintained at 34.5–35°C and monitored by a thermistor (YSI, Yellow Springs, OH). The oxygen tension (PO2) of the suffusion solution was 15–20 mmHg, as determined using a microoxygen electrode (model MI-730; Microelectrodes, Bedford, NH). The preparation was allowed to stabilize for 45 min before experiments were initiated. Microscopic observations were carried out using an Olympus microscope (model BH-2; Olympus, Lake Success, NY, equipped with a television camera ( Cohu, S5000 Series; Cohu, San Diego, CA) and a Sony U-matic video recorder (model VO5800; Sony, Teaneck, NJ).

The microvascular branching orders in the mouse cremaster have been described recently (16). Diameter and red blood cell velocity (rbc) measurements were made in A2 and A3 arterioles. Vessel luminal diameter (D) was measured on-line using an image shearing device (model 907, Instruments for Physiology and Medicine, San Diego, CA). rbc was measured along the vessel centerline using the “dual-slit” photodiode and a velocity cross-correlator (model 102 BF, Instruments for Physiology and Medicine) (36, 37). rbc measurements were made using a water immersion ×40 objective and ×6.3 eyepiece. The centerline rbc was converted to the mean rbc across the vessel diameter using a conversion factor of 1.6 (rbc/Vmean = 1.6), originally described by Baker and Wayland (2) and later validated by Seki and Lipowsky (32). Volumetric flow rates (Q) were determined from Vmean and the vessel cross-sectional area (A) as described (2). The practicality of flow estimations has been demonstrated by Lipowsky et al. (22) in studies involving nullidipillary microcirculation of SS patients, as well as in our previous studies with S+S-Antilles mice (16).

Protocol 1. In a series of experiments, arteriolar responses to topical application of ACh (10^−6 M, Sigma, St. Louis, MO) and SNP (10^−6 M, Sigma), both NO-mediated vasodilators, were compared in normal and S+S-Antilles mice. After baseline measurements, suffusion with bicarbonate Ringer was interrupted, and ACh or SNP prepared in the same medium was topically applied. Vessel diameter and Vrbc were measured after 3 min of the topical application. In pilot experiments with control mice, both ACh and SNP resulted in maximal vasodilatory effects when the concentration of each was increased from 10^−8 to 10^−4 M. Increasing the concentration to 10^−3 M caused no appreciable further increase in the diameter (data not shown). Hence, the concentration of 10^−6 M was selected in each case. Ten minutes were allowed to lapse before the next topical application. In general, diameters returned to baseline levels within 10 min.

In separate experiments, arteriolar diameter response to ACh and SNP was evaluated after blockade with indomethacin. The cremaster preparation was suffused with bicarbonate Ringer solution containing indomethacin (50 µM) for 15 min before topical application of these substances. In three
separate S+ S-Antilles mice, indomethacin (5 mg/kg) was infused via the jugular vein to evaluate its effect on mean arterial pressure (MAP).

In a series of experiments, arteriolar diameter response to topically applied forskolin (Sigma), a cAMP-mediated vasodilator, was evaluated at a concentration of 10^{-6} and 10^{-5} M prepared in bicarbonate Ringer solution.

Protocol 2. In these experiments, the effect of N^{o}-nitro-L-arginine methyl ester (L-NAME; Sigma) infusion before and after L-arginine (Sigma) administration was evaluated. After 45 min of stabilization of the cremaster preparation and measurements of resting diameters and V_{hc} (~15 min). L-NAME (20 mg/kg) was infused intravenously over 5 min. The arteriolar diameters and A2 V_{hc} were measured at 10, 20, and 40 min. L-Arginine (20 mg·kg^{-1}·min^{-1}) was then infused for 15 min, and microcirculatory variables were measured. After L-arginine, L-NAME infusion (20 mg/kg) was repeated, and microcirculatory measurements were carried out at 10 and 30 min thereafter.

Protocol 3. In a series of experiments, arteriolar diameter response to the infusion of aminoguanidine (AG; Sigma) was compared. AG (100 µM/kg) was infused over a 10-min period, and the diameters were measured at the end of the 10-min infusion and at 15 and 30 min thereafter.

Western blot analysis. Cremaster tissue protein extraction was carried out for Western blot analysis (4 controls, 4 transgenics). The cremaster tissue was excised, rinsed in saline, and homogenized in boiling lysis buffer (1% SDS in 10 mM Tris·HCl, pH 7.4). Three milliliters of buffer were used per gram of the tissue. The homogenate was boiled for 5 min, and the diameters were measured at the end of the 10-min infusion and at 15 and 30 min thereafter.

Microvascular variables under resting conditions. The resting arteriolar diameters (in µm, means ± SD) for control and S+ S-Antilles mice, respectively, were as follows and showed no significant differences: A2, 43.4 ± 7.3 (n = 44) and 46.1 ± 8.3 (n = 39) (P = 0.12); A3, 23.0 ± 6.3 (n = 43) and 24.2 ± 5.8 (n = 42) (P = 0.38).

RESULTS

Mean arterial blood pressure. Steady-state MAP ranged from 100 to 124 mmHg in control mice (n = 16) and from 80 to 105 mmHg in S+ S-Antilles mice (n = 15). The averaged MAP values (means ± SD) were significantly lower in S+ S-Antilles mice compared with control mice, i.e., 90 ± 7 vs. 113 ± 8 mmHg (P < 0.0001). Mice expressing human α- and β^-globins (75% β^- of all β^-globins, n = 4) on a mouse homozygous β^-major (mild pathology) showed an average MAP of 90 ± 7 mmHg (P < 0.005 vs. controls). On the other hand, transgenic sickle mice expressing only 30% β^-globins (n = 2) had higher MAP (99.0 and 102 mmHg) than other two transgenic lines.

Microvascular variables under resting conditions. The resting arteriolar diameters (in µm, means ± SD) for control and S+ S-Antilles mice, respectively, were as follows and showed no significant differences: A2, 43.4 ± 7.3 (n = 44) and 46.1 ± 8.3 (n = 39) (P = 0.12); A3, 23.0 ± 6.3 (n = 43) and 24.2 ± 5.8 (n = 42) (P = 0.38).

Response to Ach, an endothelium-dependent vasodilator. As shown in Fig. 1A, in control mice (n = 9), Ach (10^{-6} M) caused >50% increase in the mean diameter of A2 arterioles (n = 9) from 41.3 ± 5.5 to 64.4 ± 12.6 µm (P < 0.0001) and ~80% increase in A3 diameters (n = 9) from 21.2 ± 5.1 to 38.0 ± 11.3 µm (P < 0.001). In contrast, in S+ S-Antilles mice (n = 8), there was >10% increase in the diameter of A2 (n = 10) and A3 (n = 8) arterioles (Fig. 1A). The difference in response between control and transgenic mice was significant (P < 0.01) (Fig. 1A).

In controls (n = 5), Ach caused a twofold increase in A2 (n = 5) volumetric Q (16.5 ± 4.1 to 33.0 ± 12.2 nl/s, P < 0.03). In contrast, in transgenic sickle mice (n = 5), Ach had no effect on Q (5.2 ± 1.6 to 5.4 ± 2.3 nl/s, P > 0.8), indicating an attenuated response.

Response to SNP, an NO donor. Figure 1B shows the effect of SNP, an endothelium-independent vasodilator,
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Fig. 1. Arteriolar diameter (A2 and A3) responses to topical application of ACh (10⁻⁶ M) and sodium nitroprusside (SNP) (10⁻⁶ M) in control and S+S-Antilles (S+S-Ant) mice. A: arteriolar diameter response to ACh. For each arteriolar order, difference in response between control and transgenic groups was significant (\*P < 0.02). *P < 0.01–0.001 compared with respective pre-ACh values (paired t-test). B: arteriolar diameter response to SNP. In contrast to controls, S+S-Antilles mice showed a greatly diminished response to SNP in A2 and A3 arterioles. *P < 0.002–0.0001 compared with respective pre-SNP values; **P < 0.02–0.003 compared with control arterioles.

Fig. 2. Effect of forskolin (10⁻⁵ M) on arteriolar diameters (left: A2; right: A3) in control and S+S-Antilles mice. In both groups of mice, forskolin caused a significant and comparable arteriolar dilation. *P < 0.02 and **P < 0.003 compared with respective control values (paired t-test).

on the arteriolar diameters. In controls (n = 6), topical SNP (10⁻⁶ M) caused ~45% and 60% increases over the initial A2 and A3 diameters (n = 6 each), respectively (A2: 43.2 ± 6.5 to 63.4 ± 9.3 µm, P < 0.005; A3: 28.4 ± 4.4 to 47.1 ± 10.7 µm, P < 0.003; paired t-test). On the other hand, S+S-Antilles mice (n = 5) showed a greatly diminished response, i.e., 16 and 23% increases, respectively for A2 (n = 7) and A3 (n = 5) arterioles (A2: 40.6 ± 12.1 to 46.7 ± 5.6 µm; A3: 25.2 ± 6.7 to 30.8 ± 7.7 µm, both P < 0.01). The difference in response between control and transgenic mice was significant (P < 0.02–0.003) (Fig. 1B).

Responses after blockade with indomethacin. In a series of experiments, indomethacin (50 µM) was added to the suffusion solution to effect a blockade of cyclooxygenase activity. In controls (n = 4), indomethacin resulted in a slight increase in the mean arteriolar diameters [A2 (n = 7): 41.2 ± 4.8 to 42.0 ± 7.4 µm, P > 0.06; A3 (n = 5): 18.9 ± 7.6 to 22.4 ± 5.6 µm, P < 0.05, paired t-test]. Thereafter, topical ACh (10⁻⁶ M) caused ~40 and 70% increases in the diameters of A2 and A3 arterioles (P < 0.00001 and P < 0.011). After ACh washout, topical SNP (10⁻⁶ M) resulted in >40 and 80% increases in the diameters of A2 and A3 arterioles (P < 0.002) (data not shown). In S+S-Antilles mice (n = 3), indomethacin alone caused no significant changes in the mean arteriolar diameters [A2 (n = 4): 44.5 ± 5.2 to 45.0 ± 5.1 µm, P > 0.3; A3 (n = 7): 19.3 ± 5.8 to 18.0 ± 6.4 µm, P > 0.1, paired t-test]. Again, in S+S-Antilles mice, the arteriolar diameter responses to both ACh and SNP were greatly attenuated. ACh caused increased mean diameters of A2 and A3 arterioles by <10% (P > 0.12) and 33% (P < 0.03), respectively. Similarly, SNP induced smaller increases in the diameters of A2 and A3 arterioles, i.e., 12.8% (P > 0.3) and ~37% (P < 0.001), respectively.

Also, in S+S-Antilles mice (n = 3), intravenous infusion of indomethacin (5 mg/kg) did not result in any significant changes in MAP (mmHg) over a 30-min period (initial, 89 ± 6 mmHg; indomethacin, 90 ± 4, P > 0.1).

Responses to forskolin, a cAMP-mediated vasodilator. Forskolin (10⁻⁶ M) caused a comparable arteriolar dilation in control and S+S-Antilles mice (Fig. 2). In controls (n = 2), the mean diameter of A2 arterioles (n = 6) showed ~44% increase from 39.0 ± 8.7 to 56.3 ± 10.9 µm (P < 0.02), whereas in transgenic mice (n = 2), the diameter (n = 5) increased by ~40%, i.e., from 48.7 ± 8.4 to 58.4 ± 4.0 µm (P < 0.02). A3 arterioles showed ~72% increase in controls (n = 6; 20.6 ± 4.1 to 35.6 ± 8.2 µm, P < 0.003) and ~60% increase in transgenic mice (n = 5; 22.3 ± 4.3 to 35.6 ± 5.3, P < 0.003). Increasing the concentration of forskolin to 10⁻⁵ M did not cause any further arteriolar dilation in either group (data not shown). Similar arteriolar responses were obtained in two S+S-Antilles mice where forskolin and ACh were applied sequentially in the same preparations (data not shown).

Responses to L-NAME and L-arginine. In a series of experiments, response to intravenous infusions of L-NAME (20 mg/kg), the nonselective inhibitor of NOS was determined before and after L-arginine (20
mg·kg⁻¹·min⁻¹). Briefly, after the tissue stabilization and baseline measurements of microcirculatory variables (60 min), L-NAME was infused intravenously for 5 min, and the measurements were repeated at 70, 80, and 100 min (total, 40 min). This was followed by a 15-min infusion of L-arginine, and thereafter L-NAME infusion was repeated as indicated in Figs. 3–5 (also see MATERIALS AND METHODS, Protocol 2).

L-NAME caused comparable decreases in the mean arteriolar diameters in both control (n = 5) and S+S-Antilles (n = 4) mice (P < 0.05, ANOVA) (Fig. 3, A and B). At a 40-min interval, A2 (control, n = 10; transgenic, n = 9) and A3 (control, n = 10; transgenic, n = 13) arterioles showed almost a 40% decrease in the diameter. Infusion of L-arginine (20 mg·kg⁻¹·min⁻¹) after L-NAME resulted in significant increases in A2 and A3 diameters in both groups of mice compared with pre-L-arginine values (P < 0.05) (Fig. 3, A and B). When L-arginine was followed by L-NAME, A2 and A3 arterioles again showed significant constriction (P < 0.05) in controls, whereas the transgenic mice showed significant constriction in A3 arterioles (P < 0.05).

Figure 4 shows plots of estimated volumetric Q in A2 arterioles of control and S+S-Antilles mice during the same sequential infusions. The resting Q values were significantly lower in transgenic mice compared with control (P < 0.005) (Fig. 4). In both groups of mice, the infusion of L-NAME caused a decrease in Q (P < 0.05). In controls, Q showed a marked 80% decrease after 40 min of L-NAME infusion compared with the resting values (from 20.1 ± 8.9 to 3.1 ± 1.7 nl/s). In S+S-Antilles mice, which showed a 60% lower resting Q compared with controls, L-NAME caused a further 60% decline in Q at 40 min (i.e., from 6.8 ± 3.2 to 2.7 ± 1.9 nl/s). In both controls and transgenic mice, L-arginine infused after L-NAME caused a marked recovery in Q compared with pre-L-arginine values: control from 3.1 ± 1.7 to 14.8 ± 5.6 nl/s; transgenic from 2.7 ± 1.9 to 8.0 ± 5.5 nl/s, P < 0.05). When L-NAME was then given, L-NAME again resulted in a significant decrease in Q in control mice (P < 0.05), whereas in transgenic mice, the flow decrease was not significant.

As shown in Fig. 5, in both control (n = 5) and S+S-Antilles mice (n = 4), L-NAME caused comparable increases in MAP within 10 min (i.e., 27 and 30%, respectively; P < 0.05). Although the initial baseline MAP values were lower in transgenic mice (P < 0.05), the differences between the two groups were not significant at different time points after L-NAME infusion. Infusion of L-arginine after L-NAME caused a significant increase in MAP in both control and transgenic mice compared with the preceding values (P < 0.05). L-NAME infused after L-arginine again resulted in a significantly higher MAP in both controls and transgenic mice compared with the initial baseline values (P < 0.05).

Responses to AG. In separate experiments, arteriolar response to AG, an inhibitor of iNOS, was evaluated to delineate the role of iNOS. A 10-min infusion of AG (100 µM) was followed by arteriolar diameter measurements at 15- and 30-min intervals. There was no significant effect of AG on the arteriolar diameters of both control (n = 3) and transgenic (n = 2) mice (A2 and A3 arterioles, each n = 5) at any given interval (control

\[\text{Fig. 3. Effect of L-NAME (20 mg/kg) before and after L-Arg (20 mg·kg}^{-1}·\text{min}^{-1}) \text{ on arteriolar diameters (A: A2; B: A3) in control and S+S-Antilles mice. Experiments were initiated after 45 min of tissue stabilization and 15 min of baseline measurements (60 min total). Duration of infusions in each case is indicated by horizontal bars. *P < 0.05 denotes significant L-NAME-dependent decrease in diameters. †P < 0.05 compared with preceding values.}\]
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### DISCUSSION

The studies presented here demonstrate that: 1) transgenic sickle mice expressing human \(\alpha\)-, \(\beta^5\)-, and \(\beta^S\)-globins (\(S+S-Antilles\) mice) exhibit a lower blood pressure, and this is associated with an increased eNOS expression and a demonstrable NOS/NO activity; and 2) transgenic sickle mice show blunted arteriolar responses to NO-mediated vasodilators.

NOS/NO activity and systemic hypotension. Our results support a significant and probably greater NO production in transgenic sickle mice. First, the results of Western blotting show an elevated eNOS expression in the transgenic mouse cremaster (i.e., 1.5- to 3.5-fold increase) compared with controls. Second, we observe a lower MAP in \(S+S-Antilles\) mice as expected with continual NO/NOS stimulation. In addition, the infusion of indomethacin, a potent inhibitor of cyclooxygenase activity (40), did not result in any changes in MAP in \(S+S-Antilles\) mice, showing that prostaglandins did not contribute to hypotension.

Similarly, a less severe transgenic mouse expressing human \(\alpha\)- and \(75\% \beta^S\)-globins (9) shows a lower MAP compared with control mice and an elevated eNOS in the kidneys (3). In contrast, MAP was closer to normal values in an asymptomatic mouse line expressing human \(\alpha\)- and \(30\% \beta^S\) levels. Thus an increased eNOS activity in \(S+S-Antilles\) could result in a persistent NO production and a lower blood pressure, as was also shown for eNOS-overexpressing transgenic mice (26). In the present studies, all mice, both normal and transgenic, have a normal hematocrit, thus eliminating anemia as a contributing factor.

We confirmed NOS activity by experiments aimed at inhibiting or stimulating NO production. L-NAME, a nonselective inhibitor of NOS, resulted in comparable arteriolar constriction in both controls and \(S+S-Antilles\) mice (see Fig. 3), which is in accordance with the inhibitory effect of L-NAME on NOS activity (5, 22). The arteriolar constriction was associated with significant increases in MAP in both groups of mice (see Fig. 5), the magnitude of response being similar in both groups. L-Arginine infused after L-NAME resulted in a near complete recovery of the arteriolar diameter and MAP in both control and transgenic mice (see Figs. 3 and 5). These results are consistent with the competitive interactions between L-NAME and L-arginine. Taken together, these results support a significant NOS activity in \(S+S-Antilles\) mice.

The cremaster tissue, while positive for eNOS, was negative for iNOS. However, iNOS activity at another site, such as in the the kidneys of \(75\% \beta^S\) mice (3), could
contribute to hypotension in transgenic sickle mice. To test this possibility, we examined the responses to AG at a concentration (100 μM/kg) that causes inhibition of iNOS but not of eNOS (6). AG had no effect on MAP or arteriolar diameters in either controls or transgenic mice, suggesting that iNOS did not contribute to the observed effects on MAP and arteriolar diameters in the transgenic mouse.

Importantly, as in transgenic sickle mice, sickle cell anemia patients also show a lower blood pressure than normal subjects (11, 13, 15, 30). The factors leading to a lower blood pressure and a lower peripheral resistance (23) in sickle cell anemia are not fully defined. Postulated mechanisms include altered levels of vasoactive substances and an altered vascular reactivity to vasoactive stimuli (13). Sickle cell anemia patients show a higher NO production (28). There is evidence for an elevated renal prostaglandin synthesis in nephropathy associated with sickle cell anemia (8), but indomethacin administration to SS patients has no effect on MAP (7), similar to what was found in the present studies. These findings coupled with an elevated eNOS expression in S+S-Antilles mice strengthen the role for NO in the regulation of systemic blood pressure, with implications for sickle cell anemia.

Mechanism of blunted responses to NO-mediated vasodilators (ACh and SNP). Our results show that the microvascular diameters in S+S-Antilles mice are not significantly different compared with controls, but the ability of the vessels to respond to NO-mediated vasodilators is affected. NO derived from endothelial cells or NO donors causes vasorelaxation by activating guanylate cyclase-cGMP system in the underlying vascular smooth muscle cells. The blunted vasodilatory effect of ACh in transgenic sickle mice is consistent with an increased NO/NOS activity that would result in a depressor effect on the vascular tone. Furthermore, the diminished arteriolar response to SNP is also compatible with an increased generation of endothelial NO, resulting in continual stimulation of guanylate cyclase-cGMP activity in the vascular smooth muscle. The arteriolar responses to ACh and SNP were not altered after blockade with indomethacin, suggesting that these responses were independent of prostacycin activity. Notably, arteriolar response to forskolin was not affected in S+S-Antilles mice, showing that cAMP-mediated vasodilatory mechanism was intact. Thus, in S+S-Antilles mice, a significant vascular tone is indicated by arteriolar dilation in response to forskolin. In addition, the increased NO/NOS activity could affect dynamic modulations of vascular tone (vasomotion) and cause altered resistance to flow as is evident by lower blood pressure. Also, we cannot rule out contributions from heterogeneity of microvascular tone in different organs to systemic blood pressure of transgenic sickle mice.

These results are comparable to those reported for transgenic mice that constitutively overexpress eNOS, have increased basal levels of cGMP, and show diminished responses to these vasodilators (26). Similar results have been reported in a rat model for acute renal ischemia in which an increased NOS activity was accompanied by attenuated responses of renal blood flow to both ACh and SNP (5).

Increased NOS/NOS activity is associated with chronic hypoxia. In S+S-Antilles mice, intravascular sickling, red blood cell-endothelial interactions, and episodes of stasis and transient occlusion may cause both mechanical and hypoxic injuries to the vascular endothelial lining and result in chronic tissue hypoxia. Previous studies have shown that hypoxia associated with prolonged ischemia increases the activity of eNOS and NO production in the coronary microcirculation (39).

In conclusion, we propose a model in which the microcirculatory flow abnormalities in transgenic sickle mice could lead to chronic tissue hypoxia that triggers vascular tone modifications secondary to chronic increase in NOS/NOS activity. Thus the elevated NOS/NOS activity results in systemic hypotension associated with a depressor effect on the vascular tone. The continual stimulation of NOS/NOS activity in the endothelium significantly reduces the responses to NO-mediated vasodilators. Thus, although an increased NOS/NOS activity might compensate for flow abnormalities in transgenic sickle mice and by extension in sickle cell anemia, it may also cause pathophysiological alterations in the vascular tone, such as attenuated responses to NO-mediated vasodilators. Transgenic sickle mouse models will be a useful tool to investigate the role of NO in the pathophysiology of sickle cell anemia.

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