A Microvascular Site of Action of Sodium Nitroprusside in Striated Muscle of the Rat

David E. Longnecker, M.D.,* Richard A. Creasy, M.D.,† Donald C. Ross, Ph.D.‡

The microvascular effects of sodium nitroprusside (SNP) were studied in rat striated muscle to determine the peripheral vascular sites of action of the drug. Eleven male rats were anesthetized with pentobarbital and the cremaster muscle was prepared for television microscopy. The internal diameters of three arterioles and two venules of each animal were measured before, during, and after SNP infusion (40 µg/kg/min). First-order arterioles (123 ± 7 µm) showed no significant response to SNP, and their internal diameters correlated poorly ($r = -0.31$) with decreases in mean arterial pressure (MAP). The internal diameters of third-order arterioles (42 ± 2 µm) correlated well with MAP ($r = 0.85$), but these arterioles did not dilate significantly during SNP infusion. The internal diameters of fourth-order arterioles (15 ± 1 µm) correlated best with MAP ($r = 0.91$), and significant ($P < 0.05$) dilatation occurred promptly during SNP infusion. Thereafter, the responses of fourth-order arterioles could be divided into two subgroups. One subgroup dilated initially but promptly returned to control diameters, while the other subgroup dilated ($P < 0.05$) throughout exposure to SNP. Both third- and fourth-order arterioles constricted significantly when SNP infusion was discontinued. Venular diameters were not affected by SNP. In five additional rats, topical application of SNP (10-4 M) to the cremasteric microvasculature resulted in dilatation of fourth-order arterioles, which persisted throughout exposure to SNP, but reverted to control diameters when the drug was removed. The transient dilatation of some fourth-order arterioles, and the arteriolar constriction observed when intravenous infusion of SNP was terminated, appear to represent compensatory responses to hypotension, since these effects were not seen with topical application of SNP. The results indicate that the primary peripheral vascular site of action of SNP is in fourth-order arterioles. (Anesthetic technique: hypotension, induced, nitroprusside. Microcirculation: muscle. Muscle: striated.)

Sodium nitroprusside (SNP) is frequently employed to induce hypotension during anesthesia because of its rapid onset and short duration of action and ease of administration. In man, SNP appears to decrease arterial pressure primarily through its effects on the peripheral vasculature, since cardiac output is not significantly decreased by the drug. Unlike nitroglycerin, which is purported to alter the venous system predominantly, SNP is alleged to produce relatively equal changes in venous capacitance and arteriolar resistance. Myocardial depression by SNP has not been found.

Despite the reported changes in calculated arterial resistance and venous capacitance, there have been no systematic observations of the microvasculature to determine which vessels are affected by the drug. The purpose of this study was to determine the peripheral vascular site(s) of action of SNP by direct observation of the microvasculature. The microvasculature of the cremaster muscle of the rat was studied because of its suitability as a microvascular preparation, and because muscle vascular resistance has been shown to decrease during SNP infusion in other species.

Methods and Materials

Eleven male Sprague-Dawley rats, average weight 125 ± 5 g (SEM), were anesthetized with intraperitoneally administered pentobarbital, 50 mg/kg, initially, supplemented with half the initial dose when necessary in order to maintain satisfactory anesthesia. Tracheostomy was performed and each rat was allowed to breathe oxygen, 40 per cent in nitrogen. Mean arterial pressure (MAP), obtained from a cannula in the left common carotid artery connected to a Statham P23De transducer, was recorded continuously on a polygraph. Heart rate, measured via the ECG, was recorded continuously. The left external jugular vein was cannulated for administration of either SNP or balanced salt solution, infused at a rate of 0.54 ml/hr. SNP was prepared in dextrose, 5 per cent in water, immediately prior to infusion, and the concentration was adjusted to deliver 40 µg/kg/min. The SNP infusion rate was similar to that reported to lower MAP to 40–50 mmHg in anesthetized rats. Body temperature, measured by rectal or esophageal thermistor (Yellow Springs Instrument Co.), was maintained at 36–37.5 C by a heating pad and incandescent lamp.

The left cremaster muscle was exposed through a midline scrotal incision and gently dissected free from subcutaneous tissue. The thin striated muscle was prepared in a manner similar to that described by Baer, irrigated with a warm electrolyte solution, spread over a heated transparent pedestal, and covered with a glass coverslip. Cremasteric temperature was measured continuously and maintained at 34.5–35.5 C. The animal preparation was mounted on the stage of a compound microscope and the cremasteric microvas-
experimental periods separated by at least 30 min between successive administrations of SNP. Each experimental period consisted of a 10-min control period, a 10-min infusion period, and a 10-min post-infusion period. MAP and heart rate were measured each minute and vascular diameters were measured every 30 sec before, during, and after SNP infusion. Average values (mean ± SEM) for MAP, heart rate, and vessel diameters were calculated at each sampling interval and were compared with their respective control values using the Student t test for paired data. Linear regression analysis was performed to correlate the percentage change in vessel diameter for each group with percentage change in MAP.

The effects of topical application of SNP on fourth-order arterioles and on third-order venules were determined in five additional animals. The preparation and protocol were identical to those described above, except that either warmed (35 C) electrolyte solution, or electrolyte solution containing 10^-4 M SNP, was suffused onto the cremaster muscle throughout the experimental period. When no response to SNP was observed, adenosine, 10^-2 M, was applied to confirm reactivity of the microvessels. In these later experiments, the microvascular effects of SNP were determined in the absence of the systemic hypotension that accompanied the intravenous administration of the drug.

Results

Intravenous SNP

Control (before SNP) and experimental (during SNP) heart rate and MAP were compared by analysis of variance for each of the four infusions, and no significant difference was observed. Therefore, data for heart rate and MAP were pooled to obtain an average response for each animal. During 44 infusions in 11 rats, MAP, which averaged 95 ± 3 torr prior to SNP, decreased promptly in response to the drug (fig. 2). Maximum hypotension occurred 2 min after the start of SNP infusion, when MAP averaged 61 ± 2 per cent of control. MAP recovered slightly during the remainder of the infusion and exceeded control values when SNP infusion was discontinued. Four minutes after discontinuation of SNP, MAP was 115 ± 5 per cent of control, and it remained significantly increased during the remainder of the postinfusion period. Heart rate increased slightly but significantly during the last 6 min of SNP infusion, and returned to control values within 3 min after the drug was discontinued. The maximum heart rate response was only 107 ± 1 per cent of control at minute 5 of SNP infusion.
Fig. 2. The effects of intravenously administered SNP (40 μg/kg/min) on heart rate and mean arterial pressure, expressed as percentages (mean ± SEM) of the average control values (ACV), in 11 rats. Heart rate was increased significantly \( (P < 0.05) \) between minutes 15 and 22 (inclusive). Arterial pressure was decreased significantly \( (P < 0.01) \) between minutes 11 and 20, and increased significantly \( (P < 0.01) \) between minutes 22 and 30.

Although there was a slight tendency for first-order arterioles to constrict during infusion of SNP, no statistically significant change was observed (fig. 3). Third-order arterioles tended to dilate during SNP, but again the change (to 105 ± 3 per cent) was not significant. However, these vessels constricted (to 87 ± 4 per cent; \( P < 0.05 \)) when SNP infusion was discontinued.

Fourth-order arterioles dilated (to 124 ± 8 per cent) in response to SNP, but significant dilatation was present only during the initial 4 min of drug infusion. These vessels constricted significantly (to 76 ± 7 per cent) when SNP infusion was discontinued. All fourth-order arterioles dilated initially in response to SNP. Thereafter, the responses could be divided into two subgroups. Subgroup A remained dilated throughout SNP infusion, while the internal diameters of the vessels in subgroup B promptly returned to control after the initial dilatation (fig. 4).

Linear regression analysis revealed a strong correlation \( (r = 0.91) \) between the decrease in arterial pressure and the dilatation observed in fourth-order arterioles. Among these vessels, subgroup A had internal diameters that correlated most closely with MAP \( (r = 0.93) \), whereas internal diameters in subgroup B showed only fair correlation \( (r = 0.51) \). The correlation coefficients for first-order arterioles and third-order arterioles were −0.31 and 0.85, respectively.

Neither first-order nor third-order venular diameters were altered significantly by SNP (fig. 5).

**Topical SNP**

Sodium nitroprusside, \( 10^{-4} \) M, applied topically to the cremaster muscle caused significant arterial dilatation within 30 sec of application, and dilatation persisted for 3.5 min after SNP was removed (fig. 6). In contrast to intravenous administration, neither transient dilatation nor post-SNP constriction occurred with topical application of the drug. Third-order venular diameters were unaltered by the topical application of SNP. However, topical application of adenosine, \( 10^{-2} \) M, produced dilatation to 114 ± 5 per
percent of control ($P < 0.05$), indicating that dilatation was possible in these vessels.

**Discussion**

Since SNP is purported to lower arterial pressure by its direct effect on vascular smooth muscle, there must be additional factors that result in the increased responsiveness of fourth-order arterioles to the drug. Increased drug effectiveness in the smaller arterioles is not unique to SNP. Duling and Berne reported that maximum responses to both acetylcholine and norepinephrine occurred in the precapillary arterioles of the hamster cheek pouch, with decreasing effects in progressively larger arterioles.\(^9\) Furness and Marshall reported a similar distribution of constrictor responses to catecholamines in the rat mesenteric microvasculature.\(^10\) Hutchins and co-workers demonstrated an inverse relationship between resting arteriolar diameter and the dilatation produced by regional intra-arterial isoproterenol in the cremaster microvasculature of both normotensive and hypertensive rats.\(^11\) Finally, Eriksson and Lisander found a similar distribution of drug effectiveness in the cat tenuissimus muscle arterioles following the intra-arterial administration of either acetylcholine or papaverine.\(^12\)

Several factors could account for the increased reactivity of small arterioles to SNP. These would include the relative amounts of vascular smooth muscle in the vessel walls, neural or humoral influences on vascular tone, and the initial wall tension. While any or all of these factors may be important, the relationship between wall tension and vessel radius is an important determinant of vascular reactivity. Gore observed that vascular reactivity was most pronounced in vessels with a calculated wall stress of $1-1.5 \times 10^5$ dynes/cm\(^2\), a value that is found only in the terminal arterioles (approximately 12–25 $\mu$m in diameter) of mammalian microvasculature under resting conditions.\(^13\)

All fourth-order arterioles dilated initially during intravenous infusion of SNP. While dilatation was persistent in some arterioles (Subgroup A), it was only transient in others (Subgroup B). Topical application of SNP to the cremaster muscle permitted an evaluation of the direct effects of the drug in the absence of systemic hypotension. Under these circumstances, SNP always dilated fourth-order arterioles, and neither transient dilatation (as in Subgroup B) nor rebound constriction (after the drug) was observed. The topical-drug studies confirmed that both the transient dilatation and the rebound arteriolar constriction resulted from systemic mechanisms that were activated by the drug-induced hypotension. Baroreceptors are probably activated during deliberate hypotension, and increased sympathetic outflow could account for the

![Fig. 3. The effects of SNP (40 µg/kg/min) on first-order (n = 11), third-order (n = 11), and fourth-order (n = 10) arteriolar diameters, expressed as percentages of control. Numbers in parentheses represent control values (ACV). First and third-order arterioles were not altered significantly by SNP. Fourth-order arterioles dilated significantly ($P < 0.05$) from minute 10.5 through minute 14. Significant ($P < 0.05$) constriction occurred during minutes 21.5 through 29 in fourth-order arterioles.]
Fig. 4. The effects of SNP (40 μg/kg/min) on subgroups A (n = 5) and B (n = 5) of fourth-order arterioles, expressed as percentages of control. Numbers in parentheses represent control values (ACV). Subgroup A dilated significantly (P < 0.05) throughout SNP, while subgroup B dilated only transiently (significant only at 11.5 min).

Fig. 5. The effects of SNP (40 μg/kg/min) on first-order and third-order venular diameters, expressed as percentages of control. Venular diameters were not altered by SNP. Numbers in parentheses represent control values (ACV).
transient arteriolar dilatation and the tachycardia observed during SNP infusion, as well as for the increased MAP and arteriolar constriction that followed administration of the drug. In addition, Miller et al. documented increased plasma renin in rats made hypotensive with SNP, suggesting that the potent vasoconstrictor angiotension II is increased during SNP-induced hypotension. We attempted to further define these compensatory responses in animals with bilateral nephrectomies (to eliminate renin) or in animals with ganglionic blockade produced by pentolinium. During anesthesia, the anephric animals showed a gradual decline in arterial pressure, which always terminated in death. Ganglionic blockade resulted in marked decreases in arterial pressure, so that valid comparisons to the experimental animals were not reasonable. If possible, such experiments would clarify the compensatory mechanisms that are activated during induced hypotension.

Neither intravenous nor topical administration of SNP produced dilatation of small veins in the rat cremasteric microvasculature. In contrast, indirect studies in man suggested that venodilatation was produced by the drug. Species differences could account for the discrepancy in alleged venous effects of SNP, although both the hypotension and the tachycardia reported here closely resemble the human responses to SNP. The cremaster preparation itself does not produce maximum venodilatation and thus obscure the response to SNP, since topically administered adenosine always produces venodilatation in vessels that were not affected by SNP. Gnei and colleagues studied the effects of SNP on isolated bull metacarpal veins and were unable to demonstrate dilatation in the absence of prior constriction produced by norepinephrine.14 Our results are consistent with their findings in veins that were not previously constricted by norepinephrine. The venous effects of SNP in man have usually been observed in patients who either have recently sustained myocardial infarction or have congestive heart failure, conditions that tend to result in increased sympathetic activity and might elicit the venous effects of the drug. Although several investigators have remarked that the decrease in right atrial pressure that accompanies SNP-induced hypotension suggests venodilatation, there is ample reason to reject this assumption. Bohlen and Gore documented that both precapillary and postcapillary pressures are related to systemic arterial pressure by a simple linear relationship.15 Thus, central venous pressure would be expected to decline in response to the decrease in arterial pressure on this basis alone. Finally, the purported venodilatation may occur in other vascular beds and not in striated muscle as studied here. In any event, we did not see venodilatation due to SNP in veins that did dilate in response to adenosine.

In summary, these experiments demonstrated an inverse relationship between resting arteriolar diameter and the amount of vasodilatation produced by intravenous infusion of SNP. The study confirmed the following effects of SNP in the rat cremasteric microvasculature: 1) the primary arteriolar site of action of SNP is the fourth-order arterioles; 2) compensatory mechanisms active during SNP-induced hypotension tend to counteract the arteriolar dilatation produced by the drug; 3) neither intravenous nor topical administration of SNP produced venodilatation.

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References


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