Nitrosyl Factors Mediate Active Neurogenic Hindquarter Vasodilation in the Conscious Rat

Robin L. Davisson, Alan Kim Johnson, Stephen J. Lewis

Abstract Exposure to noxious environmental stimuli such as air-jet stress (AJS) produces a pattern of hemodynamic changes referred to as the "defense reaction." In the rat these changes include a relatively modest increase in mean arterial blood pressure (MAP), tachycardia, renal and mesenteric vasoconstriction, and a marked hindquarter vasodilation. The aim of the present study was to determine whether the AJS-induced decrease in hindquarter resistance is mediated by a sympathetic neurogenic vasodilator system that uses nitric oxide (NO) and/or related nitrosyl factors. AJS produced a small, rapid increase in MAP, which quickly returned to baseline (within 5 seconds), and a substantial increase in hindquarter blood flow and decrease in hindquarter resistance, which occurred almost instantaneously (1 to 2 seconds) and were sustained for at least 30 seconds. The intravenous injection of either bretylium (5 mg/kg), which prevents impulse propagation-mediated release of neurotransmitters/neuromodulators from sympathetic terminals, or Nω-nitro-L-arginine methyl ester (L-NAME, 25 μmol/kg), which blocks NO synthesis, essentially abolished the AJS-induced increase in hindquarter blood flow and fall in hindquarter resistance. In contrast, the hindquarter vasodilation produced by the NO donor sodium nitroprusside (4 μg/kg IV) was markedly exaggerated in the bretylium- or L-NAME-treated rats. We also found that rat lumbar sympathetic fibers projecting to the hindquarter vasculature contain NADPH diaphorase, a marker for NO synthase in paraformaldehyde-perfused tissue. The above findings demonstrate that AJS produces a rapid and sustained increase in hindquarter blood flow, which is independent of changes in MAP, and suggest that these effects are mediated by active sympathetic neurogenic vasodilation involving the release of nitrosyl factors from postganglionic sympathetic neurons and/or the vascular endothelium. (Hypertension. 1994;23[part 2]:962-966.)

Key Words • sympathetic nervous system • hind limb • nitric oxide • Nω-nitro-L-arginine methyl ester • bretylium compounds

Exposure of the rat to a noxious environmental stimulus such as air-jet stress (AJS) elicits a "defense reaction" that includes hemodynamic changes such as a modest increase in arterial blood pressure, tachycardia, vasoconstriction in the renal and mesenteric vascular beds, and a marked vasodilation in the hindquarter bed. This vasodilation of skeletal muscle vasculature plays a vital role in preparing the animal for flight. The mechanisms mediating the AJS-induced changes in hindquarter vascular resistance have not been fully defined. In particular, it is not clear whether this fall in hindquarter resistance (HQR) results from a withdrawal of sympathetic drive, the action of circulating adrenal catecholamines, or the activation of a sympathetic neurogenic vasodilator system. Active neurogenic vasodilation may result from one or a combination of mechanisms including sympathetic nerve (norepinephrine or ATP)-mediated release of vasodilator substances from perivascular nerves, mast cells, or the vascular endothelium or theoretically, a release of vasodilator factors from the postganglionic sympathetic nerves themselves.

There is now substantial evidence that sympathetically derived norepinephrine and ATP will stimulate the release of endothelium-derived nitric oxide (NO) or related nitrosyl factors (NOFs) such as S-nitrosothiols or dinitrosyl iron complexes. As such, it is possible that AJS-induced hindquarter vasodilation may involve the sympathetic nerve-mediated release of NO/NOFs from the vascular endothelium. Several immunohistochemical studies have provided evidence that nitric oxide synthase (NOS) is localized in peripheral autonomic neurons, and Toda and Okamura in 1992 provided functional evidence that nonadrenergic, noncholinergic vasodilator neurons innervating the cerebral and mesenteric vasculature may use NO as their neurotransmitter. However, although NOS has been detected in preganglionic sympathetic nerve terminals in the rat, this enzyme has not been found in sympathetic ganglia or postganglionic fibers. As such, there is no evidence that neurogenically derived NO/NOFs may mediate AJS-induced hindquarter vasodilation.

The aim of the present study was to determine whether the AJS-induced reduction in HQR in conscious rats is mediated by an active neurogenic (sympathetic) vasodilation involving the release of NOFs. We now report that AJS produced an immediate and sustained increase in hindquarter blood flow (HQB), which is independent of changes in blood pressure, and that the resulting decrease in HQR was prevented by inhibition of impulse propagation-mediated release of factors from postganglionic sympathetic nerve terminals by bretylium or inhibition of NO synthesis with Nω-nitro-L-arginine methyl ester (L-NAME). In contrast, the hindquarter vasodilator effects of epinephrine were not altered by either bretylium or L-NAME. We also report that postganglionic lumbar sympathetic nerve fibers projecting to the hind limb vasculature of the rat contain NADPH diaphorase, a marker for NO in paraformal-
dehydro-perfused tissues. These results suggest that AJS-induced hindquarter vasodilation may be mediated by active sympathetic neurogenic vasodilation rather than a withdrawal of sympathetic drive or the actions of adrenal catecholamines. The active neurogenic vasodilation may involve sympathetic nerve-mediated release of NOFs from the vascular endothelium, or alternatively the postganglionic sympathetic nerves themselves may release NOFs.

Methods

In Vivo Studies

All experiments were performed on conscious, freely moving male Sprague-Dawley rats (Harlan, Madison, Wis) weighing 300 to 400 g (n=28). The animals were individually housed in Plexiglas cages and maintained on a daily 12-hour photoperiod with Purina rat food and tap water freely available at all times except during testing procedures.

Surgical Procedure

Rats were anesthetized with ketamine (120 mg/kg) and acepromazine maleate (12 mg/kg) via intraperitoneal injection and surgically implanted with femoral arterial and venous catheters for the measurement of pulsatile (PP) and arterial blood pressure and heart rate (HR) and for the administration of drugs, respectively. Immediately after catheterization, a midline laparotomy was performed, and a miniature pulsed Doppler flow probe was placed around the lower abdominal aorta for the measurement of HQF. The probe was sutured in place, the leads and catheters were tunneled subcutaneously and exteriorized between the scapulae, and the wounds were closed. To protect the probe wires and polyethylene tubing while allowing animals unrestricted movement during recovery and experimental testing, the free ends of the catheters and Doppler leads were led through a stainless steel skin button spring-swivel assembly that was mounted to a ring stand clamp and suspended above the cage. The skin button was attached to the skin incision in the sacral region using stainless steel sutures. Details of the Doppler technique, including construction of the probes, the reliability of the method for the estimation of flow velocity, and quantitative determination of percent changes in HQR, have been described previously.

Protocol

After a 5-day recovery period, animals were connected to a Beckman Dynograph coupled pressure transducer (Cobe Lab, Inc) and Doppler flowmeter (Bioengineering, University of Iowa) for recording HR, PP, and mean arterial pressure (MAP), and for recording HQF, respectively. The AJS consisted of a 1-second standardized burst of compressed air (Tech Duster, Techni-Tool) directed at the top of the head. The effects of AJS on the hemodynamic parameters were determined after intravenous pretreatment with either saline (0.9% NaCl, n=7), the inhibitor of impulse propagation-mediated release of neurotransmitters/neuromodulators from sympathetic nerve terminals bretylium tosylate (5 mg/kg, n=7), or the NO synthesis inhibitor L-NAME (25 μmol/kg, n=6). AJS was applied 10 minutes after reaching steady MAP and blood flow values after drug pretreatment, and cardiovascular responses were measured continuously throughout the administration of and recovery from the AJS. The hypotensive and hindquarter vasodilator effects of the NO donor sodium nitroprusside (4 μg/kg IV, n=20) as well as epinephrine (0.5 mg/kg IV, n=8) were also examined in saline-, bretylium-, and L-NAME-treated rats.

Histochemical Studies

Male Sprague-Dawley rats (n=4) were killed with an overdose of sodium pentobarbital and perfused intracardially with 500 mL of an ice-cold solution of 4% paraformaldehyde in 0.1 mol/L sodium phosphate buffer (pH 7.4) for 1 hour. The lumbar sympathetic chains and iliac arteries were removed, postfixed at 4°C for 1 hour, and rinsed in 0.1 mol/L Tris buffer overnight. Tissues were sectioned at 20 μm and mounted onto Superfrost Plus electrostatically charged slides (Fisher Scientific Co). Sections were then treated at 37°C for 60 minutes with 0.1 mol/L Tris buffer containing 0.3% Triton X-100, 0.1 mg/mL nitroblue tetrazolium, 1.2 mg/mL sodium malate, and 1.0 mg/mL β-NADPH for demonstration of NADPH diaphorase activity.

Drugs

All drugs used in this study were obtained from Sigma Chemical Co, except bretylium tosylate (Research Biochemicals, Inc).

Statistical Analysis

Data are expressed as mean±SEM and were analyzed by repeated-measures ANOVA followed by Student's modified t test with Bonferroni correction for multiple comparisons between means, using the modified error mean square term from the ANOVA.

Results

A summary of the effects of L-NAME and bretylium on baseline HR, MAP, HQF, and HQR values is shown in Table 1. L-NAME produced a significant pressor response and reductions in baseline HR and HQF, resulting in a marked increase in resting HQR. Bretylium caused an immediate "adrenergic effect," including increased HR and MAP and hindquarter vasodilation, because of its initial sympathetic neurotransmitter releasing action. However, the cardiovascu-
FIG 1. Representative tracings show examples of the effects of air-jet stress on heart rate (HR), mean arterial pressure (MAP), pulsatile pressure (PP), and hindquarter blood flow (HQF) of saline (0.9% NaCl IV)-treated, bretylium (5 mg/kg IV)-treated, and N\(^{\text{G}}\)-nitro-L-arginine methyl ester (L-NAME, 25 \(\mu\)mol/kg IV)-treated conscious Sprague-Dawley rats.

lar parameters had returned to preinjection values by 20 minutes (Table 1). Typical examples of the effects of AJS on HR, MAP, PP, and HQF in rats pretreated with either saline (panel A), bretylium (panel B), or L-NAME (panel C) are shown in Fig 1. AJS produced a small increase in HR, a moderate increase in MAP, which quickly returned to baseline (by 5 seconds), and a substantial increase in HQF, which occurred almost instantaneously and was sustained for at least 30 seconds.

The pattern and magnitude of the AJS-induced cardiovascular responses could be elicited repeatedly (at least six episodes given 5 minutes apart), with no diminution of the responses in saline-treated rats (data not shown). In rats pretreated with either bretylium (panel B) or L-NAME (panel C), AJS produced a small transient decrease in MAP but no changes in HQF. This resulted in decreases in HQR similar to those found in saline-treated rats immediately after AJS (1 to 2 seconds) but no falls in HQR at 5 to 10 seconds and 30 seconds, respectively.

A summary of the effects of AJS on HR, MAP, HQF, and HQR in rats pretreated with either saline, bretylium, or L-NAME is shown in Figs 2 and 3, respectively. In saline-treated rats, AJS caused an immediate (1 to 2 seconds) small increase in MAP, which returned to baseline by 5 to 10 seconds, and a marked instantaneous increase in HQF, which was sustained for at least 30 seconds. These changes resulted in significant AJS-induced reductions in HQR at the times examined. After the intravenous injection of bretylium (Fig 2) or L-NAME (Fig 3), AJS produced a small transient hypotension but no changes in HQF. This resulted in decreases in HQR similar to those found in saline-treated rats immediately after AJS (1 to 2 seconds) but no falls in HQR at 5 to 10 seconds and 30 seconds, respectively.

A summary of the hypotensive and vasodilator effects of the NO donor sodium nitroprusside in animals pretreated with saline, bretylium, or L-NAME is shown in Table 2. Sodium nitroprusside caused significantly greater reductions in MAP and HQR in bretylium- and L-NAME-treated rats compared with those pretreated with saline. In contrast, the hindquarter vasodilator effects of epinephrine were not altered by either bretylium (before treatment versus after treatment, \(-37\pm8\% \text{ versus } -30\pm8\%, n=3, P>0.05\)) or L-NAME (before treatment versus after treatment, \(-38\pm5\% \text{ versus } -46\pm5\%, n=5, P>0.05\)).

Histochemical staining of rat lumbar sympathetic nerve fibers for NADPH diaphorase activity is shown in Fig 4. These paraformaldehyde-fixed neurons stained heavily for NADPH diaphorase. In addition, we observed NADPH diaphorase-positive sympathetic nerve terminals but not perivascular nerves in the iliac artery (R.L.D., A.K.J., S.J.L., unpublished data, 1994).

**Discussion**

The present study demonstrates that AJS induces a rapid vasodilation in the hindquarter vasculature of the conscious rat, which is sustained well after the transient AJS-induced increase in MAP subsides. This vasodilation is markedly diminished by blockade of impulse propagation-mediated release of neurotransmitters/neuromodulators from postganglionic sympathetic nerve terminals with bretylium or by inhibition of NO synthesis with L-NAME. Despite the possibility that AJS-induced release of epinephrine from the adrenal glands may contribute to the maintenance of the hind
TABLE 2. Percent Changes in Mean Arterial Pressure, Hindquarter Blood Flow, and Hindquarter Resistance in Conscious Sprague-Dawley Rats Produced by Intravenous Injection of Sodium Nitroprusside (4 µg/kg) Before and After Treatment With Saline, Bretylium, or N°-Nitro-L-Arginine Methyl Ester

<table>
<thead>
<tr>
<th>Parameter</th>
<th>MAP, mm Hg</th>
<th>HQF, kHz</th>
<th>HQR, mm Hg/kHz</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before Saline (0.9% NaCl IV, n=7)</td>
<td>-17±2</td>
<td>-11±4</td>
<td>-5±2</td>
</tr>
<tr>
<td>After Saline (0.9% NaCl IV, n=7)</td>
<td>-19±3</td>
<td>-13±4</td>
<td>-6±2</td>
</tr>
<tr>
<td>Before Bretylium (5 mg/kg IV, n=7)</td>
<td>-18±3</td>
<td>-17±4</td>
<td>-6±3</td>
</tr>
<tr>
<td>After Bretylium (5 mg/kg IV, n=7)</td>
<td>-50±5*</td>
<td>41±7*</td>
<td>-64±9*</td>
</tr>
<tr>
<td>Before L-NAME (25 µmol/kg IV, n=6)</td>
<td>-19±3</td>
<td>-13±4</td>
<td>-6±3</td>
</tr>
<tr>
<td>After L-NAME (25 µmol/kg IV, n=6)</td>
<td>-31±4*</td>
<td>44±7*</td>
<td>-52±9*</td>
</tr>
</tbody>
</table>

L-NAME indicates N°-nitro-L-arginine methyl ester; MAP, mean arterial pressure; HQF, hindquarter blood flow; and HQR, hindquarter resistance. Values are mean±SEM.

*P<.05, after versus before treatment.

limb vasodilation,2 the finding that bretylium, which does not inhibit the release of adrenal catecholamines,11 markedly attenuates the AJS-induced vasodilation probably discounts a major role for circulating epinephrine in this response. Taken together, these results suggest that the AJS-induced hindquarter vasodilation, especially that observed after the transient pressor response, is mediated by an active neurogenic process involving the release of NOFs rather than the baroreceptor afferent-mediated withdrawal of sympathetic tone. The finding that the brief initial pressor response (which is accompanied by transient vasoconstriction of the renal and mesenteric vasculature) is reversed by bretylium suggests that AJS produces a generalized activation of the sympathetic nervous system. The sustained nature of the AJS-induced hindquarter vasodilation may be due to prolonged neurogenic input to the hindquarter vasculature or a brief burst of sympathetic input that causes the release of a vasodilator substance with a biological half-life of at least 30 seconds. As such, it is possible that the factor mediating the vasodilation may not be NO per se but an NOF whose synthesis depends on the production of NO. Interestingly, Gaston et al16 recently demonstrated that the endogenous S-nitrosothiol S-nitrosoglutathione has a half-life of approximately 30 seconds. These studies cannot determine the site(s) from which NOFs may be released by AJS. However, the AJS-induced neurogenic vasodilation may result from sympathetic nerve (norepinephrine or ATP)–mediated release of NOFs from the vascular endothelium or perivascular nerves or from the direct release of NOFs from the postganglionic sympathetic nerves (see below).

Although bretylium or L-NAME virtually abolished hindquarter vasodilation 5 to 10 and 30 seconds after AJS, a significant relaxation occurred in these animals 1 to 2 seconds poststressor. This transient reduction in resistance may be due to incomplete blockade of sympathetic vasodilator mechanisms, coupled with exaggerated responsiveness to the vasorelaxant effects of NOFs. This is supported by the observation that sodium nitroprusside produces a markedly exaggerated hindquarter vasodilation in bretylium- and L-NAME–treated rats. Although the exaggerated hindquarter vasodilation to sodium nitroprusside in bretylium-treated rats may be due to a loss of baroreflex-mediated vasoconstriction, the enhanced vasodilation in L-NAME–treated rats is probably due to an upregulation of vascular smooth muscle soluble guanylate cyclase after the L-NAME–induced inhibition of tonic NO synthesis/release.17 Alternatively, the remaining transient (1 to 2 seconds) AJS-induced hindquarter vasodilation in L-NAME–treated rats may be due to a release of other sympathetically neurotransmitters/neuromodulators such as epinephrine,18 which may cause an NOF-independent vasodilation. Indeed, we found that systemically injected epinephrine causes a profound hindquarter vasodilation that is not altered by either bretylium or L-NAME. These results suggest that neurogenically derived epinephrine may initiate the AJS-induced hindquarter vasodilation but is unlikely to be involved in maintaining the NO-dependent phase of this response.

Our histochemical findings that paraformaldehyde-fixed postganglionic sympathetic fibers innervating the
hindquarter vasculature contain NADPH diaphorase suggest that these neurons may produce NOS. In preliminary studies we confirmed these findings immunohistochemically using a purified antibody specific for the neuronal form of constitutive NOS. Taken together, these findings suggest that active NOF-mediated sympathetic vasodilation may involve one or a combination of three major processes (Fig 5). These processes include (1) a release of NOFs from a subpopulation of postganglionic sympathetic vasodilator neurons, (2) the sympathetic (norepinephrine, ATP)-mediated release of NOFs from perivascular nerves, and (3) the sympathetic (norepinephrine, ATP)-mediated release of NOFs from the vascular endothelium.

Although these in vivo studies cannot in themselves determine which of these mechanisms is involved, our finding that there are virtually no NADPH diaphorase-positive perivascular nerves in the hindquarter vasculature may rule out this possibility, although there may be more of these nerves in the small resistance vessels. We recently completed a series of in vitro studies in which we examined the sympathetic neurotransmitter/neuromodulator-releasing effects of bretylium and tyramine on tension in endothelium-denuded rabbit thoracic aorta precontracted with either prostaglandin E2 or phenylephrine (R.L.D., A.K.J., S.J.L., unpublished data). We found that bretylium and tyramine produced a marked relaxation of the endothelium-denuded vessels and that these effects were diminished by L-NAME and the inhibitor of the activation of soluble guanylate cyclase, and methylene blue, respectively.

The present study suggests that centrally (AJS) mediated activation of the sympathetic nervous system results in active neurogenic hindquarter vasodilation, which may involve the release of NOFs from the endothelium and perhaps from a subpopulation of postganglionic sympathetic neurons. As such, NOFs may play a critical role in mediating the hemodynamic adjustments in response to noxious environmental stimuli.

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References

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R L Davisson, A K Johnson and S J Lewis

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