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Arteriovenous Differences in Plasma Concentrations of Catechols in Rats with Neuropathic Pain

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Background: **Alterations in cutaneous temperature, sweating, and cutaneous blood flow in patients with pain states, such as reflex sympathetic dystrophy and causalgia, have been interpreted as evidence for exaggerated sympathetic outflow. It was determined whether pain behavior in a rat model of sympathetically maintained pain is associated with alterations in regional sympathoneural function.**

Methods: **Peripheral neuropathy was induced in 29 Sprague-Dawley rats by ligation of the left L5 and L6 spinal nerves. Sixteen other rats had sham surgery (nerve exposure without ligation). Animals were tested for behavioral signs of allodynia (decreased paw withdrawal thresholds to mechanical stimuli) at 2 and 4 weeks after the surgery. Arterial and iliac venous blood samples (left, affected; right, control) were obtained at 2 weeks (NP2, n = 14) and 4 weeks (NP4, n = 15) after neuropathic or sham (n = 8 at 2 and 4 weeks) surgery. Plasma concentrations of dihydroxyphenylalanine, dihydroxyphenylacetic acid, dopamine, norepinephrine, and the intraneuronal norepinephrine metabolite, 3,4-dihydroxyphenylglycol, were analyzed in arterial and left and right iliac venous samples.**

Results: **A decrease in paw withdrawal threshold was observed in neuropathic (NP2 and NP4) but not sham-operated rats. Affected and control limbs did not differ in arteriovenous differences in concentrations of dihydroxyphenylalanine, dihydroxyphenylacetic acid, dopamine, or 3,4-dihydroxyphenylglycol. No differences were observed between sham-operated and neuropathic animals in these arteriovenous increments. In contrast, affected limbs of NP2 rats had a reduced arteriovenous increment in norepinephrine concentrations, compared to that in the control side ($P < 0.05$).**

Conclusions: **No neurochemical evidence of sympathetic hyperactivity is observed in the rat model of neuropathic pain; if anything, norepinephrine release is decreased in the affected limb. Autonomic disturbances in neuropathic pain are therefore more likely the result of receptor supersensitivity than increased local sympathoneural traffic. (Key words: Pain, neuropathic: reflex sympathetic dystrophy; sympathetically maintained pain. Sympathetic nervous system: catecholamines; 3,4-dihydroxyphenylglycol; norepinephrine.)**

PERIPHERAL nerve injuries may lead to chronic neuropathic pain states, such as causalgia, which are characterized by sensory and autonomic abnormalities.[1–3] A subset of patients with causalgia and reflex sympathetic dystrophy have a sympathetically maintained pain state that is considered to result from an abnormal interaction between sympathetic efferent and somatic afferent systems.[4–6] Alterations in cutaneous temperature, sweating, and cutaneous blood flow in patients with reflex sympathetic dystrophy and causalgia have been interpreted as evidence for exaggerated sympathetic outflow.[7]

Drummond *et al.* investigated sympathetic outflow indirectly in patients with reflex sympathetic dystrophy by measuring venous concentrations of norepinephrine and its intracellular metabolite, 3,4-dihydroxyphenylglycol (DHPG), in the affected and unaffected extremities.[8] Plasma DHPG and norepinephrine concentrations were less in the painful extremity of patients with allodynia, suggesting that autonomic disturbances in reflex sympathetic dystrophy result not from sympathetic overactivity but from adrenoceptor supersensitivity. These observations are consistent with the lack of mi-

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croneurographic evidence for increased sympathetic outflow in patients with reflex sympathetic dystrophy.[9]

To examine the mechanisms of pain after nerve injury, several animal models of neuropathic pain have been developed.[10–12] Bennett and Xie described a model of painful neuropathy in the rat after chronic constriction injury of the sciatic nerve.[10] These rats were often observed to have pronounced abnormalities in skin temperature.[13] However, Wakisaka *et al.* found that the abnormal skin temperature of the affected paw did not reflect the level of sympathetic vasomotor activity as assessed by norepinephrine histofluorescence.[14] Sympathectomy, before or immediately after nerve ligation, partially reversed the hyperalgesic behavior in this model.[15] Two other rat models of neuropathic pain have been developed with either partial ligation of the sciatic nerve or tight ligation of the L5 and L6 spinal nerves unilaterally.[11,12] Although all three models result in allodynia and hyperalgesia in the distribution of the sciatic nerve, the Kim and Chung model of tight ligation of spinal nerves is considered to result in a more consistent and reproducible nerve injury. Chemical or surgical sympathectomy reversed the pain behavior in the latter two models of neuropathic pain.[16,17] Hence, these animal models of neuropathic pain have features that resemble sympathetically maintained pain states in humans.

We wished to determine, in a rat model of neuropathic pain that is considered to be sympathetically maintained, whether pain behavior is associated with alterations in regional sympathoneural function, as evidenced by differences in arterial and regional venous concentrations of catechols. Concentrations of different catechols in arterial and venous plasma reflect related but distinct aspects of sympathoneural function.[18] Arteriovenous differences in concentrations of dihydroxyphenylalanine (DOPA) and dihydroxyphenylacetic acid (DOPAC) provide indexes of regional synthesis of catecholamines. Arteriovenous differences of norepinephrine reflect more directly exocytotic release of norepinephrine from the sympathetic terminals. Plasma DHPG reflects intraneuronal metabolism of norepinephrine. Regional spillover of catechols into the circulation can be influenced by local blood flow. Hence, comparisons of blood flow in the injured and control limbs were made.

Methods and Materials

Peripheral neuropathy was induced by ligation of the left L5 and L6 spinal nerves, as described by Kim and Chung,[11] in 29 Sprague-Dawley rats (150 to 200 g). Sixteen additional rats had sham surgery, *i.e.,* nerve exposure but no ligation. The animals were tested for behavioral signs of allodynia at 2 and 4 weeks after the nerve ligation or sham surgery. Under general anesthesia with halothane (inspired concentration of 1%), arterial blood samples (0.3–0.5 ml) were obtained from the abdominal aorta or axillary artery. Venous blood samples were obtained from right (control side) and left (affected side) iliac veins at 2 weeks (NP2, n = 14) or 4 weeks (NP4, n = 15) after neuropathic or sham (n = 8 at 2 and 4 weeks) surgery. Blood samples were obtained by cannulating the inferior vena cava 3–5 mm above the junction of the iliac veins, using a 22-G cannula and directing the catheter caudally into the iliac veins. The order of sampling from the left and right iliac veins was randomized. Regional blood flow, measured using radiolabeled microspheres, was compared in the left and right hind paws in six other rats 2 weeks after neuropathic surgery.

Behavioral Tests. Paw withdrawal thresholds to mechanical stimuli were determined in both hind paws of sham and neuropathic animals. An ascending series of von Frey filaments was applied to the plantar surface of the rat paw, and paw elevation or licking was considered a withdrawal response. The von Frey filament just eliciting a consistent response—withdrawal with at least two of three stimuli—was considered the threshold. The mean of three threshold tests was determined.

Biochemical Assays for Catechols. Simultaneous measurements of plasma concentrations of DOPA, DOPAC, dopamine, and norepinephrine and its metabolite, DHPG, were made in the arterial and venous plasma from the nerve-injured and control extremities. A schematic representation of the synthesis and fate of norepinephrine at sympathetic nerve terminals is shown in figure 1. The sources of catechols in venous blood also are indicated. Plasma concentrations of catechols were assayed by batch alumina extraction, followed by liquid chromatography with electrochemical detection.[19,20] The validity and sensitivity of the assay technique were reported previously by us.[19,20] For a sample volume of 0.3–0.5 ml, the limits of detection would be about 20 pg/ml for DOPA, DHPG, and norepinephrine; 50 pg/ml for dopamine; and 100 pg/ml for DOPAC.

Regional Blood Flow Studies. Previous studies indicated that the local blood flow influences the regional spillover of norepinephrine.[21–23] In general, increased flow due to vasodilation is associated with increased spillover of catecholamines and decreased arteriove-

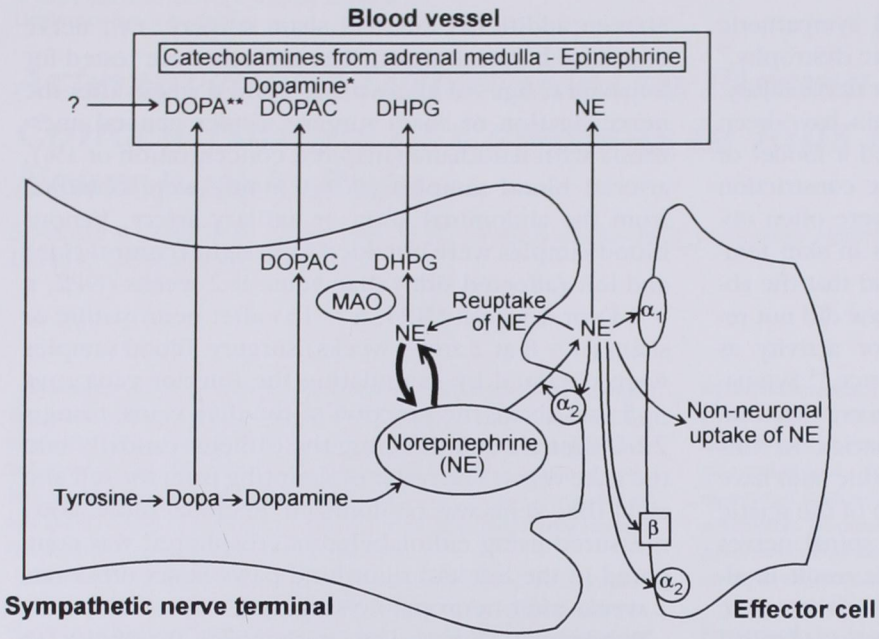

Fig. 1. Schematic representation of synthesis and fate of norepinephrine at sympathetic nerve terminals. The source of catechols in venous blood is indicated. DHPG = 3,4-dihydroxyphenylglycol; DOPA = dihydroxyphenylalanine; DOPAC = dihydroxyphenylacetic acid; MAO = monoamine oxidase; α_1, α_2, and β = adrenoceptors. *Sources of free dopamine in plasma are less well understood. **Plasma DOPA is, at least in part, derived from sympathetic nerves. However, other sources for circulating DOPA have been suggested. (See reference 18 for details.)

nous differences in plasma norepinephrine concentrations. Regional blood flow was measured, using radiolabeled microspheres, in the injured and control limbs of six neuropathic rats 2 weeks after surgery.

Rats were anesthetized with halothane and tracheally intubated, and their lungs were mechanically ventilated (Harvard Rodent Ventilator model 683, Southnatick, MA). Anesthesia was maintained with 1.25% halothane (inspired concentration) throughout the experiment. Polyethylene catheters (PE-50) were inserted into the tail artery for monitoring blood pressure, into the right axillary artery for obtaining the microsphere reference sample, into the right jugular vein for blood replacement, and into the right axillary vein for phentolamine administration. Another catheter (PE-10) was placed into the left cardiac ventricle *via* the right carotid artery for microsphere injection. Arterial pressure transducer was referenced to the atrium. Pressure was measured with a Statham P23 Db transducer (Oxnard, CA) and recorded continuously on a polygraph (Gould-Brush, Cleveland, OH). Rectal temperature was monitored and maintained at 38.5 ± 1.1°C with a heating blanket. Arterial blood *p*H, p_{O_2}, and p_{CO_2} were measured at 37°C immediately after withdrawal using a Radiometer ABL3 analyzer (Copenhagen, Denmark). Arterial oxygen saturation, hemoglobin, and oxygen content were measured spectrophotometrically with a Radiometer Hemoximeter OSM3. The animals' arterial *p*H, p_{CO_2}, p_{O_2}, hemoglobin, and oxygen saturation were maintained within normal limits during the blood flow studies (*p*H 7.41 ± 0.02, p_{CO_2} 37 ± 1 mmHg, p_{O_2} 150 ± 6 mmHg, hemoglobin 12.6 ± 1 G/dl, oxygen saturation 97 ± 0.1%).

Blood flow was measured with microspheres (15 μm in diameter) labeled with either ^{153}Gd or ^{113}Sn (Dupont-NEN Products, Boston, MA) in random sequence. A dose of approximately 3×10^5 microspheres suspended in 0.45 ml of saline was injected into the left ventricle over a 1-min period. The microsphere reference sample was withdrawn from the right axillary artery at a rate of 0.7 ml/min starting 30 s before the microsphere injection and ending 1.5 min after the injection was completed (Harvard Infusion/Withdrawal Pump model 940, Milles, MA). Transfusion of blood from a donor rat anticoagulated with heparin was started at 0.5 min after microsphere injection. The blood from the donor rat was infused into the right jugular vein at a rate of 0.7 ml/min simultaneously with the withdrawal of arterial reference blood. At the end of the experiment, the heart was arrested by potassium chloride injection. The hind limbs were cut proximally and divided into skin, muscle, bone, and hind paw. The kidneys also were excised. Radioactivity in tissue and blood was measured in a gamma scintillation counter, and counts were corrected for spectral overlap of isotopes. Blood flow was calculated as the product of corrected tissue counts and the arterial reference withdrawal rate divided by counts in the arterial

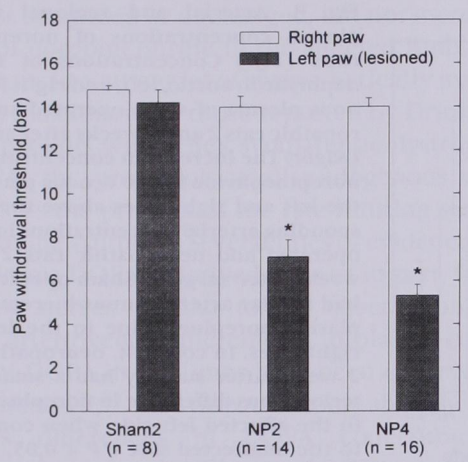

Fig. 2. Paw withdrawal thresholds to mechanical stimuli in sham-operated and neuropathic rats. A decrease in paw withdrawal threshold was observed in the nerve-injured side of neuropathic rats (*left*) at 2 and 4 weeks after surgery. The neuropathic rats thus exhibited allodynia to mechanical stimuli that persisted for at least 4 weeks. *Right *versus* left; $P < 0.05$; 1 bar = 10^6 dynes/cm^2 or 10^3 g/cm^2; SHAM2, NP2, and NP4 correspond to sham-operated and neuropathic rats 2 and 4 weeks after surgery.

reference sample. At least 15 min after completion of surgery, blood flow was measured (baseline). Blood flow studies were repeated 10 min after phentolamine (2 mg/kg intravenous over 5 min) administration.

Data Analysis. The results of the behavioral data and the plasma concentrations of catechols in sham and neuropathic animals were compared using analyses of variance. A P value less than 0.05 was considered significant. Differences between sham and neuropathic animals were further analyzed using Duncan's multiple comparisons. Comparisons between the nerve-ligated and control limbs within each group were made using paired t tests. All data are expressed as mean ± SEM.

Results

Behavioral Studies. A unilateral decrease in paw withdrawal threshold was observed in the nerve-ligated side of neuropathic rats at 2 and 4 weeks after surgery (fig. 2). The neuropathic rats thus exhibited allodynia to mechanical stimuli.

Biochemical Assays. The concentrations of DOPA, DOPAC, dopamine, and DHPG in the arterial and venous plasma of sham-operated and neuropathic rats are presented in table 1. Arterial concentrations of DOPA, DOPAC, and dopamine were similar. Affected (left) and control (right) limbs of sham-operated and neuropathic animals (NP2 and NP4) were similar in arteriovenous differences in concentrations of DOPA, DOPAC, and dopamine.

Concentrations of norepinephrine in the arterial plasma of sham-operated and neuropathic animals were similar (fig. 3). Sham-operated rats had similar arteriovenous differences in plasma norepinephrine concentrations in the left and right sides. The arteriovenous

Table 1. Arterial and Venous Plasma Concentrations of Catechols in Sham-operated and Neuropathic Rats

	Sham 2 wk	NP 2 wk	Sham 4 wk	NP 4 wk
DOPA (pg/ml)				
Aortic	651 ± 52	539 ± 21	682 ± 20	586 ± 18
Rt iliac vein	738 ± 31	635 ± 21	786 ± 23	687 ± 24
Lt iliac vein	726 ± 37	621 ± 23	814 ± 29	668 ± 15
DOPAC (pg/ml)				
Aortic	1,316 ± 159	1,322 ± 112	1,231 ± 27	1,157 ± 80
Rt iliac vein	1,757 ± 144	1,709 ± 91	1,517 ± 38	1,527 ± 118
Lt iliac vein	1,609 ± 152	1,581 ± 117	1,410 ± 49	1,459 ± 75
Dopamine (pg/ml)				
Aortic	29 ± 26	28 ± 8	38 ± 7	30 ± 7
Rt iliac vein	37 ± 14	51 ± 13	83 ± 12	55 ± 11
Lt iliac vein	50 ± 18	52 ± 15	61 ± 12	39 ± 11
DHPG (pg/ml)				
Aortic	1,260 ± 204	1,356 ± 128	1,202 ± 48	1,204 ± 55
Rt iliac vein	1,825 ± 183	2,083 ± 175	1,662 ± 60	1,734 ± 95
Lt iliac vein	1,686 ± 183	1,888 ± 195	1,725 ± 111	1,659 ± 89

Lt = left (nerve-injured side); Rt = right (control side); DOPA = dihydroxyphenylalanine; DOPAC = dihydroxyphenylacetic acid; DHPG = dihydroxyphenylglycol; NP 2 wk and NP 4 wk = neuropathic rats 2 and 4 weeks, respectively, after surgery.

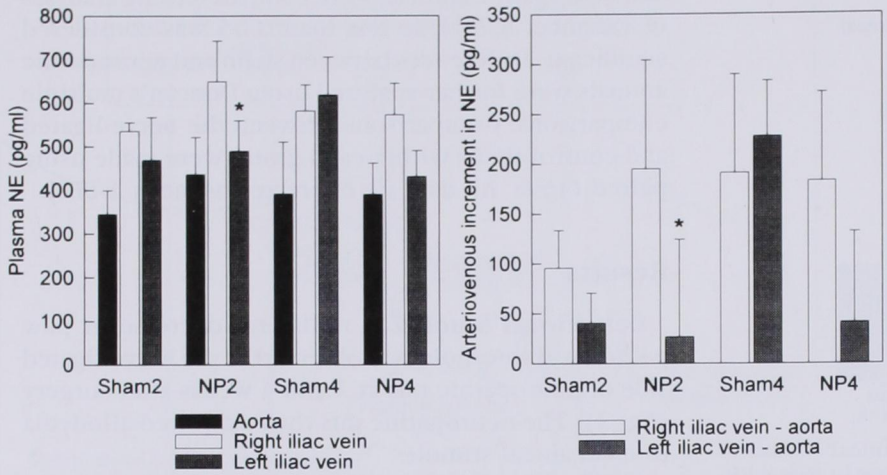

Fig. 3. Arterial and regional venous plasma concentrations of norepinephrine. *(Left)* Concentrations of norepinephrine in aortic, left, and right iliac venous plasma of sham-operated and neuropathic rats 2 and 4 weeks after surgery. *(Right)* The increase in concentrations of norepinephrine in the venous plasma on the left and right sides above the corresponding arterial concentrations in sham-operated and neuropathic rats 2 and 4 weeks after surgery. Sham-operated rats had similar arteriovenous increments in plasma norepinephrine in the left and right sides. In contrast, neuropathic rats 2 weeks after surgery had a smaller arteriovenous difference in norepinephrine in the affected left side when compared to the unaffected side (*$P < 0.05$, left *vs.* right side). These data suggest decreased exocytotic release of norepinephrine in the affected limb.

differences in concentrations of norepinephrine in the control limb of neuropathic rats were not significantly different from those in sham-operated animals. In contrast, neuropathic rats 2 weeks after surgery had a smaller arteriovenous increment of norepinephrine in the affected side when compared to the unaffected side ($P < 0.05$). The arteriovenous difference in norepinephrine was normalized in the neuropathic rats 4 weeks after surgery.

Arterial and venous DHPG concentrations were similar in the sham-operated and neuropathic rats, and the arteriovenous differences were similar in the affected and unaffected sides (table 1).

Blood Flow Studies. Blood flows to the skin, muscle, bone, paw, and the entire hind limb were similar in the nerve-ligated and the control limbs of the neuropathic rats (table 2). In addition, the renal blood flows were similar and within the normal range, indicating good mixing of the microspheres. The increase in blood flow to the muscle, bone, and hind paw after systemic administration of phentolamine was similar in the nerve-ligated and control limbs. Although there was a trend toward a lower blood flow to the nerve-injured hind paw when compared to the control paw, these values were not statistically significant.

Discussion

In an animal model of sympathetically maintained pain, there was a smaller increase in arteriovenous concentrations of plasma norepinephrine in the affected side 2 weeks after nerve injury when compared to the unaffected side. These differences were, however, normalized at the 4-week period. These results

Table 2. Tissue Blood Flow (ml · min^{-1} · 100 g^{-1}) in the Nerve-injured (Left) and Control (Right) Hind Limbs

Tissue (N = 6)	Left (baseline)	Left (postphentolamine)*	Right (baseline)	Right (postphentolamine)
Skin	5.9 ± 0.6	5.6 ± 0.5	5.9 ± 0.4	5.7 ± 0.3
Muscle	6.6 ± 0.4	19.6 ± 2.3†	6.2 ± 0.6	22.1 ± 3†
Bone	18.1 ± 0.8	29.3 ± 3.3‡	18.8 ± 1.2	27.9 ± 3.3‡
Hind paw	8.1 ± 1.2	36.4 ± 12.8‡	11.6 ± 2.1	48.8 ± 15‡
Hind limb	8.3 ± 0.1	20.3 ± 2.4†	8.2 ± 0.5	23.4 ± 3.0†
Kidney	376 ± 25	331 ± 37	362 ± 24	316 ± 31

* A 2-mg/kg dose of phentolamine was administered iv over 5 min after the baseline blood flow measurements and the second set of measurements was made 10 min later.

† $P < 0.005$, postphentolamine *versus* baseline.

‡ $P < 0.05$, postphentolamine *versus* baseline.

suggest decreased or unchanged, but not increased, release of norepinephrine in the affected limb. Our observations in a rat model of sympathetically maintained pain are analogous to those reported by Drummond *et al.* in patients with reflex sympathetic dystrophy.[8]

DOPA, the precursor of all endogenous catecholamines, is the product of the rate-limiting step in catecholamine synthesis. Several lines of evidence indicate that plasma DOPA is derived, at least in part, from sympathetic nerves. Similar to the observations in this study, arteriovenous increments in plasma concentrations of DOPA in the limbs have been observed in rats and humans,[24,25] and sympathectomy abolishes arteriovenous differences in DOPA concentrations.[24,25] Changes in regional spillover of DOPA, therefore, are considered to provide an index of changes in regional synthesis of norepinephrine in sympathetic nerve terminals.[18] Because the arteriovenous differences in concentrations of DOPA were similar in the nerve-injured and control limbs of neuropathic rats, the synthesis of norepinephrine seems to be unaffected in this model.

DOPAC is a deaminated metabolite of dopamine (fig. 1). Plasma DOPAC is derived, at least partly, from metabolism of dopamine in sympathetic nerves. Thus, inhibition of catecholamine synthesis by α-methyl tyrosine markedly decreases plasma DOPAC concentrations,[25] and patients with pure autonomic failure have low concentrations of plasma DOPAC.[26] The lack of differences in the arterial and venous concentrations of DOPAC between sham-operated and neuropathic animals is consistent with DOPA data and also suggests that norepinephrine synthesis is unaffected in the neuropathic extremity.

Sources of plasma-free (unconjugated) dopamine and basis for changes in regional dopamine concentrations are poorly understood.[18,27] The current results showed consistent arteriovenous differences in plasma dopamine, which agree with the notion that the increments reflect release of dopamine from sympathetic terminals. Although no significant effects of nerve injury on these differences were noted for dopamine, the concentrations were quite low—often at or less than the limit of detection of the assay—and there was substantial interindividual variability in the data. Thus, the dopamine results cannot be applied to the main hypothesis of the current study.

Plasma norepinephrine concentrations indirectly reflect sympathetically mediated exocytosis, whereas plasma epinephrine concentrations mainly reflect adrenomedullary secretion.[18] Several clinical studies have used venous plasma concentrations of norepinephrine, the principal neurotransmitter of the sympathetic nervous system, as an index of overall sympathetic "activity."[28–30] In an innervated vascular bed, the regional arteriovenous increment in norepinephrine concentrations probably mainly reflects exocytotic release of norepinephrine from local sympathetic nerve terminals. In general, venous concentrations of plasma norepinephrine agree well with directly recorded sympathetic neural activity.[31,32] Plasma norepinephrine concentrations should be interpreted with caution, however. For example, plasma norepinephrine concentrations depend not only on the rate of exocytotic release from the sympathetic terminals but also on the rate of reuptake of norepinephrine back into the terminals. We observed a decrease in the regional arteriovenous difference in norepinephrine in the affected limb of neuropathic rats. This is unlikely to result from alterations in reuptake, as one would have to postulate an increase in norepinephrine uptake by the sympathetic terminals.

Circulating concentrations of norepinephrine also are influenced by anesthetics and regional blood flow.[22,33] Unlike other anesthetic agents, such as pentobarbital, ketamine, and diethylether, halothane minimally affects plasma catechol concentrations.[33] In addition, it is unlikely that the anesthetic would have differential effects on one extremity. Direct measurements under halothane anesthesia failed to demonstrate regional blood flow differences between nerve-ligated and control limbs. The effects of halothane on blood flow in a neuropathic extremity, however, have not been determined. Hence, a differential effect of halothane on the blood flow to the nerve-injured extremity cannot be excluded as a confounding factor influencing the regional release of norepinephrine.

A potential limitation of this study is that, if altered sympathoneural function is limited to a small region of the extremity, such as the paw (10% of extremity mass), measuring regional changes in catechol concentrations in the venous effluent of the extremity may not be a sensitive index. In the rat, the uninjured spinal nerve (L4) innervates not only the foot but also a significant portion of the dorsum of the extremity. It is unlikely that changes in sympathetic activity in the intact nerve after ligation of the L5 and L6 spinal nerves would be restricted to the paw. Clinically, the region of pain, altered cutaneous sensitivity, and autonomic changes usually are not localized to the distribution of an injured nerve but often are of a "glove and stocking"

distribution. A localized region of sympathetic hyperactivity may be missed in this study. However, such a possibility does not explain the observations of a decreased norepinephrine exocytosis in the nerve-ligated extremity.

Wakisaka *et al.* observed a gradual loss of norepinephrine-containing sympathetic efferent fibers starting within a week after nerve injury.[14] Chung *et al.* reported a similar decrease in sympathetic fibers in the sciatic nerve, using the same model as in this study.[33] Thus, it is surprising that, despite a possible decrease in the number of sympathetic fibers innervating the neuropathic extremity, the values for neurochemical indices of catecholamine synthesis were similar in the control and neuropathic limbs. A possible explanation is that the neuropathic injury results in a compensatory increase in catecholamine synthesis in the remaining fibers.

A significant portion of the norepinephrine released from the sympathetic terminals is taken up by the sympathetic neurons and subsequently either restored or metabolized by monoamine oxidase to form DHPG (fig. 1). During sympathetic activation, increases in plasma norepinephrine concentrations are associated with increases in plasma DHPG concentrations.[34,35] The spillover of DHPG into the blood reflects intraneuronal metabolism of norepinephrine in the sympathetic nerve terminals. In the current study, the concentrations of DHPG were similar in the venous plasma from the affected and unaffected sides, indicating that the overall turnover of norepinephrine was not affected in the neuropathic rats. Thus, there was a discrepancy between the decreased arteriovenous increment in norepinephrine concentrations in the neuropathic limb and the lack of decrease in the arteriovenous increment in DHPG concentrations. This discrepancy can be explained by the previous observations that plasma DHPG is derived from two sources.[18] Intraneuronal leakage of norepinephrine from the storage vesicles is the main source of plasma DHPG, and norepinephrine reuptake from the extracellular fluid is only a minor source under resting conditions. Thus, whereas the norepinephrine data indicated decreased sympathetically mediated exocytosis, which could occur if sympathetic axons were damaged and nerve traffic decreased, the DHPG, DOPA, and DOPAC data indicated approximately normal overall turnover and synthesis of norepinephrine in the sympathetic terminals. The neurochemical findings therefore suggest that the nerve injury does not result in increased nerve traffic, and the sympathetic terminals are intact. The study, however, was confined to measurements of catechols in the resting, anesthetized state and did not investigate possible differences in regional responses to stress.

Tissue blood flow studies revealed a trend toward decreased flow in the nerve-injured paw when compared to the control hind paw. Phentolamine was administered systemically to the rats to examine possible bases for asymmetry in blood flow, if it existed. However, the results revealed a similar increase in blood flow in both paws after the phentolamine administration.

Our observations in the neuropathic rat are consistent with previous studies in patients with reflex sympathetic dystrophy that failed to demonstrate an increase in sympathetic "activity" in the affected region. Neurophysiologic recordings of sympathetic outflow to the affected skin have revealed normal responses to arousal stimuli.[9,36,37] Patients with reflex sympathetic dystrophy do not have increased venous norepinephrine concentrations in the affected limb.[38] In addition, reflex vasoconstrictor responses in the symptomatic extremity seem reduced rather than increased, suggesting sympathetic denervation.[39,40] The above have led to the postulate that receptor supersensitivity, rather than excessive norepinephrine release, may be the mechanism for the hyperalgesia and autonomic disturbances associated with sympathetically maintained pain.[8,41] Arnold *et al.* provided direct evidence for increased responsiveness of venous α adrenoceptors to exogenously administered norepinephrine in patients with reflex sympathetic dystrophy.[42] Another explanation for the current findings and previous reports is that the nerve injury results in partial denervation, with compensatory increases in synthesis in the remaining terminals. The denervation could augment vasoconstrictor responses, not only by upregulation of postsynaptic receptors but also by decreasing neuronal reuptake of catecholamines in the sympathetic neuroeffector junctions. This would help to explain the lack of side-to-side differences in blood flow after phentolamine administration in animals with unilateral nerve injury.

In conclusion, the rat model of neuropathic pain resembles the clinical condition of sympathetically maintained pain states such as reflex sympathetic dystrophy. In both, regional venous concentrations of indexes of norepinephrine synthesis, turnover, reuptake, and release are not increased; the current results indicated decreased exocytosis of norepinephrine. These observations suggest that the autonomic disturbances

in patients with neuropathic pain states are more likely to result from sympathetic denervation than from sympathetic hyperactivity.

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