Enhanced neurally evoked responses and inhibition of norepinephrine reuptake in rat mesenteric arteries after spinal transection

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Brock, James A., Melanie Yeoh, and Elspeth M. McLachlan. Enhanced neurally evoked responses and inhibition of norepinephrine reuptake in rat mesenteric arteries after spinal transection. Am J Physiol Heart Circ Physiol 290: H398–H405, 2006. First published September 2, 2005; doi:10.1152/ajpheart.00712.2005.—In patients with severe high cord lesions, resting arterial blood pressures are usually low immediately after the injury, presumably because of a marked decrease in ongoing sympathetic activity below the lesion (28, 30, 33). Partial recovery of arterial blood pressure during the first few weeks after the injury is thought to be due to adaptation of the renin-angiotensin system (21). However, patients with lesions above T5 also develop autonomic dysreflexia (15). In the present study, we have investigated the effects of spinal transection at T4 on neuroeffector responses of isolated second-order mesenteric arteries. In the rat, spinal transection at T4 removes virtually all bulbospinal inputs to the sympathetic preganglionic neurons supplying the rat tail artery, markedly enhanced the amplitude and duration of contractile responses of isolated segments of this cutaneous artery to sympathetic nerve stimulation in vitro (35). When the preganglionic neurons that project to the rat tail artery were silenced (decentralized) by transection of their preganglionic inputs, neurovascular transmission was similarly augmented (36). These findings show that a decrease in sympathetic nerve activity after spinal cord injury leads to hyperreactivity of the cutaneous vasculature to neural activation. The brief reflex sympathetic bursts initiated in spinal patients (33) were associated with >30-s decreases in cutaneous blood flow. In normal subjects, similar bursts of activity decrease skin blood flow for ~10 s, indicating that reflex vasoconstriction is prolonged in the skin of patients with spinal lesions. This change would play a major role in the genesis of autonomic dysreflexia, if it applies in many vascular beds below the lesion.

In patients, autonomic dysreflexia is normally not expressed unless the lesion is above T6, i.e., when the loss of baroreflex control includes most of the splanchnic vascular bed. Rats with spinal transection at or above T4 also develop autonomic dysreflexia (15). In the present study, we have investigated the effects of spinal transection at T4 on neuroeffector responses of isolated second-order mesenteric arteries. In the rat, spinal transection at T4 removes virtually all bulbospinal inputs to the sympathetic preganglionic neurons supplying the splanchnic vascular bed while damaging <5% of these neurons directly (2, 29).

Mesenteric arteries in the rat are innervated by axons that originate from sympathetic postganglionic neurons and peptidergic primary afferent neurons with cell bodies in dorsal root ganglia (14, 19). Electrical activation of sympathetic terminals produces contraction of second-order mesenteric arteries through the combined action of norepinephrine (NE) at α1-adrenoceptors and ATP at P2X1 purinoceptors (3, 12, 27). In contrast to the rat tail artery (35), α2-adrenoceptors do not contribute to the postjunctional actions of released NE (3, 27). Calcitonin gene-related peptide released from the activated primary affer-
ent axons opposes the effects of sympathetic axon activation
(1). When investigating the effects of spinal transection on the
mechanical responses to sympathetic nerve activation, we
therefore pretreated the arteries with capsaicin to avoid the
effects of activating the afferent axons. We determined the
effects of the \(\alpha_1\)-adrenoceptor antagonist prazosin and the
P2-purinoceptor antagonist suramin on neurally evoked con-
tractions. In addition, we evaluated the effects of spinal tran-
section on responses of the mesenteric arteries to the \(\alpha_1\)-
adrenoceptor agonists phenylephrine (PE) and methoxamine,
to the P2X-purinoceptor agonist \(\alpha_1\)-methylene ATP (\(\alpha_1\)-mATP),
and to depolarization evoked by raised extracellular K\(^+\) con-
centration (\(K^{+}\)o).

MATERIALS AND METHODS

All experimental procedures conformed to the Australian Code of
Practice for the Care and Use of Animals for Scientific Purposes and
were approved by the University of New South Wales Animal Care
and Ethics Committee.

The spinal cord was transected in female inbred Wistar rats (~7 wk
of age) under anesthesia with intraperitoneally injected ketamine (60
mg/kg) and xylazine (10 mg/kg). After infiltration of the back muscles
with 0.3 ml of the long-acting local anesthetic bupivacaine hydrochlo-
ride (5 mg/ml; Marcain, AstraZeneca), the spinal cord was exposed by
a laminectomy over the T4 segment, and the cord was cut with fine
scissors. A piece of gelatin foam was placed between the cut surfaces
and another over the laminectomy site before oxytetraycline powder
was applied and the incision was closed. Warm saline (\(\approx 2\) ml, 0.9%
NaCl) was injected intraperitoneally, and oxytetracycline (100 mg/kg)
and benzylpenicillin (90 mg/kg) were injected subcutaneously.
The urinary bladder was emptied before and at frequent intervals after
spinal transection. The animals were operated on a heating blanket;
respiration and rectal temperature were monitored frequently until the
animals recovered consciousness. After surgery, the animals recov-
ered quickly, moving about their cages within 2 h and eating and
drinking wool onto the next day. Urinary bladders were emptied
manually at least four times a day for the first 1–2 wk until they
emptied automatically, and the animals were then monitored at least
twice a day. The animals (n = 25) were caged in groups of two to four
and maintained for 7.5 wk (SD 0.6). During this period, the animals
appeared healthy; they recovered body weight over the 1st wk and
gained weight thereafter. In sham-operated controls, the laminectomy
was performed to expose the spinal cord. The sham-operated animals
(n = 19) were maintained for 7.0 wk (SD 0.6). In addition, unoperated
animals (n = 25) age matched to the spinalized and sham-operated
animals were also used as controls.

The animals were exsanguinated under deep anesthesia with pen-
tobarbitone (80–100 mg/kg ip), and the ileum and attached mesentery
were removed and maintained in physiological saline of the following
composition (in mM): 150.6 Na\(^+\), 4.7 K\(^+\), 2 Ca\(^{2+}\), 1.2 Mg\(^{2+}\), 144.1
Cl\(^{-}\), 1.3 H\(_2\)PO\(_4\), 16.3 HCO\(_3\), and 7.8 glucose. The solution was
gassed with 95% O\(_2\)-5% CO\(_2\) (to pH 7.2) and warmed to 35–36°C.
Second-order arteries supplying the distal 20 cm of ileum (measured
from the point of entry into the cecum) were isolated.

Mechanical responses. Segments of second-order mesenteric artery
(1.15–2.00 mm long [1.55 mm (SD 0.17)] were mounted isometrically
between stainless steel wires (50 \(\mu\)m diameter) in a four-chamber
myograph (Multi Myograph model 610M, Danish Myo Technology).
Each chamber of the myograph contained 6 ml of physiological saline
that was exchanged at intervals of 6–15 min throughout the recording
period. To normalize the basal conditions and the isometric contrac-
tions, Laplace’s equation was used to convert the measured force to
the effective pressure exerted on the luminal surface of the artery (24).
Initially, the inner circumference at a distending pressure of 13.3 \(\times\)
10\(^3\) N/m\(^2\) (100 mmHg) was determined, and the circumference was
adjusted to 90% of that determined at 13.3 \(\times\) 10\(^3\) N/m\(^2\). At this
setting, mesenteric arteries are at the peak of their length-force
relation (24). Output from the myograph was recorded by using a
PowerLab recording system (ADInstruments).

After they were mounted, the vessels were allowed to equilibrate
for 30 min; during this period all arteries were treated with 1 \(\mu\)M
capsaicin for 10 min to remove the effects of the peptidergic primary
afferent axons (10). The vessels were then exposed to four 6-min
applications of 3 \(\mu\)M PE, each separated by an 8-min washout
interval. During this period, the amplitude of the contractions to each
successive application of PE increased to reach a plateau level.

Electrical stimulation. Electrical stimuli (0.2-ms pulse width, 30 V)
supplied by a Grass SD9 stimulator were applied through platinum
plate electrodes mounted on either side of the artery along its length.
In preliminary experiments, these stimuli produced supramaximal
responses that were abolished by 0.5 \(\mu\)M tetrodotoxin, indicating that
the electrically evoked contractions were due entirely to release of
neurotransmitter from the perivascular nerves.

The arteries were stimulated with single trains of 100 stimuli at 1,
2, 3, and 5 Hz, with each train separated by a 5-min interval. These
long trains of stimuli were chosen to mimic the effects of ongoing
nerve activity in vivo. In arteries from unoperated animals, contrac-
tions to stimulation frequencies \(<1\) Hz were too small to measure
accurately.

In the experiments investigating the effects of blocking \(\alpha_1\)-adreno-
ceptors and P2 purinoceptors, the tissues mounted in two chambers
were stimulated at 4-min intervals with trains of 20 stimuli at 10 Hz,
with the averaged response to the third and fourth trains being used for
control data. These trains of relatively high-frequency stimuli were
used because they produced fast transient contractions of sufficient
amplitude to enable accurate assessment of the effects of antagonists.
After the fourth train of stimuli, the \(\alpha_1\)-adrenoceptor antagonist
prazosin (10 nM) was added to one chamber and the P2X purino-
ceptor antagonist suramin (0.1 mM) was added to the other. Each drug
was left in contact with the tissues for four trains of stimuli, and the
response to the fourth train was used to determine its effect. Both
tissues were then exposed to a combination of prazosin and suramin
at these concentrations for another four trains of stimuli, with the
response to the last train used to determine the combined effect of
both agents.

Chemical stimulation. Noncumulative concentration-response curves
for the \(\alpha_1\)-adrenoceptor agonists PE (0.01–30 \(\mu\)M) and methoxamine
(0.01–30 \(\mu\)M) were determined by applying concentrations that in-
creased by half-log increments, with the tissue exposed to each
concentration for 5 min and then washed for 7 min before the addi-
tion of the next concentration. During the wash, the tissue relaxed
completely to basal tension. In one set of experiments, concentration-
response curves to PE were determined in the presence and absence
of desmethylimipramine (DMI) to determine the effects of blocking the
neuronal NE transporter. In another set of experiments, after the
highest concentration of PE, the artery was exposed to 3 \(\mu\)M \(\alpha_1\)-mATP
for 3 min. For groups of arteries from unoperated and spinal-
ized rats, concentration-response curves to 0.03–10 \(\mu\)M \(\alpha_1\)-mATP
were determined by applying concentrations that increased by half-
log increments, with the tissue exposed to each concentration for 3 min
and then washed for 30 min before the addition of the next
concentration. These experiments showed that 3 \(\mu\)M \(\alpha_1\)-mATP produces a
near-maximal contraction.

Responses to 60 mM extracellular K\(^+\) were recorded in the pres-
ence of 10 nM prazosin to prevent the excitatory actions of NE
released from the sympathetic nerves. The tissues were exposed to
three applications of 60 mM K\(^+\)-physiological saline solution
(equimolar substitution of KCl for NaCl) for 3 min, each separated by
5–10 min washes. The average responses to the second and third
applications of K\(^+\) were analyzed.

In a separate series of experiments, concentration-response curves for K\(^+\)
were determined for arteries from sham-operated and spinal-
animals. These arteries were treated with 10 nM prazosin and exposed for 5 min to physiological saline solutions containing 0, 20, 40, 60, 80, and 100 mM K+ (equimolar substitution of KCl for NaCl), with 10-min washout periods between application of each concentration.

Data analysis. Peak amplitudes of the contractions to trains of electrical stimuli, to applications of PE, methoxamine, or α,β-mATP, or to changes in [K+], were measured. For the contractions evoked by 100 stimuli at 5 Hz, the rise time was the interval between 10% and 90% of the peak contraction and 50% decay was the interval for the contraction at the end of the stimulus train to decline by 50%. The 50% decay time of high-K+-induced contraction was measured as the time from removal of the high-K+ solution until the amplitude of the contraction had fallen to 50% of its value at the start of washout. When data were obtained from more than one arterial segment from one animal, the mean value was used for statistical comparisons (i.e., n = number of animals studied).

SPSS 11 for Mac OS X (SPSS, Chicago, IL) was used for all statistical comparisons. For all nerve stimulation data and 50% decay time data for the K+-contractions, Mann-Whitney U tests were used for pairwise comparisons and Kruskal-Wallis tests for multiple comparisons because of unequal variance between the groups of data (determined by F tests). When multiple pairwise comparisons were made, P for the Mann-Whitney U-tests was adjusted using the Dunn-Sidak method. For all other multiple comparisons, one-way ANOVA and Tukey-Kramer post hoc tests were used. Other pairwise comparisons were made with two-tailed paired or unpaired Student’s t-tests. EC50 and slope of the concentration-response curves for PE and methoxamine were estimated by fitting the data to the Hill equation using Igor Pro (Wavemetrics, Lake Oswego, OR). The degree of association between the response of arteries to electrical activation and their sensitivity to PE was assessed using Spearman’s coefficient of rank correlation. P < 0.05 was taken as a significant difference. The nerve stimulation data and the 50% decay time data for the K+-contractions are presented as medians and interquartile range (IQR), and all other data are presented as means (SD).

Drugs. l-Phenylephrine HCl (PE), prazosin HCl, methoxamine HCl, desmethylimipramine HCl (DMI), α,β-mATP, and suramin were supplied by Sigma Chemical. Prazosin was prepared as a 1 mM stock solution in 10% (vol/vol) dimethyl sulfoxide in water. PE, methoxamine, DMI, and α,β-mATP were prepared as stock solutions in water, and suramin was made up directly in the physiological saline.

RESULTS

Basal conditions. The luminal diameter of the vessels and basal distending pressures measured after the equilibration period did not differ significantly between unoperated [286 μm (SD 27) diameter, 6.7 × 103 N/m² (SD 0.7 × 103) pressure, n = 25], sham-operated [277 μm (SD 28) diameter, 7.3 × 103 N/m² (SD 0.8 × 103) pressure, n = 19], and spinalized [276 μm (SD 32) diameter, 6.9 × 103 N/m² (SD 1.0 × 103) pressure, n = 25] arteries (P = 0.14 by ANOVA for diameter, P = 0.10 by ANOVA for basal distending pressure).

Responses to neural stimulation. Figure 1A shows the contractile response of a sham-operated and a spinalized artery to trains of 100 stimuli at 1, 3, and 5 Hz. Arteries from spinalized animals produced much larger responses to all frequencies of stimulation than arteries from unoperated and sham-operated animals (Fig. 1B). The magnitude of this effect was similar over the range of frequencies of stimulation, the response of the spinalized arteries being increased approximately five- to sixfold compared with the sham-operated arteries. However, the peak contractile response of sham-operated arteries to stimulation at 3 and 5 Hz was smaller than that of unoperated arteries (Fig. 1B).

Time course of neurally evoked responses. The effects of spinal transection on the time course of contractions to 100 stimuli at 5 Hz were assessed. The 10–90% rise time of the contractions did not differ between unoperated, sham-operated, and spinalized arteries: 12.9 s (IQR 10.6–14.2), 13.9 s (IQR 13.2–16.3), and 13.0 s (IQR 10.5–14.2), respectively (P = 0.20 by Kruskal-Wallis test). There was no significant difference between the 50% decay times for unoperated [2.6 s (IQR 2.2–3.0)] and spinalized [3.0 s (IQR 2.5–3.7)] arteries (P = 0.15 by Mann-Whitney U-test). However, the 50% decay time for sham-operated arteries [2.3 s (IQR 2.0–2.6)] was reduced compared with that for spinalized arteries (Mann-Whitney U-test, P < 0.01).

Effects of α1-adrenoceptor and P2-purinoceptor blockade on neurally evoked contractions. The effects of the α1-adrenoceptor antagonist prazosin (10 nM) and the P2-purinoceptor antagonist suramin (0.1 mM) were investigated on contractions evoked by 20 stimuli at 10 Hz. The increase in pressure produced by this train of stimuli did not differ between unoperated [7.2 × 103 N/m² (IQR 5.3–9.4), n = 14] and sham-operated [5.6 × 103 N/m² (IQR 4.0–6.6), n = 13, P = 0.13 by Mann-Whitney U-test] arteries, but the response of the spinalized arteries was increased compared with both of these groups.

Fig. 1. Enhanced contractions to perivascular nerve stimulation after spinal transection. A: contractions of arteries from sham-operated and spinalized animals to stimulation of perivascular nerves with 100 stimuli at 1, 3, and 5 Hz. B: peak increase in pressure produced by 100 stimuli at 1, 3, and 5 Hz in arteries from unoperated (open bars, n = 14), sham-operated (hatched bars, n = 13), and spinalized (solid bars, n = 14) animals. Values are median and interquartile range. Statistical differences (Mann-Whitney U-tests) between responses to each frequency of stimulation: **P < 0.01; ***P < 0.001.
of arteries \([14.5 \times 10^3 \text{ N/m}^2 \text{ (IQR 12.6–17.0)}, n = 12, P < 0.001\] for both comparisons by Mann-Whitney \(U\)-test).

Figure 2, A and B, shows the reduction in pressure of the neurally evoked responses produced by suramin and prazosin, respectively. Despite the marked difference in the peak amplitude of the neurally evoked responses, the net reduction in the pressure produced by suramin was similar for unoperated and spinalized arteries (Fig. 2A). The reduction in pressure produced by suramin was smaller for sham-operated than for spinalized arteries but did not differ from that for unoperated arteries (Fig. 2A). In contrast, the decrease in pressure produced by prazosin was much greater in spinalized than in unoperated and sham-operated arteries (Fig. 2B). These findings indicate that the increase in the neurally evoked response of spinalized arteries is due primarily to an increase in the prazosin-sensitive (noradrenergic) component of contraction.

Figure 2, C and D, shows the percent block of the neurally evoked contractions produced by suramin and prazosin, respectively. The percent block produced by suramin varied greatly between the groups of arteries, with the contraction of sham-operated arteries being most affected by this agent and that of spinalized arteries being least affected (Fig. 2C). In contrast, the percent block produced by prazosin was similar (80–85%) for all groups of arteries (Fig. 2D), although there was a small but significant difference between unoperated and sham-operated arteries. In all groups of arteries, the combined application of suramin and prazosin reduced the peak force of contraction by ~95%: 96% (IQR 96–98) for unoperated, 95% (IQR 86–96) for sham-operated, and 97% (IQR 95–99) for spinalized arteries.

**Responses to PE.** Figure 3A shows the concentration-response curves for PE in unoperated, sham-operated, and spinalized arteries. Compared with unoperated and sham-operated arteries, spinalized arteries showed a leftward shift in their concentration-response curve for PE (Fig. 3A; \(P < 0.01\) for both comparisons by Tukey-Kramer tests). In addition, compared with unoperated arteries, the PE concentration-response curve for sham-operated arteries was significantly shifted to the right (Fig. 3A; \(P < 0.01\) by Tukey-Kramer test). The EC\(_{50}\) for PE also differed between the three groups of arteries, but there was no difference between the slopes of the Hill equation fits (Table 1). There was no difference in the maximal increase in pressure produced by PE between unoperated, sham-operated, and spinalized arteries (Table 1).

A significant negative correlation was found between the response of arteries to neural activation (100 stimuli at 5 Hz) and their EC\(_{50}\) for PE \((r = -0.82, P < 0.001)\).

**Changes in effects of the neuronal NE transporter.** The leftward shift in the concentration-response curve to PE could result from a postjunctional increase in reactivity of the vascular smooth muscle to \(\alpha_1\)-adrenoceptor activation and/or a reduction in its uptake by the nerve terminals. To determine the

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**Fig. 2.** Effects of 0.1 mM suramin and 10 nM prazosin on contractions of arteries from unoperated (Un-op, \(n = 13\)), sham-operated (\(n = 13\)), and spinalized (\(n = 12\)) animals evoked by 20 stimuli at 10 Hz. A and B: reduction in pressure of nerve-evoked responses produced by suramin and prazosin. C and D: percent block of nerve-evoked responses produced by suramin and prazosin. Values are median and interquartile range. Statistical differences (Mann-Whitney \(U\)-tests) between groups of arteries: \(*P < 0.05\); \(**P < 0.01\).
Fig. 3. Increased sensitivity of arteries to phenylephrine, but not methoxamine, after spinal transection. A: concentration-response curves for phenylephrine in arteries from unoperated (●, n = 19), sham-operated (▲, n = 13), and spinalized (■, n = 19) animals. B: concentration-response curves for methoxamine in arteries from unoperated (●, n = 12), sham-operated (▲, n = 6), and spinalized (■, n = 12) animals. Curves are best fits to Hill equation. Values are means (SD).

Table 1. Properties of concentration-response relations for PE and methoxamine

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>EC50, μM</th>
<th>Slope of Fit to Hill Eq</th>
<th>Maximum Increase in Pressure, 10^3 N/m²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unoperated</td>
<td>19</td>
<td>1.3 (0.8)†‡</td>
<td>0.52 (0.14)</td>
<td>37.4 (6.4)</td>
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<tr>
<td>Sham-operated</td>
<td>13</td>
<td>2.1 (0.7)‡‡</td>
<td>0.44 (0.10)</td>
<td>40.6 (6.0)</td>
</tr>
<tr>
<td>Spinalized</td>
<td>19</td>
<td>0.7 (0.4)‡‡</td>
<td>0.52 (0.19)</td>
<td>38.8 (6.2)</td>
</tr>
<tr>
<td>Methoxamine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unoperated</td>
<td>12</td>
<td>1.2 (0.5)</td>
<td>0.33 (0.10)</td>
<td>38.9 (5.8)</td>
</tr>
<tr>
<td>Sham-operated</td>
<td>6</td>
<td>0.9 (0.2)</td>
<td>0.34 (0.06)</td>
<td>33.3 (4.4)</td>
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<tr>
<td>Spinalized</td>
<td>12</td>
<td>1.2 (0.7)</td>
<td>0.35 (0.12)</td>
<td>34.7 (8.9)</td>
</tr>
</tbody>
</table>

Values are means (SD). PE, phenylephrine. †‡ Significant differences (Tukey-Kramer test) between unoperated, sham-operated, and spinalized arteries (P < 0.01).

Table 2. Properties of concentration-response relations for PE with and without DMI

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>EC50, μM</th>
<th>Slope of Fit to Hill Eq</th>
<th>Maximum Increase in Pressure, 10^3 N/m²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unoperated</td>
<td></td>
<td>1.7 (0.6)‡‡</td>
<td>0.46 (0.14)</td>
<td>36.5 (6.5)</td>
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<td>Spinalized</td>
<td></td>
<td>0.7 (0.3)‡‡</td>
<td>0.47 (0.12)</td>
<td>30.8 (6.6)</td>
</tr>
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</table>

Values are means (SD); n = 6. DMI, desmethylimipramine. *Significant differences (unpaired t-test) between EC50 values for unoperated and spinalized arteries without DMI (P < 0.01). Significant differences (paired t-tests) for unoperated and spinalized arteries between EC50 values without and with DMI: † P < 0.01; ‡ P < 0.001.

role of PE uptake into nerve terminals, the effects of blocking the neuronal NE transporter on the reactivity to PE were evaluated in pairs of arteries from the same animal, one being treated with DMI. In a second series of experiments, concentration-response curves to the α1-adrenoceptor agonist methoxamine, which is not a substrate for the neuronal NE transporter (31), were constructed.

The maximum increase in pressure produced by PE did not differ between unoperated and spinalized arteries in the absence or presence of 30 nM DMI (Table 2). In unoperated and spinalized arteries, DMI decreased the EC50 for PE without changing the slopes of the Hill equation fits (Table 2). Importantly, although in the absence of DMI the EC50 values for PE differed significantly between unoperated and spinalized arteries, in the presence of this agent the EC50 values were similar (Table 2).

The concentration-response curves for methoxamine did not differ between unoperated, sham-operated, and spinalized arteries (Fig. 3B; P = 0.59 by ANOVA). There was also no difference between the EC50 values, the slope of the Hill equation fits, or the maximum increase in pressure produced by methoxamine for these groups of arteries (Table 1).

Responses to αβ-mATP. Application of 3 μM αβ-mATP produced a contraction that peaked shortly after its addition and then waned. The peak increase in pressure produced by αβ-mATP did not differ significantly between unoperated [23.8 ± 10^3 N/m² (SD 3.2 ± 10^3), n = 8] and spinalized [25.4 ± 10^3 N/m² (SD 2.8 ± 10^3), n = 7] arteries, but the response of the sham-operated arteries [12.2 ± 10^3 N/m² (SD 5.9 ± 10^3), n = 7] was ~50% smaller (P < 0.01 by Tukey-Kramer test for comparisons with unoperated and spinalized arteries). For unoperated (n = 5) and spinalized (n = 5) arteries, there was no difference between the concentration-response curves (P = 0.37 by ANOVA) or the EC50 values [0.5 μM (SD 0.2) for unoperated and 0.5 μM (SD 0.1) for spinalized, P = 0.79 by unpaired t-test] for α,β-mATP.

Responses to high [K^+]. The peak increase in pressure produced in 60 mM extracellular K^+ did not differ between unoperated [32.0 ± 10^3 N/m² (SD 4.0 ± 10^3), n = 8], sham-operated [32.0 ± 10^3 N/m² (SD 4.8 ± 10^3), n = 7], and spinalized [33.1 ± 10^3 N/m² (SD 2.3 ± 10^3), n = 7] arteries (P = 0.84 by ANOVA). On return to normal bathing solution, the 50% decay of the K^+-evoked contraction was slower in spinalized [9.3 s (IQR 8.6–12.4)] than in unoperated [7.4 s (IQR 7.1–7.8), P < 0.05 by Mann-Whitney U-test] and sham-operated [6.8 s (IQR 6.0–7.8), P < 0.05 by Mann-Whitney U-test] arteries.

In a separate series of experiments, there was no difference in the K^+ concentration-response curves between sham-operated and spinalized arteries (Fig. 4; P = 0.21 by ANOVA).

DISCUSSION

Spinal transection at T4 in the rat produced a marked increase in the contractile response of second-order mesenteric arteries to electrical stimulation of the perivascular sympathetic nerves. This change was attributable to increased α1-adrenoceptor activation and was correlated with an increased sensitivity to the α1-adrenoceptor agonist PE. The latter appeared to be due to a decrease in PE removal by the neuronal NE transporter. For unoperated and spinalized arteries, there was no difference between the concentration-response curves (P = 0.37 by ANOVA) or the EC50 values [0.5 μM (SD 0.2) for unoperated and 0.5 μM (SD 0.1) for spinalized, P = 0.79 by unpaired t-test] for α,β-mATP.
transporter. The responses of spinalized arteries to the P2X-purinoceptor agonist α,β-mATP and to raised [K\(^+\)]\_o were unchanged compared with unoperated arteries. There was also no change in the neurally evoked increase in pressure that was due to released ATP. Together, these findings suggest that the noradrenergic component of the nerve-evoked response in the mesenteric artery is selectively increased after spinal cord transection.

NE and ATP are known to play a role in nerve-evoked contractions of rat mesenteric arteries. In the present study, prazosin reduced the contractions of the unoperated and sham-operated arteries evoked by 20 stimuli at 10 Hz by ~80%, whereas suramin reduced these contractions by 40–70%. Together, prazosin and suramin virtually abolished the responses to nerve stimulation. The nonadditive nature of the blockade produced by prazosin and suramin suggests that NE and ATP released from the sympathetic nerves act synergistically to produce activation of the vascular smooth muscle. This suggests that increased reactivity of the spinalized arteries to PE. In second-order mesenteric arteries, the majority of sympathetic varicosities do not make close neuroeffector junctions with the arterial smooth muscle. Two findings indicate that increased reactivity to PE is accounted for by a reduction in its removal by the neuronal NE uptake transporter: 1) Blockade of neuronal uptake with DMI produced a leftward shift in the concentration-response curve for PE in unoperated and spinalized arteries and abolished the supersensitivity to PE. 2) The sensitivity of spinalized arteries to the α\(_1\)-adrenoceptor agonist methoxamine was the same as in controls; methoxamine, in contrast to PE, is not a substrate for the neuronal NE uptake transporter. This suggests that the efficacy of uptake by the transporter is modified by the levels of ongoing nerve activity.

Sympathetic neuroeffector transmission in the rat tail artery was augmented after spinalization by quite different mechanisms. Nerve-evoked contractions of the tail artery involve the synergistic interactions of α\(_1\)- and α\(_2\)-adrenoceptor activation (5, 35). After spinalization, the increased responses to nerve stimulation were correlated with an increased sensitivity to the α\(_2\)-adrenoceptor agonist clonidine and raised [K\(^+\)]\_o. This suggests that the α\(_2\)-adrenoceptor-mediated component of contraction and the overall reactivity of the tail artery were enhanced (35). Tail arteries displayed an increased reactivity to PE at 2 wk, but not at 8 wk, after spinal transection, when neurally evoked contractions were still markedly potentiated. For all measured parameters, there were no differences between tail arteries from unoperated and sham-operated animals. Thus the mechanisms underlying the augmentation of the neurally evoked contractions after spinal transection differ markedly between the cutaneous and splanchnic vascular beds. In the

Fig. 4. Absence of change in reactivity to raised K\(^+\) concentration ([K\(^+\)]) after spinal transection, shown as concentration-response curves for K\(^+\) in arteries from sham-operated and spinalized animals. Values are means (SD); n = 6.

(20). If some of the neurally released NE originates from noncontacting varicosities, then NE diffusing from the sites of transmitter release might act similarly to exogenously applied NE. In support of this idea, the increase in NE concentration detected at the adventitial surface of second-order mesenteric arteries in response to trains of 10 stimuli at 10 Hz peaked at >0.1 μM (9) and the EC\(_{50}\) for bath-applied NE at α\(_1\)-adrenoceptors is ≈0.8 μM (8). This indicates that the concentration of endogenous NE in the adventitia is adequate to activate the same receptors that are activated by exogenous NE.

It is possible that NE release is enhanced after spinalization, in accord with disuse, increasing neurotransmitter release at other synapses (25). There is no direct evidence for this possibility, but an increase in NE release is likely to contribute to the augmented responses of tail arteries from spinalized animals (35). If it is assumed that NE and ATP are coreleased (7), the fact that there was no change in the neurally evoked increase in pressure attributable to ATP in mesenteric arteries argues against an increase in transmitter release in this vessel.

The above-mentioned arguments do not address the mechanisms that underlie the increased sensitivity of the spinalized arteries to PE. In general, supersensitivity after decentralization of sympathetic postganglionic neurons is nonspecific, with tissues displaying increased sensitivity to a wide range of agents (11). Because of this lack of specificity, postfunctional hyperreactivity is believed to result from an alteration in smooth muscle function, rather than from a change in receptor expression. However, because the sensitivity of the spinalized arteries to α\(_1\)-mATP and high [K\(^+\)]\_o was unchanged compared with unoperated arteries, their increased sensitivity to PE was not due to a generalized increase in the reactivity of the smooth muscle. Two findings indicate that increased reactivity to PE is accounted for by a reduction in its removal by the neuronal NE uptake transporter: 1) Blockade of neuronal uptake with DMI produced a leftward shift in the concentration-response curves for PE in unoperated and spinalized arteries and abolished the supersensitivity to PE. 2) The sensitivity of spinalized arteries to the α\(_1\)-adrenoceptor agonist methoxamine was the same as in controls; methoxamine, in contrast to PE, is not a substrate for the neuronal NE uptake transporter (31). This suggests that the efficacy of uptake by the transporter is modified by the levels of ongoing nerve activity.
cutaneous artery the changes are mostly postjunctional, whereas in the mesenteric artery the changes are prejunctional.

Surprisingly, compared with the unoperated mesenteric arteries, responses to nerve stimulation were reduced in the sham-operated arteries. This change was associated with an increased EC$_{50}$ for PE. The decreased reactivity of sham-operated arteries to PE, relative to unoperated arteries, was most probably due to increased neuronal uptake of this substance, because the sensitivities of these arteries to methoxamine were similar. It should be noted that the 50% decay times of the neurally evoked contractions of sham-operated arteries were faster than those of spinalized arteries, which may reflect the more rapid removal of released NE. The sham-operated arteries also were less reactive to α,β-mATP but not to raised [K$^+$]. These findings suggest that neurally evoked responses of the sham-operated arteries are selectively reduced. Indeed, the changes in responses to nerve stimulation and PE in sham-operated arteries were the opposite of those observed for the spinalized arteries. This might indicate that the sympathetic outflow to the mesenteric arteries increased after the sham operation. Increased sympathetic nerve activity and/or catecholamine release from the adrenal medulla might be produced by operative stress, but this is unlikely to have lasted for 7 wk. In all operated animals, reflex changes in nerve activity could result from inflammation of dorsal root ganglia near the lesion (23), but again this is unlikely to persist. Regardless of the reason for the changes in the arteries of sham-operated animals, the fact that their responsiveness was reduced relative to unoperated animals indicates that the effects of spinal transection are even more marked than indicated by the differences from unoperated controls.

We have assumed that the changes observed in the spinalized mesenteric arteries, as for the tail arteries, result from silencing of ongoing sympathetic nerve activity after spinal transection (35). If this is the case, the changes should apply equally well to arterial vessels in patients with lesions at any transection (35). If this is the case, the changes should apply equally well to arterial vessels in patients with lesions at any transection (35). If this is the case, the changes should apply equally well to arterial vessels in patients with lesions at any transection (35). If this is the case, the changes should apply equally well to arterial vessels in patients with lesions at any transection (35). If this is the case, the changes should apply equally well to arterial vessels in patients with lesions at any transection (35).

In conclusion, the findings of the present study provide further evidence that increased reactivity of the vasculature to sympathetic nerve activation is likely to contribute prominently to the etiology of autonomic dysreflexia. Therefore, interventions that inhibit or prevent the development of vascular hyperactivity after spinal injury have considerable therapeutic potential. Our data indicate that decreases in sympathetic activity modify vascular reactivity by distinct mechanisms in different arterial beds and that this might be exploited to alleviate different symptoms. Whether hyperreactivity contributes to modified vascular perfusion under resting conditions in spinally injured humans is not clear. Furthermore, the findings suggest that the ongoing level of sympathetic activity in intact humans may modulate the effectiveness of neurovascular transmission.

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