Peripheral changes above and below injury level lead to prolonged vascular responses following high spinal cord injury

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Laird AS, Finch AM, Waite PM, Carrive P. Peripheral changes above and below injury level lead to prolonged vascular responses following high spinal cord injury. Am J Physiol Heart Circ Physiol 294: H785–H792, 2008. First published November 30, 2007; doi:10.1152/ajpheart.01002.2007.—Autonomic dysreflexia (AD) is a debilitating disorder producing episodes of extreme hypertension in patients with high-level spinal cord injury (SCI). Factors leading to AD include loss of vasomotor baroreflex control to regions below injury level, changes in spinal circuitry, and peripheral changes. The present study tested for peripheral changes below and above injury level 6 wk after a transection at the fourth thoracic spinal level. Changes in vascular conductance were recorded in the femoral, renal, brachial, and carotid arteries in response to intravenous injections of two α-adrenergic agonists, phenylephrine (PE; 0.03–100 μg/kg) and methoxamine (Meth; 1–300 μg/kg). Unlike PE, Meth is not subject to neuronal reuptake. Ganglionic blockade (0.6 mg/kg chlorisondamine) was used to eliminate the central component of the cardiovascular response. After ganglionic blockade, SCI animals exhibited prolonged vasoconstriction in response to PE in all blood vessels measured compared with those in intact animals (all, P < 0.035). However, the PE dose-response curves obtained after ganglionic blockade revealed no significant difference in the potency between the two groups (all, P > 0.06), indicating that the prolonged vasoconstriction was not due to supersensitivity to PE. In contrast to PE, vascular responses to Meth did not vary between intact and SCI groups (all P > 0.108). These results show the development of a widespread peripheral change producing prolonged vasoconstriction in response to PE, but not Meth, possibly due to reduced neuronal reuptake of PE after SCI. This is the first study to report such a change in blood vessels not only below but also above injury level. Interventions to correct this reduced reuptake may help limit the development of AD.

autonomic dysreflexia; spinal cord transection; phenylephrine; methoxamine

Besides paralysis and loss of sensation, spinal cord injury (SCI) causes a range of autonomic dysfunctions. Just as the motor reflexes within the body are known to be affected by SCI, so are many autonomic reflexes below the injury level (24). For example, a hyperreflexic detrusor often evolves, thermoregulation is impaired, and cardiovascular problems such as autonomic dysreflexia (AD) can also develop in patients with injuries in the high thoracic or cervical spinal segments (9, 10, 24). This autonomic dysfunction can produce just as much morbidity as motor and sensory deficits and, thus, deserves research attention. In particular, AD is a disorder that occurs in ~85% of patients with high SCI and can lead to permanent deficits or death due to cerebrovascular accidents and myocardial infarction (8, 19). The condition evolves from exaggerated sympathetic reflexes that are triggered by afferent stimulation from areas below the injury level. Common triggers include an overfull bladder and constipation (12, 15, 29). Exaggerated sympathetic activity causes increased vasoconstriction below the injury, resulting in dangerous high blood pressure, which leads to headaches, bradycardia, and cold extremities. Additional symptoms occur due to compensatory baroreflex-mediated vasodilation in blood vessels above the injury level, inducing flushing and sweating (10).

To understand the AD condition, it is important to consider the types of changes that may lead to the development of the disorder. First, there is the obvious loss of supraspinal control pathways, which occurs following SCI. This loss impairs the function of the vasomotor baroreceptor reflex, which would otherwise maintain normal blood pressure through a reduction of sympathetic drive to vascular beds. In addition, following SCI, changes are known to occur within the spinal cord itself, both on preganglionic sympathetic neurons and afferent inputs, with extensive sprouting of CGRP fibers below the level of injury (35). It is thought that this plasticity may also contribute to the exaggerated reflexes seen in the disorder. Second, peripheral vascular changes may also develop, especially due to the ongoing low sympathetic tone, which results from a high SCI.

Peripheral changes that may contribute to AD include adrenergic supersensitivity (1, 18, 22), altered adrenoceptor density (28), smooth muscle reactivity (37), increased norepinephrine (NE) release (21, 37), and decreased synaptic reuptake of NE (3). In addition, there may be changes in other control mechanisms such as those involving angiotensin and nitric oxide (13, 38).

This paper aims to investigate peripheral vascular changes that develop by 6 wk after a transection at the fourth thoracic spinal level (T4) in the rat, at a time when AD is well established. We have compared the vascular response with the α-adrenergic agonist phenylephrine (PE) before and after ganglionic blockade to separate peripheral from centrally mediated changes. These responses were studied in blood vessels above and below the injury level to assess changes resulting from a direct loss of supraspinal control (blood vessels below injury level) compared with widespread, systemic effects, such as chronic low mean arterial pressure (MAP) (blood vessels both above and below injury level). This is the first time that such a comparison has been made following ganglionic blockade. Finally, the responses to PE were compared with methoxamine (Meth), another α-adrenergic agonist that, unlike PE, is not a

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substrate for neuronal reuptake. This was used to test for any alteration in NE reuptake mechanisms.

METHODS

Experiments were performed on 14 adult male albino Australian Wistar rats (Biological Resource Centre, Sydney, New South Wales). All experimental procedures were approved by the Animal Care and Ethics Committee of the University of New South Wales in accordance with the guidelines of the National Health and Medical Research Council of Australia for animal research.

Spinal cord transection. Rats in the SCI group (n = 7) were rendered paraplegic by a complete transection of the spinal cord at T4 under anesthesia (ketamine and xylazine, 100 mg + 50 mg/kg ip). After the dissection of the superficial and deep muscles of the back, a laminectomy of the third thoracic vertebra was performed to reveal the T4 spinal segment, which was cut with microscissors. Complete transection was confirmed with a scalpel blade and by observation of the cut cord surfaces under the dissecting microscope. After transection, a gap of ~2 mm was present between the rostral and caudal ends of the cord. A piece of gelfoam was inserted to fill the gap and reduce bleeding. Gelfoam was also placed on the dorsal surface of the cord. Finally, the muscle tissue and skin were sutured in layers.

Postoperative care. Rats received an antibiotic (Keflin, 100 mg/ml sc) twice daily until no sign of urinary infection was seen for 3 consecutive days. The animals also received an analgesic (carprofen, 50 mg/kg sc) daily for 3 days and saline solution (5 ml of 0.9% saline sc) to provide hydration until each rat could drink following the recovery period. At this point AD, in response to colorectal distension, bowel impaction occurred. Dietary supplements were given to treat any lesion or swelling. Any rat with a severe lesion was euthanized.

SCI rats underwent the terminal experiment. The procedure was conducted under pentobarbitone sodium anesthesia involving an induction and adaptation to housing.

Experimental protocol. After SCI, all rats were allowed a 6-wk recovery period. At this point AD, in response to colorectal distension, was present (change in MAP of at least 25 mmHg) (20). Intact rats (n = 7) were also housed during this period to serve as controls for age and adaptation to housing.

Terminal experiment. At the 6-wk time point, both the intact and SCI rats underwent the terminal experiment. The procedure was conducted under pentobarbitone sodium anesthesia involving an initial intraperitoneal injection (45 mg/kg) and maintenance with con- tinuous intravenous infusion (15 mg·kg⁻¹·h⁻¹). Animals were main- tained at 37°C on a homeostatic heating pad. The room temperature was maintained at 27–28°C for 7 days, and rat temperature was measured regularly until a normal temperature was maintained. Other interventions included administering a laxative (Caltax; Novartis Australasia) if bowel impaction occurred. Dietary supplements were given to treat weight loss. The rats were weighed daily and monitored for skin lesions or swelling. Any rat with a severe lesion was euthanized.

Analysis of dose-response curves to compare sensitivity with adrenergic agonists. The maximum decreases in vascular conductance were extracted from the response to each dose of PE and Meth and expressed as a percentage of baseline. These values were then expressed against the log agonist dose to produce sigmoidal dose-response curves using nonlinear regression analysis within Prism 4.0 (GraphPad) software. The log EC₅₀ and maximal response values were determined from these dose-response curves. The mean log EC₅₀ and standard error for intact and SCI groups were calculated as well as the geometric mean of the EC₅₀, and these values were compared by t-tests, where P values of <0.05 were taken to indicate a significant difference between the intact and SCI parameters.

RESULTS

Cardiovascular responses to PE injection without ganglionic blockade. The average responses of intact and SCI animals to PE injection are shown in Fig. 1. It can be seen that SCI animals had reduced baseline HR and MAP compared with those of intact animals while under anesthesia. The difference was statistically significant for both variables (P < 0.047). In both groups, an intravenous PE (10 µg/kg) injection produced a marked increase in MAP and corresponding decreases in vascular conductances in all four arteries examined. There was also a decrease in HR, as expected (Fig. 1). Overall, the graph...
suggests that the response to PE was more pronounced in SCI than in intact animals in both magnitude and duration. This overall effect was confirmed by repeated-measures ANOVAs for MAP and conductance in each vascular bed (all, $P < 0.035$). Further analysis of the duration of the response also revealed significantly longer $T_{75}$ for each vascular bed in SCI animals (all, $P < 0.033$). However, peak changes (extracted from individual responses) were not significantly different between the two groups (all, $P > 0.11$; one-way ANOVA). This first experiment shows that in SCI animals all the vascular beds examined took longer to recover from PE, even those above the injury level, which have intact sympathetic innervation and baroreflex control.

**Cardiovascular responses to PE injection under ganglionic blockade.** The previous experiment was then repeated under ganglionic blockade to isolate the responses from any central or reflex influence (Fig. 2). As expected, ganglionic blockade reduced the baseline HR and MAP of intact animals down to the same level observed in SCI animals (HR, from 308 to 261 beats/min; and MAP, from 108 to 86 mmHg). Ganglionic blockade had no significant effect on the baseline HR and MAP of SCI animals. Also as expected, the PE (10 $\mu$g/kg) injection caused increases in MAP and corresponding decreases in vascular conductance with no change in HR. However, although the difference between intact and SCI animals was not as marked as before, the overall response still appeared more pronounced in SCI animals. Repeated-measures ANOVAs revealed that the MAP response was actually not significantly different between the two groups ($P = 0.09$), but the four conductance responses, both above ($P < 0.001$) and below injury level ($P < 0.01$), were different. Once again, the SCI group showed longer periods of decreased vascular conductance ($T_{75}$; all, $P < 0.038$) and no significant difference in the peak parameter changes (all, $P > 0.13$), except for the carotid artery conductance ($P = 0.046$; Fig. 2). This second experiment now clearly shows that the difference observed previously between SCI and intact animals was of mainly peripheral origin and that all vascular beds were affected.

Further analysis of the vascular responses was then performed by constructing dose-response curves from the maxi-

Fig. 1. Time course of the changes in heart rate, mean arterial pressure (MAP), and vascular conductance (carotid, brachial, renal, and femoral arteries) in response to a bolus injection of phenylephrine (PE; 10 $\mu$g/kg). The injection was made at time point 0, and the vascular conductances are expressed as percentages of the preinjection baseline. Values are means ± SE. *$P < 0.05$, vascular conductance responses significantly different for the 2 groups, as determined by a repeated-measures ANOVA. SCI, spinal cord injury; Cond, conductance; bpm, beats/min.
The curves are shown in Fig. 3, and the EC₅₀ and maximum response values are listed in Table 1. The analysis revealed that the log EC₅₀ values were not significantly different for any of the blood vessels tested, suggesting that no change in sensitivity to PE had developed (P > 0.2; Fig. 3 and Table 1). The maximal response of the carotid artery after SCI was significantly higher than that of the intact response (P < 0.05). This suggests that PE was a more effective agonist in the carotid artery of SCI than intact animals, although this was not because of an increased sensitivity to PE.

Cardiovascular responses to Meth injection under ganglionic blockade. The same experiment described in METHODS was then repeated using Meth instead of PE, also while under ganglionic blockade (Fig. 4). Baseline HR and MAP were not significantly different from the level recorded before the PE injection following ganglionic blockade (P > 0.21). As expected, a 100 µg/kg injection of Meth produced an increase in MAP associated with decreased vascular conductance in all blood vessels (Fig. 4). However, in contrast with PE, the overall response was very similar between intact and SCI animals, except in the carotid artery. Statistical analysis with a repeated-measures ANOVA revealed that the difference in the carotid response was in fact not significant (P = 0.2), nor were the other three arteries (P > 0.1; Fig. 4). The T₇₅ analysis also confirmed that the duration of the vascular responses was not significantly different between SCI and intact animals (all, P > 0.052). This experiment shows that the peripheral response to Meth is the same in SCI and intact animals, in contrast to PE. The difference between these two α-adrenergic agonists may be attributed to the fact that Meth is not subject to neuronal reuptake.

The vascular responses to Meth were also analyzed from dose-response curves, as with PE. The parameters extracted from these dose-response curves show no difference between intact and SCI animals for any of the blood vessels (Table 1). The maximal response of the carotid artery response to Meth after SCI was not significantly higher than the intact value, unlike the difference seen in response to PE.
WIDESPREAD PERIPHERAL CHANGES FOLLOWING SCI

The primary finding of this study is that SCI animals exhibit longer-lasting vasoconstriction in blood vessels above and below injury level in response to PE injection. This prolonged vasoconstriction must be due to peripheral changes since it is still observed after ganglionic blockade. Supersensitivity of adrenergic receptors can be ruled out because the dose-response curves did not undergo a rightward shift. However, the fact that no difference was observed between the intact and SCI responses to Meth strongly suggests that the peripheral change is related to a decrease in the neuronal reuptake of PE, at least in the femoral, renal, and brachial vasculature. The fact that peripheral changes occurred throughout the body, not just below the injury level, indicates that the effect is widespread and suggests an adaptation to the long-term consequences of SCI.

Changes in baseline blood pressure after SCI. Under anesthesia, SCI animals had a markedly reduced baseline MAP compared with intact animals. This observation is consistent with previous reports of lowered basal MAP in SCI animals (20) and patients (2, 23), particularly following anesthetic administration (11, 34). This hypotension is a result of reduced vasomotor tone following SCI due to a lower level of basal sympathetic tone below the level of the transection (23, 30). Accordingly, ganglionic blockade had little effect on SCI animals (which were already partially decentralized) but markedly reduced MAP in intact animals, bringing the two groups down to the same baseline level. Although it could be desirable to perform the study in conscious animals to avoid the effect of an anesthetic on cardiovascular reflexes and vasomotor tone, the use of ganglionic blockade has made the two groups comparable.

DISCUSSION

The first experiment showed that intravenous injections of PE in SCI animals produced greater and longer-lasting vasoconstrictions than in intact animals. This effect is not surprising for vascular beds located below the level of the lesion since the vasomotor preganglionic sympathetic neurons that control these beds have lost the supraspinal connections that mediate the baroreceptor reflex. This leads to the vasoconstriction in the femoral and renal arteries being greater and longer due to the loss of reflex withdrawal of sympathetic tone in response to the rise in blood pressure. However, the same effect was also observed in the brachial and carotid arteries, which were above the level of the lesion and would have had intact baroreflex modulation. This surprising observation raised the possibility that the long-lasting vasoconstriction was, perhaps, not of central but of peripheral origin.

The peripheral origin of the prolonged vasoconstriction was confirmed after ganglionic blockade because PE stimulation still resulted in longer-lasting vasoconstriction in SCI compared with intact animals, despite the complete removal of central vasomotor regulation. Furthermore, the effect was observed in all four vasculatures, suggesting that a general, widespread hyperreactivity to PE had developed in chronic SCI animals.

To better characterize the prolonged vasoconstriction, dose-response curves to PE under ganglionic blockade were constructed. Except for an increase in the maximal response in the SCI carotid artery, the curves were the same in intact and SCI animals.

Table 1. Parameters of the Hill equation used to model the curves

<table>
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<tr>
<th></th>
<th>n</th>
<th>( \log EC_{50} \pm SE )</th>
<th>( EC_{50} \mu g/kg )</th>
<th>Maximal Response, %</th>
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<td><strong>PE + BLK</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Carotid</td>
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<td>7</td>
<td>0.95 \pm 0.12</td>
<td>8.91</td>
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<tr>
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<td>0.71 \pm 0.16</td>
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<tr>
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<tr>
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<td>9.12</td>
<td>90.39 \pm 8.00</td>
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<td><strong>Meth + BLK</strong></td>
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<td>120.30 \pm 11.88</td>
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Values are means \( \pm SE \); n, number of animals. The maximal response is the maximum decrease of vascular conductance expressed as a percentage of the preinjection baseline conductance for responses of phenylephrine following ganglionic blockade (PE + BLK) and methoxamine following ganglionic blockade (Meth + BLK). SCI, spinal cord injury. *P < 0.05.

Fig. 3. Dose-response curves compiled from the effect of increasing PE doses on the carotid, brachial, renal, and femoral arteries under ganglionic blockade. In these curves, the decrease in vascular conductance is expressed as a percentage of the preinjection baseline. *P < 0.05, where the maximal response of intact and SCI curves was significantly different.
animals. The analysis revealed no significant change in EC50 between the two groups for any of the blood vessels tested, indicating that the exaggerated blood vessel responses were not due to an increased sensitivity to PE. The increase in the maximal response observed in the carotid artery may reflect changes in the relative proportion of each of the α1-adrenoceptor subtypes expressed in the SCI carotid artery. In particular, this increase may be due to changes in the ratio of α1A- to α1B-adrenoceptors since it has been shown in in vitro studies that coexpressing α1A- and α1B-adrenoceptors leads to a synergistic increase in PE maximal response (but not Meth maximal response) compared with the expression of the α1A-adrenoceptor alone (14).

Exaggerated responses to PE or NE have previously been shown in spinally injured tetraplegic patients (1, 18, 25). Studies have found that PE causes exaggerated pressor responses in SCI patients compared with intact subjects (18, 25), and one study reported greater vasoconstriction of the dorsal foot vein following the application of NE (1). Although Arnold et al. (1) conclude that the exaggerated responses seen in their study are due to the development of supersensitive adrenoceptors following SCI, their results could also result from a decreased level of NE reuptake following SCI. The use of the α-adrenergic agonist Meth, which unlike NE and PE, is not a substrate for the NE transporter NET1 (33), or the inhibition of NET1 with a selective NET1 inhibitor (e.g., desipramine) would be necessary to ascertain whether the exaggerated responses reported are due to adrenoceptor changes or impaired neuronal reuptake of the agonist.

Extensive studies by McLachlan and colleagues (3, 36, 37) have previously examined this issue using an in vitro dissected artery preparation. Studies by this group have reported the development of adrenoceptor supersensitivity of the mesenteric (3) but not tail (36, 37) arteries following chronic spinal cord transection and sympathetic denervation. A recent study by this group has also used the adrenergic agonist Meth and the NET1 blocker desipramine to remove the variable of neuronal agonist reuptake from the responses of intact and denervated arteries. They reported that although the mesenteric artery of spinally transected animals showed a leftward shift of PE dose-response curves, dose-response curves produced for
Meth, and PE following NET1 blockade, did not differ between intact and transected arteries (3).

To distinguish between these variables in our study, we also tested Meth in place of PE for the final measurements. In response to Meth, no significant difference was observed between the intact and SCI groups. There are two differences between the actions of Meth and PE in producing vasoconstriction; these are that Meth is not a substrate for NET1 (33) and that although PE is known to act with similar potencies at \( \alpha_{1A}^- \), \( \alpha_{1B}^- \), and \( \alpha_{1D}^- \) adrenoceptors, Meth is 20 times more potent at the \( \alpha_{1A}^- \)-subtype receptors than \( \alpha_{1B}^- \) or \( \alpha_{1D}^- \)-subtype receptors (27). The fact that, following ganglionic blockade, brachial, femoral, and renal arteries of SCI animals showed a longer duration of vasoconstriction (longer \( T_{75} \)) than intact animals while having similar peak conductance changes suggests a slower rate of recovery following PE in SCI subjects. This supports the hypothesis of a decrease in neuronal reuptake of PE following SCI, leading to a prolonged saturation of postsynaptic adrenoceptors and prolonged vasoconstriction. However, this may not be the case in the carotid artery if, as postulated before, the increased response was due to a relative increase in the density of the \( \alpha_{1D}^- \)-receptor, since Meth has little effect on the \( \alpha_{1B}^- \)-receptor. This is supported by the fact that in the carotid artery response to PE after ganglionic blockade, the size of the increase in \( T_{75} \) was the same as the increase in peak change in conductance (1.9- and 2.3-fold difference, respectively), indicating that the rate of change of the response was actually the same in intact and SCI responses. It appears that in that case, the prolonged \( T_{75} \) is a result of the greater peak change in conductance rather than an altered degree of neuronal reuptake. Further experiments could use a NET1 inhibitor during responses to PE to test the hypothesis that reduced neuronal NE reuptake is the mechanism of the change in the brachial, femoral, and renal arteries within our study.

Although our study found prolonged vascular conductance responses to PE following SCI, we did not find significantly different PE dose-response curves for SCI and intact arteries like Brock et al. (3) did. Possible reasons for the lack of adrenoceptor supersensitivity found in our study compared with that found in Brock et al. (3) include our in vivo systemic injection protocol compared with the direct agonist application technique used by Brock et al. (3). In addition, the artery type examined may affect results, considering that McLachlan and colleagues have reported that although the mesenteric artery shows supersensitivity to PE at week 7 (3), the tail artery does not (36, 37).

A widespread effect. This is the first study to report vascular changes both above and below injury level following high-level SCI. In fact, no other study has compared the peripheral responses of blood vessels above and below injury level following SCI after ganglionic blockade in vivo or in vitro. Most of the studies that have previously compared the responses of blood vessels above and below injury level in spinal patients (without the use of ganglionic blockade) have found that although vascular adaptations develop in regions below the injury level, they do not necessarily occur above the injury (6, 7). However, most of the subjects in these studies had injuries below T6, which is generally considered the critical level for development of AD and lowered basal MAP. One study of ultrasound images taken of the radial and posterior tibial arteries of SCI patients with injuries ranging from T2 to T11 (half with injuries below T6) reported that both arteries have impaired vascular relaxation in response to increased blood flow following occlusion compared with able-bodied subjects (31). This suggests that peripheral changes can occur in blood vessels above the SCI lesion, even in cases of relatively low-level SCI.

In a study of SCI patients with impaired sympathetic control (injuries from C7 to T4), Karlsson et al. (16) examined the blood pressure, NE spillover, NE clearance, and blood flow in the arm and leg at baseline and during bladder stimulation to produce AD. They found no significant difference in baseline levels between SCI and control groups; however, during bladder stimulation, SCI patients exhibited elevated blood pressure and leg NE spillover with significantly decreased leg blood flow. Interestingly, although NE spillover in the arm did not change during bladder stimulation, arm blood flow still decreased by the same relative amount as the leg. The fact that this decrease did not reach significance may be due to greater variability in the responses of the arm. Nevertheless, it suggests that blood vessels above the level of injury in high-SCI patients can respond vigorously to circulating catecholamines even though they have an intact vasomotor baroreflex.

The fact that enhanced PE responses were observed in blood vessels above and below injury level after SCI in our study suggests that the mechanisms producing the peripheral changes must be acting on vasculature throughout the body. These mechanisms include an impaired reuptake of PE and, in some beds such as the carotid vascular bed, a possible change in \( \alpha_{1}^- \)-receptor subtype density. The origin of these peripheral changes is likely to be systemic and secondary to the consequences of SCI on the entire organism. The most obvious and likely explanation would be that these changes are an adaptation to compensate for the lower basal blood pressure and reduced level of circulating NE in the vascular system. Although this adaptation may be beneficial at rest to help maintain MAP, it also causes hyperreactivity to circulating catecholamines that may be released during episodes of AD.

Conclusions. Taken together, our results suggest that widespread peripheral changes, including a decreased level of presynaptic reuptake of NE, occur following SCI, leading to exaggerated responses to NE. These widespread changes probably develop due to the lower basal MAP and NE levels present following high-level SCI and are likely to contribute to the development of AD in this group of patients. Further studies are needed to confirm the cause and time course of the development of the reduced reuptake and, hence, establish its role in the development of AD in animals and patients. Furthermore, such reduced reuptake could be a target of future interventions aimed at alleviating the symptoms and morbidity produced by AD.

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