

# Adrenergic Sensitivity of Uninjured C-fiber Nociceptors in Neuropathic Rats

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## Abstract

We investigated the adrenergic sensitivity of afferent fibers in the L4 dorsal roots of rats with a unilateral ligation of the L5–L6 spinal nerves. About 12% of nociceptive fibers on the affected side were excited by sympathetic stimulation or by intra-arterial injection of norepinephrine which did not affect A $\beta$ -fiber activity. Sympathetic excitation of nociceptive fibers was suppressed by  $\alpha_1$ -antagonist prazosin, while it was unaffected by  $\alpha_2$ -antagonist yohimbine. Most of these fibers were excited by intra-arterial injection of  $\alpha_1$ -agonist phenylephrine, without being affected by an injection of  $\alpha_2$ -agonist clonidine. Sympathetic excitation was blocked by lidocaine applied near the receptive fields of recorded fibers. The results suggested that some nociceptors remaining intact after partial nerve injury become sensitive to sympathetic activity by the mediation of  $\alpha_1$ -adrenoceptors in the peripheral endings.

**Key Words:** Peripheral nerve injury, neuropathy, causalgia, nociceptor, alpha-1 adrenoceptor

## INTRODUCTION

Partial peripheral nerve injury often leads to neuropathic pain states that are exacerbated by sympathetic efferent activity. A classic example is causalgia, which is characterized by spontaneous burning pain, hyperalgesia and allodynia.<sup>1</sup> The nerve injury-induced interaction between sympathetic efferent fibers and primary afferent neurons has been proposed as the basis for causalgia.<sup>2</sup> One such interaction is thought to occur within the dorsal root ganglion, and this has been supported by ample evidence provided by morphological as well as electrophysiological studies.<sup>3–6</sup> A second possible interaction could take place at the peripheral terminal region of afferent neurons. This possibility has been supported by clinical observations in causalgia patients that pain relieved by a sympathetic block was

induced again by the injection of norepinephrine into the previously painful skin.<sup>7</sup>

A controversy exists as to which adrenoceptor subtypes could be involved in mediating the sympathetic-sensory interaction at the peripheral terminal region. Clinical data suggest that the  $\alpha_1$ -receptor plays a pivotal role in mediating such an interaction. This is seen in causalgia patients where pain relieved by topically-applied  $\alpha_2$ -agonist clonidine was rekindled when  $\alpha_1$ -agonist phenylephrine was applied into the clonidine-treated area.<sup>8</sup> On the other hand, electrophysiological study has shown that following partial nerve section in the rabbit ear, some of the remaining intact nociceptive fibers developed adrenergic sensitivity at the receptive terminal region, which was blocked by  $\alpha_2$ -antagonists.<sup>9,10</sup>

It has been shown that rats with a tight ligation of L5–L6 spinal nerves develop behavioral signs of various forms of pain like those observed in causalgia, and that sympathectomy relieves these signs, indicative of a good model for causalgia.<sup>11,12</sup> In this rat model, the L4 spinal nerve contains afferent neurons with axons remaining uninjured and projecting into the skin of the hind paw.

The present study was undertaken using this rat model to see if afferent neurons with uninjured axons had developed adrenergic sensitivity and, if so, to

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determine which adrenoceptor subtypes are responsible for the mediation of this sensitivity.

## MATERIALS AND METHODS

Young adult male rats (Sprague-Dawley, 150–200 g) were used in this study. For the experimental group, the left L5 and L6 spinal nerves were isolated and tightly ligated with 6–0 silk thread under halothane anesthesia. For the control group, a sham operation was performed on the left side with the same surgical procedure as above except for spinal nerve ligation. The mechanical sensitivity of both hind paws of the animals was examined with a von Frey filament (30 mN) 2–4 weeks after surgery. The frequency of foot withdrawal was measured by counting the number of foot liftings in response to 10 applications of filament.

The rats were anesthetized with sodium pentobarbital (40 mg/kg i.p.) and prepared for acute electrophysiological recording. The right external jugular vein and trachea were cannulated. A laminectomy was performed to expose the spinal roots of T13–L5. The animal was mounted prone in a spinal frame, paralyzed with Flaxedil (10 mg/kg, i.v.) and artificially respirated. The end-tidal CO<sub>2</sub> level was maintained between 3.5 and 4.5%, and rectal temperature was maintained at 37 ± 0.5°C.

For single fiber recording, the L4 dorsal root was cut near the root entry zone. A bundle of nerve fibers separated from the distal cut end of the dorsal root was teased until a fine nerve filament was isolated. Recordings were made from an isolated nerve filament that was placed on a platinum hook recording electrode, with the reference electrode pinned to nearby tissue. Nerve impulses were amplified for display on an oscilloscope and for the construction of peristimulus time histograms of the discharge rates. Conduction velocity of the recorded fiber was determined by dividing the propagation distance between the stimulating and recording electrodes by the response latency to single pulses (0.3 ms, 0.05–3 mA, 1 Hz), where a stimulating electrode was placed across the sciatic nerve. For sympathetic stimulation, pulse-train stimuli (0.3-ms pulses, 0.5–4.0 mA, 30–50 Hz) were applied for 10 sec with a second stimulating electrode placed across the T13 and/or L1 ventral roots. This stimulation was reported to elicit

pre- and hence postganglionic sympathetic axons projecting into the hind limb.<sup>4,13</sup>

Adrenergic agents were administered by intravenous or intra-arterial injection. The latter was done via a catheter inserted into a side branch of the femoral artery at the popliteal region with a 100- to 200- $\mu$ l single bolus followed by a 100- $\mu$ l saline flush. Agents used were norepinephrine bitartrate, phentolamine mesylate, propranolol HCl, prazosin HCl, yohimbine HCl, phenylephrine HCl, and clonidine HCl.

Changes in the discharge rate after agonist injection, or after the onset of sympathetic stimulation, were obtained at times when the discharge rate had deviated from the basal impulse rate. When changes were greater than 30% of the basal rate, afferent fibers were considered responsive. Responses to sympathetic stimulation were compared before and after application of the antagonist. When the difference was more than 30%, the applied antagonist was considered to exert an effect.

## RESULTS

Rats with spinal nerve ligation showed a higher foot withdrawal frequency on the nerve-injured side than on the uninjured side in response to 10 applications of a von Frey filament with 30-mN bending force (6.3 ± 0.93 and 0.11 ± 0.12, respectively, n=34). This stimulation never evoked foot withdrawals from sham-operated animals. Our sampled afferents included 191 fibers isolated from the left L4 dorsal

Table 1. Proportion of Afferent Fibers Excited by Sympathetic Stimulation in Sampled Fibers from L4 Spinal Nerves of Nerve-Injured and Sham-Operated Rats

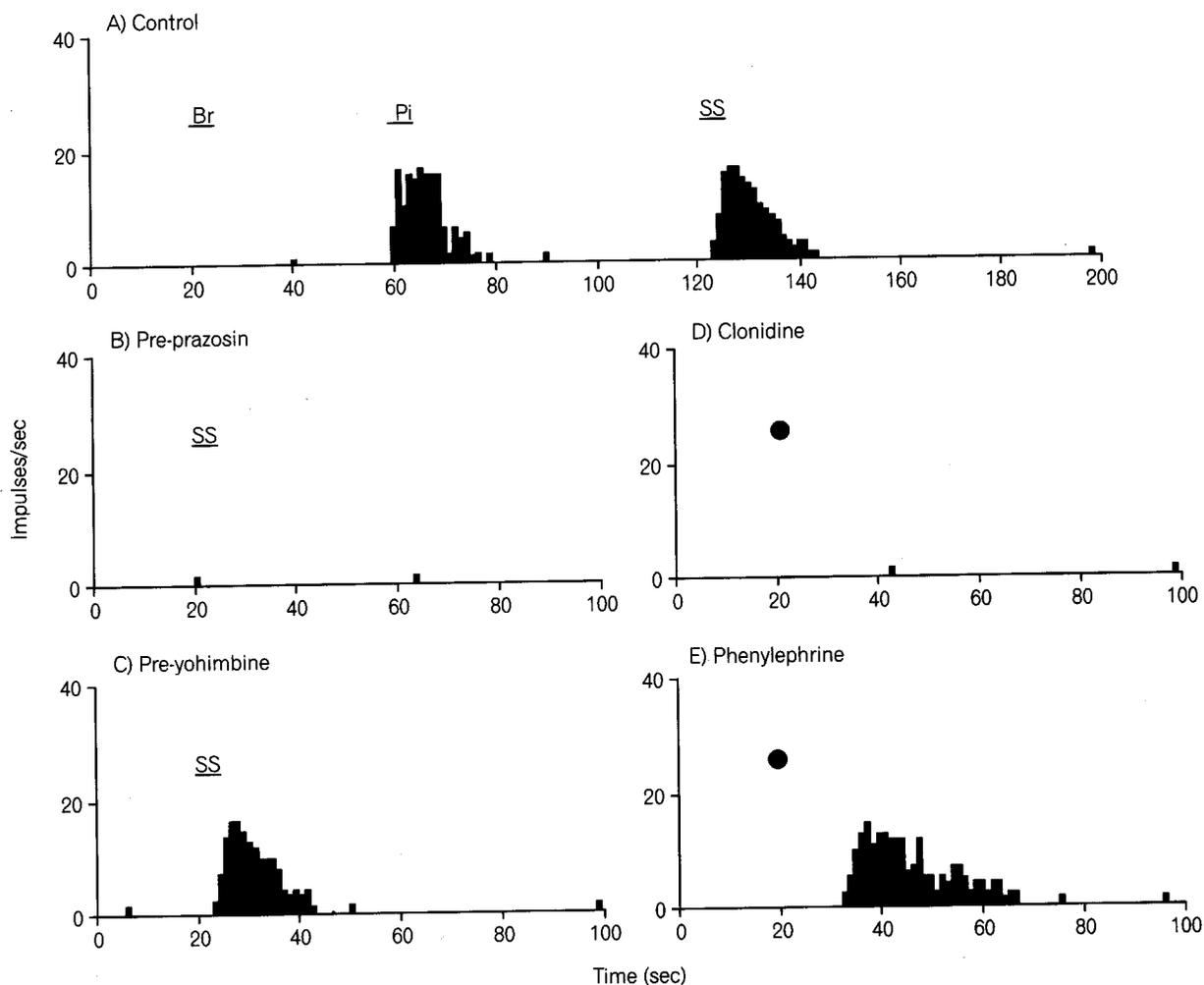
Fiber type	Nerve-injured rats		Sham-operated rats	
	N	Excited	N	Excited
A $\beta$	58	0	32	0
A $\delta$	38	5	21	0
C	95	11	25	0

Fiber types were categorized on the basis of the conduction velocity (A  $\beta$ , >25 m/sec; A  $\delta$ , 2.5–25 m/sec; C, <2.5 m/sec). N, number of sampled afferent fibers; Excited, number of afferent fibers responding with increased impulses to sympathetic stimulation.

roots of rats with spinal nerve ligation and 78 fibers from those of sham-operated rats (Table 1). These fibers were divided into 3 groups on the basis of the conduction velocity: A  $\beta$ - (n=58, range; 25.4–45.7 m/s, mean; 32.8 m/s), A  $\delta$ - (n=38, range; 4.3–14.1 m/s, mean; 9.9 m/s) and C-fibers (n=95, range; 0.45–1.98 m/s, mean; 1.33 m/s).

We examined sampled afferent neurons to determine whether their nerve activity was influenced by sympathetic nerve stimulation performed by electrically stimulating the T13 and/or L1 ventral roots. Among sampled afferent fibers from the L4 dorsal

roots of nerve-injured rats, all A  $\delta$ - and C-fibers were nociceptive neurons because they were responsive to pinching, but not to touching, the skin of the receptive field. About 12% of these nociceptive fibers (5 of 38 A  $\delta$ - and 11 of 95 C-fibers) were excited by sympathetic stimulation, which produced no effects on A  $\beta$ -fiber activity. All sympathetically-excited nociceptive fibers were also excitable when norepinephrine (0.5–1  $\mu$ g/kg) was injected arterially into a side branch of the femoral artery in the popliteal region. On the other hand, none of the afferent fibers from corresponding dorsal roots of



**Fig. 1.** A typical example of alpha-1 receptor-mediated adrenergic excitation of an afferent neuron with uninjured axon. A: This afferent fiber having the conduction velocity of 1.37 m/sec was responsive to pinching (Pi) but not to brushing (Br) the skin of the receptive field and was also excited by sympathetic stimulation (SS). B: When prazosin was administered intravenously (1 mg/kg) 3 min prior to sympathetic stimulation, this fiber was no longer excited by sympathetic stimulation. C: Prior-administration of yohimbine (1 mg/kg, i.v.) did not prevent this afferent fiber from being excited by sympathetic stimulation. D and E: The same afferent fiber was unresponsive to intra-arterial injection of clonidine (10  $\mu$ g/kg) while being excited by phenylephrine injection (10  $\mu$ g/kg). Dots indicate the time points of intra-arterial injection.

Table 2. Actions by Alpha Antagonists and Agonists on Afferent Fibers Showing Sympathetic Excitation

Antagonist	Sympathetic excitation		Agonist	Background activity	
	Blocked	Unchanged		Excited	Unchanged
Prazosin	13	3	Phenylephrine	13	3
Yohimbine	1	15	Clonidine	0	16

Data indicate the number of afferent fibers affected in their sympathetically-evoked excitation by systemic administration of alpha antagonists or in their background activity by intra-arterial injection of alpha-agonists. Unchanged, afferent fibers in which the response to sympathetic stimulation or background activity were changed less than 30%.

sham-operated rats were responsive to sympathetic stimulation or norepinephrine injection. For 5 nociceptive fibers showing sympathetic excitation in nerve-injured rats, excitatory responses were examined to see if they were affected by subcutaneous injection of lidocaine near the cutaneous receptive field. Excitatory responses in all tested fibers disappeared as a result of this conduction blockade, suggesting that the generation of excitation occurred at the receptive terminal region.

We investigated the type of adrenoceptors responsible for mediating the sympathetic excitation of nociceptive fibers observed in nerve-injured rats. We first tested with nociceptive fibers showing sympathetic excitation to determine whether this excitation was affected by  $\alpha$ - or  $\beta$ -adrenergic antagonist injected intravenously prior to sympathetic stimulation. Phentolamine (100  $\mu$ g/kg), the  $\alpha$ -antagonist, suppressed nociceptor excitation elicited by sympathetic stimulation, whereas the same dose of  $\beta$ -antagonist propranolol was without effect (4 nociceptive fibers tested). Based on this finding, attempts were made to determine which  $\alpha$ -receptor subtype was responsible for the mediation of sympathetic excitation. As shown in Fig. 1, this fiber, which was unresponsive to brushing, responded to pinching the skin of the receptive field with vigorous discharges that continued for about 15 seconds even after the pinching ceased. This fiber was also excited by sympathetic stimulation. When  $\alpha_1$ -antagonist prazosin was administered intravenously (1 mg/kg) 3 min prior to sympathetic stimulation, this fiber was no longer excited by sympathetic stimulation. On the other hand, prior-administration of  $\alpha_2$ -antagonist yohimbine (1 mg/kg, i.v.) did not prevent this fiber from being excited by sympathetic stimulation. The same fiber was unresponsive to intra-arterial injection

of  $\alpha_2$ -agonist clonidine (10  $\mu$ g/kg) into a side branch of the femoral artery, while it was excited by  $\alpha_1$ -agonist phenylephrine injection (10  $\mu$ g/kg, i.a.). Table 2 shows the summary data on this type of experiment performed with nociceptive fibers excited by sympathetic stimulation. Prazosin administered intravenously prior to sympathetic stimulation blocked 81% of nociceptive fibers tested (13 of 16) in their sympathetically-induced excitation, with 3 fibers remaining unaffected. On the other hand, prior-administration of yohimbine was without effect on sympathetic excitation for all nociceptive fibers except one, in which sympathetic excitation was a little suppressed. Intra-arterial injection of phenylephrine produced an excitatory response in 13 of 16 nociceptive fibers tested, whereas clonidine injection elicited no response for all tested fibers.

## DISCUSSION

The present study demonstrated that some nociceptive fibers in the peripheral nerve trunk, which remained intact after the adjacent axons were injured, developed adrenergic sensitivity at the receptive regions. This adrenergic sensitivity was blocked or mimicked by  $\alpha_1$ -antagonist or agonist, respectively, while being unaffected by  $\alpha_2$ -adrenergic agents, suggesting  $\alpha_1$ -adrenoceptor involvement in mediating this type of sensitivity. Our results are comparable to clinical data on causalgia patients, in which pain relieved by topically-applied  $\alpha_2$ -agonist clonidine was rekindled when  $\alpha_1$ -agonist phenylephrine was applied into the clonidine-treated area.<sup>8</sup> This led us to hypothesize that after partial nerve injury, uninjured nociceptors develop sensitivity to norepinephrine (NE) through the expression of  $\alpha_1$ -receptors in their

terminals. In this situation, sympathetic efferent activity would lead to ongoing activity in the nociceptors, perhaps contributing to the ongoing pain observed in patients with partial nerve injury, as is the case in causalgia.

Although the expression of adrenoceptors was reported to occur in somata of the rat primary afferent neuron following peripheral nerve injury,<sup>14</sup> direct evidence of their expression in receptive terminals of primary afferent neurons is lacking. The clinical data has shown an increased density of  $\alpha_1$ -adrenoceptors in the hyperalgesic skin of causalgia patients.<sup>15</sup> It is tempting to speculate that  $\alpha_1$ -adrenoceptors developed in nociceptive nerve terminals after nerve injury in our rat preparations. The development of adrenergic sensitivity in nociceptors raises the possibility that sympathetic efferent activity exacerbates the pain state produced by nerve injury. In support of this, we observed that spontaneous pain behavior produced in rats by spinal nerve ligation was exacerbated in a cold state and that this cold stress-induced pain behavior was alleviated by phentolamine treatment.<sup>16</sup>

Excitation of nociceptors by NE seen in this study may be due either to the direct action of NE on  $\alpha_1$ -adrenoceptors in the membrane of nociceptors or to the indirect action of NE. In the latter case, NE may act initially on the sympathetic endings which secondarily release such local mediators as prostanoids, leading to the activation of nociceptors. Evidence in favor of the direct action of NE was provided by the observation that the responsiveness of injured afferent fibers to systemically applied NE persists in rats with a prior sympathectomy.<sup>17</sup> However, the possibility of the indirect action of NE could not be excluded since in our rat preparations, the L4 spinal nerve was intact, and thus sympathetic postganglionic endings in the affected hind paw were present. Tracey et al. obtained evidence of a dual contribution of  $\alpha_1$ - and  $\alpha_2$ -adrenoceptors to hyperalgesia after partial transection of the rat sciatic nerve, in which the exacerbation of hyperalgesia by NE is likely mediated by  $\alpha_2$ -adrenoceptors located on sympathetic postganglionic terminals.<sup>18</sup>

Our data showed that some nociceptors which were uninjured and had developed adrenergic sensitivity produced a post-discharge to noxious stimulation that was indicative of being sensitized. A possible explanation for this sensitization is due to actions by

neuropeptides, such as substance-P, released at the endings of adjacent-injured nociceptive fibers as a result of antidromic impulses produced in these fibers by injury to the L5 and L6 spinal nerves. This possibility is supported in part by clinical observation in causalgia patients where local capsaicin application, which locally depletes substance-P in nociceptive fibers, relieved pain.<sup>19</sup> A recent study on rats with loose ligation of the sciatic nerve demonstrated that increased release of neuropeptides and inflammatory responses were induced in the affected hindpaw.<sup>20</sup> We speculate that injury to nociceptive fibers causes neuropeptide release from the peripheral terminal to induce neurogenic inflammation that leads to increased excitability of adjacent nociceptors. In regard to adrenergic sensitivity that was attained by sensitized nociceptive fibers, inflammatory mediators and/or neuropeptides may also play a substantial role in its development. This possibility is supported by the finding that endogenous NE release, which did not influence thermal threshold in the normal skin, was able to markedly increase heat sensitivity in skin previously sensitized by topical application of capsaicin, and that this enhanced thermal hyperalgesia was mediated by  $\alpha$ -adrenoceptors.<sup>21</sup>

In conclusion, our results indicate that after partial peripheral nerve injury, some cutaneous nociceptors which remain intact develop an abnormal excitability to either sympathetic stimulation or intra-arterial injection of norepinephrine. Activation of  $\alpha_1$ -adrenoceptors is involved in the mediation of this type of excitation. The  $\alpha_1$ -receptor-mediated adrenergic excitation of nociceptors may contribute in part to sympathetically-related pain as seen in causalgia.

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