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21. In other experiments, ethanol and polyethylene glycol enhanced the activity of these ribozymes.
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Adrenergic Excitation of Cutaneous Pain Receptors Induced by Peripheral Nerve Injury

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The mechanisms by which peripheral nerve injuries sometimes lead to causalgia, aberrant burning pain peripheral to the site of nerve damage, are uncertain, although the sympathetic nervous system is known to be involved. Whether such syndromes could be the result of the development of responsiveness by some cutaneous pain receptors (C-fiber nociceptors) to sympathetic efferent activity as a consequence of the nerve injury was tested in an animal model. After nerve damage but not in its absence, sympathetic stimulation and norepinephrine were excitatory for a subset of skin C-fiber nociceptors and enhanced the responsiveness of these nociceptors to tissue-damaging stimulation. These effects were demonstrable within days after nerve lesions, occurred at the cutaneous receptive terminal region, were manifest in sensory fibers that had not degenerated after the injury, and were mediated by α_2-adrenergic–like receptors.

CAUSALGIA, A DEBILITATING SYNdrome that develops after some peripheral nerve injuries, is characterized by severe burning pain initially localized to the skin innervated by the injured nerve (*1*). Frequently, causalgic pain is associated with sympathetic nervous system effects such as regional alterations in cutaneous blood flow and perspiration. This association, and the relief that can be provided by interruption of the sympathetic nervous supply to the affected body region, have led to characterization of causalgia and its pain as a reflex sympathetic dystrophy (*2*). Injury-induced interactions between sympathetic efferent postganglionic axons and cutaneous sensory fibers have been proposed as the basis of causalgic pain (*1*); however, sympathetic stimulation (SS) and sympathetic chemical mediators neither excite nor enhance the activity of pain receptors (nociceptors, that is, noxious stimulus receptors) of normal skin (*3*). Because SS does increase the responsiveness of some sensory units associated with nonpainful cutaneous mechanoreception (*3*), some proposals suggest central neural processes rather than peripheral interactions between efferent sympathetic neurons and specific sensory receptors for pain as the basis for causalgia (*2, 4*). We examined the influence nerve lesions have on the way nociceptors respond to sympathetic action, to clarify the relation of presumed pain receptors to causalgia.

The great auricular nerve was exposed with sterile surgical procedures in anesthetized New Zealand white rabbits and damaged in one of three ways: (i) a partial cut with scissors; (ii) two ligatures separated by 5 mm tied tightly enough to decrease blood flow through the nerve (*5*); (iii) a 30-s stretching of the connective tissue surrounding the nerve sufficient to interfere with blood flow. The animals were then monitored for 4 to 148 days; no signs of trophic changes in the ear or discomfort were apparent (*6*). Terminally, recordings were made with platinum hook electrodes from fine filaments of the nerve 15 to 20 mm central to the nerve injury; a filament was dissected until discharges were identified from a single cutaneous afferent fiber conducting <1.5 m/s (C-fiber) across the injury site (*7*). Sensory units identified as C-fiber polymodal nociceptors of the skin (CPMs), which have been linked to cutaneous pain, were selected for analysis (*8*). C-fiber sensory units of the great auricular nerve do not regenerate and show recognizable afferent properties for at least 30 days after nerve crush or transsection (*9*). Most of our observations were made on nerves injured less than 30 days previously, and therefore they represented recordings from fibers spared from degeneration by the injury. We stimulated the units with a heating and cooling sequence delivered by a counterbalanced 50-mm^2 thermode (*10*). The CPMs of hairy skin typically sensitize on repeated exposure to moderately noxious heat, that is, on a second test they generate more impulses to a given heat stimulus and the threshold temperature for heat decreases (*8, 10*). Activation and sensitization of the CPM fibers by heat are indices of the responsiveness of their peripheral receptive terminals to skin stimulation (*10*).

In control animals, CPM units sensitized as expected (*8, 10*): the mean number of impulses produced to a second heat cycle doubled compared to values from the initial trial (Table 1). SS rostral to the superior cervical ganglion in control animals (20 stimuli per second for 30 s, 5 min before beginning of the thermal cycle, causing visible vasoconstriction in the ear and a 0.5°C drop in temperature at the receptive field) produced no difference in threshold or in the mean response of CPM units to the initial thermal stimulation (*3*). SS before a second thermal test cycle suppressed the increased response expected during the active heating stage (Table 1).

In control animals, SS by itself did not excite CPM units (*3*). In contrast, after all three kinds of nerve injury, some CPM units were directly excited by SS before the first heat exposure (Table 2). This response was a low-frequency discharge during electrical stimulation of the ascending cervical sympathetic trunk and for a short period afterward (*11*). Direct excitation by SS occurred in about 20% of the units from damaged nerves, as early as 7 days after injury. Of 65 units tested 24 days or less after nerve injury, 10 were directly excited. Close arterial injections of norepinephrine (NE) also never excited CPM units of control animals (*11*). On the other hand, such NE injections activated 27 of 65 units from animals within 30 days or less after nerve injuries (Table 2 and Fig. 1). These evoked responses to SS and NE had long latencies (*11*), suggesting

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an indirect mechanism. The excitation by SS or NE did not appear to be related to vasoconstriction or decrease of blood flow because equivalent vasoconstriction and skin temperature decreases produced by the hormone vasopressin introduced by the same route did not activate CPM units (Fig. 1). The injected NE acted at the peripheral terminal region to excite the units rather than at the site of nerve injury, since its excitation could be reversibly blocked by a local anesthetic (Fig. 1D).

Sympathetic adrenergic actions in cutaneous tissues are generally produced through activation of membrane receptors (*12*). To test the involvement of adrenergic receptors in the direct excitatory effects, the SS and NE excitation was challenged by selective adrenergic antagonists. Yohimbine and rauwolsine, agents that are more potent at α_2- than α_1- or β-adrenergic receptors, blocked SS and NE excitation of CPM units of injured nerves in doses that produced modest decreases (20 mmHg) in central arterial pressure. Prazosin, an antagonist that is more effective at α_1- than α_2-adrenergic receptors, was less effective in interfering

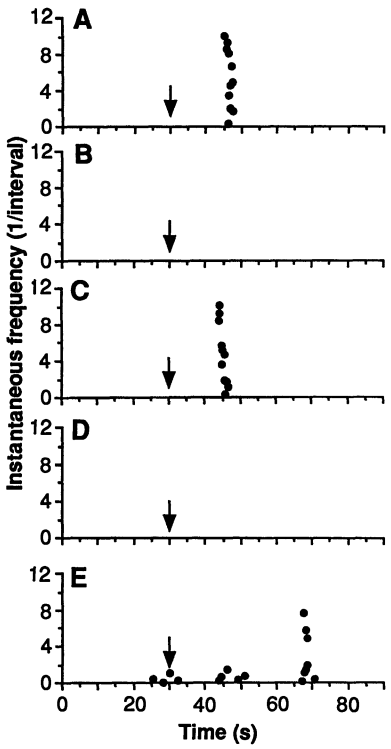

Fig. 1. Responses by CPMs in the great auricular nerve to close arterial injection of NE 17 days after partial cut injury. Arrow indicates time of injection of NE (200 ng/0.2 ml). (**A**) Initial injection, (**B**) 15 min after yohimbine (1.0 mg/kg) intraverously, (**C**) 66 min after yohimbine dose (recovery), (**D**) 5 min after local infiltration of mechanically responsive receptive field with 0.25% lidocaine, (**E**) 91 min after (D) (recovery from receptive field infiltration with lidocaine). The unit was not excited by vasopressin (0.6 units) injected intraarterially.

Table 1. Responses (mean number of impulses ± SEM) of CPMs from normal (control) and injured nerves to a thermal stimulus (*7, 8, 10*). Total indicates impulses recorded during the preliminary (Pre), stimulatory (Stim), and recovery phase during a 250-s stimulus cycle (*10*). *P* values obtained by one-way ANOVA and Fisher's test.

Nerve injury (*n*)	Phase	Stimulus 1 (no. of impulses)	Stimulus 2 (no. of impulses)
Control (28)	Pre	0.43 ± 0.17	0.71 ± 0.23
	Stim	16.79 ± 1.35	32.79 ± 4.88*
	Total	34.54 ± 3.54	53.86 ± 8.62
Control + SS (12)	Pre	0.25 ± 0.18	0.33 ± 0.19
	Stim	14.67 ± 1.33	15.58 ± 2.38
	Total	27.25 ± 4.52	30.00 ± 6.60
Partial cut + SS (24)	Pre	0.71 ± 0.35	1.21 ± 0.40
	Stim	14.83 ± 1.21	29.79 ± 4.45*
	Total	35.83 ± 5.26	57.33 ± 8.69*
Stretch + SS (14)	Pre	0.50 ± 0.36	1.14 ± 0.54
	Stim	12.14 ± 0.79	23.36 ± 4.67
	Total	36.57 ± 7.38	57.29 ± 10.25*
Ligature + SS (12)	Pre	1.17 ± 0.44	0.83 ± 0.30
	Stim	18.25 ± 2.29	33.25 ± 3.70*
	Total	46.08 ± 5.52*	67.25 ± 8.58*

*$P < 0.05$ versus control + SS.

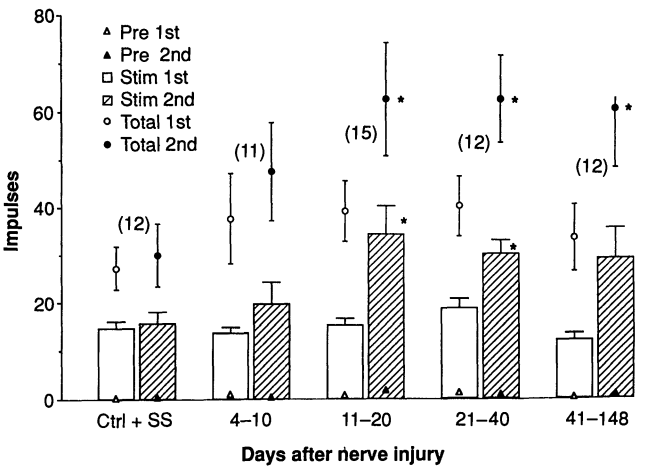

Fig. 2. Effects of time after nerve lesion on average response of CPMs to a stereotyped thermal stimulation (three-stage heating-cooling cycle). Open bars and open symbols, first heating cycle; closed bars and closed symbols, second heating cycle. The ipsilateral sympathetic trunk was stimulated for 30 s, 5 min before beginning of the heating cycle (Pre). Total indicates the impulses recorded during the preliminary, stimulatory (Stim), and recovery phase (Table 1) (*10*). Number of units for each category are in parentheses. Ctrl + SS, units from uninjured nerves. Days 4–10, 11–20, 21–40, and 41–148, units recorded after the period indicated from the time of a nerve lesion. Data from animals with different kinds of nerve lesions were pooled for these comparisons; the number of units at different survival times from each type of lesion was too small for valid comparison. Shown as mean ± SEM. *$P < 0.05$ versus control + SS.

Table 2. Direct excitation of CPM units by SS or close arterial injection of NE and the effects of α-adrenergic receptor antagonists. *n*, Number of units tested before heat stimulation; excited, number of units exhibiting discharges within 180 s after SS or NE (2 × SEM greater than background). Control, units from unoperated animals; lesion, units from nerves with lesions, preponderantly the partial cut type injury, 15 to 40 days after the nerve was damaged. The bottom of the table shows the number of excited units for which a reversible complete (Ab.) or partial (Decr. > 50%) block of discharge evoked by SS or NE was produced. Un, unchanged. Agents were given intravenously, producing 20 to 30 mm Hg decreases in systemic arterial pressure [yohimbine (0.3 to 1 mg/kg), rauwolsine (1 mg/kg), and prazosin (0.1 mg/kg)]. Most units directly excited by SS were also tested with NE injections.

Type	Sympathetic stimulation			Norepinephrine		
	n	Excited		*n*	Excited	
Control	27	0		15	0	
Lesion	101	20		71	.31	
Agent	Ab.	Decr. >50%	Un.	Ab.	Decr. >50%	Un.
Yohimbine	4	1	0	14	0	0
Rauwolscine				4	0	0
Prazosin				0	2	1

with NE excitation (Table 2) even though it produced equal or greater decreases in arterial pressure.

After nerve injury, SS failed to suppress sensitization in CPMs; the average SS-conditioned sensitization in units from injured nerves was significantly ($P < 0.05$) greater than SS-conditioned activity from units of uninjured nerves (Table 1). Absence of SS suppression of sensitization could represent loss of sympathetic efferent action caused by block of conduction of sympathetic fibers by nerve injury; however, the parallel appearance of direct SS and NE excitation suggested an active process. Furthermore, the effects of SS on thermal sensitization varied with time after the lesion. The degree of sensitization in the presence of SS was greater and more consistent for units studied 11 days or more after nerve lesion than in the group analyzed after 4 to 10 days (Fig. 2).

In normal animals, with SS the number of the CPM units showing smaller or greater responses to the second thermal stimulus sequence were about equal (Fig. 3). In contrast, after each type of nerve injury, with SS more units showed a greater response in the second heat test than the control group. Further, some units from lesioned nerves exhibited greater sensitization with SS conditioning than any of the controls (Fig. 3). Thus, in damaged nerves, a marked excitatory effect of SS on sensitization was evident in a fraction of the CPM population, corresponding to the fraction directly excited by SS and NE.

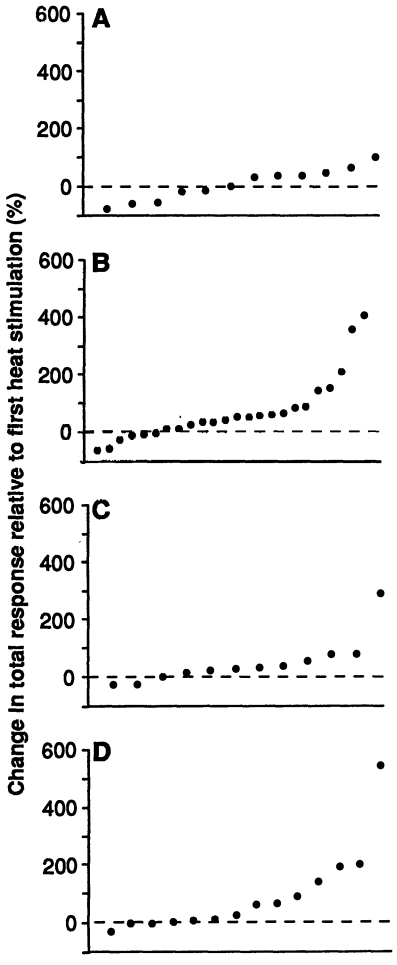

Fig. 3. Effects of SS on response of individual CPMs to a repeated thermal (heat) stimulation. For each unit the total response to the first stimulation cycle was taken as 0% with plotted point representing the response to the second stimulation cycle relative to it. Units arbitrarily arranged within each group from least to greatest difference. (**A**) Control + SS. (**B**) Partial cut + SS. (**C**) Ligature + SS. (**D**) Stretch + SS.

Our data show that partial injury of a mixed peripheral nerve initiates circumstances in which sympathetic activity and NE excite or enhance the responsiveness of a proportion of C-fiber sensory units putatively involved in cutaneous pain (*13*), effects that depend on α_2-adrenergic receptors. The appearance of adrenergic excitation of afferent fibers in our experiments was not at nerve injury sites (*14*), but was manifest at the receptive terminals. Moreover, the affected units had been spared serious damage by the nerve injury. The involvement of adrenergic receptors and the time course of the effects is similar to the supersensitivity that follows removal of sympathetic innervation from effector organs (*15*). The great auricular nerve, like many peripheral nerves, contains postganglionic sympathetic efferent fibers (*16*) and a variety of sensory fibers. Thus, the number of α_2-adrenergic receptors in some element of CPM excitation may increase as a consequence of partial sympathetic denervation by the nerve injury. Our data do not permit differentiation of effects stemming from injury of primary afferent or sympathetic efferent fibers or to determine whether the mediating α_2-adrenergic receptors were located on the CPM peripheral nerve terminals.

Thus, interuption of conduction in some fibers of a nerve can evoke an induction or up-regulation of α_2-adrenergic receptors or their actions in otherwise normal sensory nerve terminals. Our observations implicate alterations in responsiveness of cutaneous nociceptors as an etiological factor in causalgic syndromes, but they do not eliminate central nervous mechanisms in the pattern of the disease (*1, 2, 4*).

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