Sympathetically Correlated Activity of Dorsal Horn Neurons in Spinally Transected Rats

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Chau, David, Namjin Kim, and Lawrence P. Schramm. Sympa- and linear coherence, neurons have been identified in the brain stem with ongoing activity that is highly correlated to rhythms of simultaneously recorded, ongoing, peripheral sympathetic activity (Barman and Gebber 1981; Barman et al. 1995; Gebber et al. 1995; Zhong et al. 1993, 1995). These sympathetically correlated neurons are generally considered to be putative “presympathetic neurons,” 1) members of networks that generate or inhibit sympathetic activity or 2) neurons projecting brain stem-generated, sympathoexcita- or sympathoinhibitory information to spinal levels.

Spinal systems that affect sympathetic activity have received much less attention than brain stem sympathetic systems. Indeed, this laboratory has conducted the only simultaneous recordings of sympathetic nerve activity and the activity of spinal interneurons in spinally transected animals (Poree and Schramm 1992). Spinal sympathetic systems, however, deserve detailed investigation, for they may generate significant levels of sympathetic nerve activity in intact animals (Taylor and Weaver 1993), and, more importantly, they are responsible for all of the sympathetic nerve activity that is generated caudal to serious spinal cord injuries (Mathias and Frankel 1983).

Sympathetic preganglionic neurons do not themselves generate ongoing sympathetic activity in vivo (Dembowsky et al. 1985; McLachlan and Hirst 1980). Therefore sympathetic activity must be generated by the synaptic antecedents of preganglionic neurons. Logically, after spinal cord transection these antecedents reside only in the spinal cord. We anatomicoly identified candidates for spinal presympathetic neurons in experiments in which we injected pseudorabies virus into the kidneys of rats (Schramm et al. 1993). Dorsal horn neurons (DHNs) became infected via retrograde trans- port of the virus across their synapses either on renal sympa-thetic preganglionic neurons or on other synaptic antecedents of renal sympathetic preganglionic neurons (Schramm et al. 1993). The segmental distribution of infected DHNs corre-sponded exactly to that of infected renal sympathetic pregan- glionic neurons, and the modes for both distributions were located in the T10 spinal segment. The close correlation be-tween the longitudinal distributions of infected renal sympa-thetic preganglionic neurons and infected DHNs suggested the hypothesis that the infected subset of DHNs might play a role in the generation of renal sympathetic activity, espe-cially after spinal cord transection. By analogy with experi-ments conducted on brain stem generators of sympathetic activity, we reasoned that this hypothesis would be supported
if the ongoing activity of a subpopulation of DHNs was closely correlated with simultaneously recorded, ongoing renal sympathetic nerve activity (RSNA). Further, we reasoned that if these neurons played a role in generating or conveying input to sympathetic preganglionic neurons, then maneuvers that altered their activity should concurrently alter RSNA.

We identified a population of DHNs, restricted to ipsilateral, caudal thoracic segments, whose ongoing activity was strongly correlated with ongoing bursts of RSNA. Somatic stimulation that increased the activity of these neurons synchronously increased RSNA. Somatic stimulation that decreased the activity of most of these neurons simultaneously decreased RSNA. Taken together, these data provide the first electrophysiological evidence for a subpopulation of DHNs that consists of excitatory synaptic antecedents to renal sympathetic preganglionic neurons.

METHODS

Surgery

Adult male Sprague-Dawley rats (Taconic Farms and Charles River), each weighing 250–350 g, were used in these experiments. All procedures for these experiments were approved by the Animal Care and Use Committee of the Johns Hopkins University School of Medicine (protocol 94M242). Pretreatment with atropine (0.05 mg/kg sc) reduced nasal and tracheal mucus secretion. Anesthesia was induced by ether inhalation and continued by α-chloralose (100 mg/kg iv) delivered via the right femoral vein. Rats were shaved and marked with a reference grid for mapping responses to somatic stimulation (Fig. 6). The right femoral artery was cannulated for measuring arterial pressure. The trachea was intubated for artificial respiration. Rats were mounted in a stereotaxic frame and paralyzed with gallamine triethiodide (Flaxedil, 40 mg/kg iv). Rats recovered from the effects of Flaxedil every 30–60 min, permitting an assessment of the depth of anesthesia. Anesthetic (α-chloralose) was supplemented (25 mg/kg) as necessary to keep rats at a surgical plane throughout the experiments. Body temperature was monitored with a rectal probe and maintained at 37°C with a heating pad and heat lamp.

The upper cervical spinal processes and the dura matter were removed to expose the C1–C3 spinal segments for spinal cord transection. Immediately after complete spinal cord transection between C1 and C2, 1 ml of human serum albumin (Baxter Healthcare) was injected (25% solution, 12.5 g/ml 50 ml iv) to return arterial pressure to ±90 mmHg. A second laminectomy was performed to expose the spinal cord at the level of the neuronal recording (i.e., at either T2, T8, T10, T13, or L2). The exposed spinal cord was covered by a pool of warm mineral oil. A bilateral pneumothorax was performed to reduce respiratory movements. The spinal cord was stabilized by clamping two processes: one just rostral to the level of the neuronal recording and the other at sacral levels. The left kidney was approached via a left flank laparotomy. After the kidney was retracted, the adrenal gland and fat covering the psoas and the paraspinal muscles were deflected away from the renal nerve, which was usually located at the juncture of the aorta and the renal artery. The nerve was then dissected from the surrounding tissues, placed on a bipolar hook electrode, and covered with warm mineral oil.

Extracellular and renal nerve recording

The extracellular recordings were made with single-barrel carbon fiber microelectrodes (impedance 2–4 MΩ) connected to a high-impedance probe (Grass HIP5). The resulting signal was filtered (300–3,000 Hz half-power cutoff frequencies) and amplified 50,000 times (GRASS P5 AC amplifier). The single neurons’ action potentials were discriminated by a dual-window discriminator (BAR Electronics). Neurons selected for recording were spontaneously active. We are confident that these neurons represented DHNs, rather than sympathetic preganglionic neurons or the axons of primary afferents, for the following reasons. First, we rarely recorded at depths at which sympathetic preganglionic neurons are found. Second, the intraburst interspike intervals of neurons located at even our most ventral recording sites were much shorter than those reported for sympathetic preganglionic neurons in rats (Gibbey et al. 1986) or cats (Gebber and McCall 1976). Finally, the durations of these neurons’ action potentials ranged from 1 to 1.5 ms, substantially longer than the 0.3- to 0.7-ms duration of action potentials we recorded from primary afferents in Lissauer’s tract, the lateral funiculus, or the dorsal columns. In most electrode tracks, the first neuron encountered was selected for recording, after which the recording site was marked with an electrolytic lesion (60–90 μm diam) by delivery of an anodal current through the recording electrode (15 μA for 10–15 s). In the remaining electrode tracks, all neurons encountered in a single track were recorded, and a lesion was made at the deepest recording site. For these tracks, more superficial recording sites were reconstructed from this lesion based on readings from the microdrive.

RSNA was recorded with bipolar electrodes constructed from 60-μm stainless steel wire. Afferent renal nerve activity was minimal in most of our preparations, for the activity recorded after crush of the nerve proximal to the electrode at the end of experiments (to obtain 0 nerve activity) was negligible. Therefore the renal nerve distal to the bipolar electrode was often left intact. The observed correlations between renal nerve activity and the activity of DHNs could not have been due to the activation of DHNs by renal afferents, for correlated bursts of activity in DHNs clearly preceded activity in the renal nerve.

Renal sympathetic activity was amplified 10,000 times (GRASS P15 AC and GRASS P5 AC amplifiers). Filters were set at 100–3,000 Hz half-power cutoff frequencies on both amplifiers. At the end of each experiment, conduction in the renal nerve was abolished by crush or transection to determine the zero level of RSNA. Arterial pressure, RSNA, single-neuron activity, and a cutaneous stimulation indicator were recorded on VHS video tapes for offline analysis.

Somatic stimulation

Innocuous somatic stimulation (light brush with a cotton applicator) and noxious somatic stimulation (pinch with toothed forceps, maintained for 5–10 s) were delivered to sites delineated by the reference grid (Fig. 6). A response in either the RSNA or the single-neuron activity was considered excitatory or inhibitory if it represented an increase or decrease, respectively, of ±10% from the prestimulus control level.

Classification of neurons’ afferent modalities

Neurons were classified as low threshold (LT) if they were excited by brush and not further affected by pinch, wide dynamic range (WDR) if they were excited by brush and excited more by pinch, and high threshold (HT) if they were excited only by pinch. Six DHNs were inhibited by either innocuous or noxious stimulation of their (normally excitatory) primary dermatomes, or they responded to neither brush nor pinch. We classified these neurons as “other”.

Spike-triggered averaging

Spike-triggered averages of ongoing RSNA were calculated for all recorded neurons. On detection of a neuron’s action potential,
an epoch of renal nerve activity (rectified and filtered, time constant = 0.04 s) was extracted from the continuous renal nerve recording. The epoch began 300 ms before the occurrence of the action potential and lasted 700 ms after the action potential. Further unit detection was disabled for ≥1 s to avoid the overlapping of RSNA epochs. At least 300 epochs were collected and averaged for each neuron. We calculated a control or ‘dummy’ average for the same RSNA, using as a trigger an electronically generated pseudorandom signal with a frequency approximately equal to that of the recorded neuron.

A ‘correlation index’ was calculated from each spike-triggered average as the ratio of 1) the largest peak in the spike-triggered average occurring <100 ms after the onset of DHN action potentials to 2) the largest peak-to-peak deflection occurring at any time in the respective dummy average. For purposes of classification, neurons were considered correlated to RSNA (henceforth ‘sympathetically correlated’) if the correlation indexes ≥ 2 (Fig. 2) and spike-triggered averages generated from nonoverlapping subsections of their recordings had similar peaks at similar latencies.

Interspike interval analysis

Interspike interval histograms (ISIHs, maximum interval 1 s, bin size 10 ms) were generated for all neurons. The histograms were computed using either 4 min of recordings of ongoing neuronal discharges or 1,000 action potentials, whichever occurred first. The degree of skewness (Sokal and Rohlf 1969) and the mode of each ISIH were computed as measures of the degree to which neurons’ action potentials occurred in bursts.

Histology

At the end of experiments, rats were perfused transcardially with buffered saline, followed by 10% buffered formaldehyde. The relevant spinal cord segments were removed and stored in sucrose-formaldehyde solution (30% sucrose in 10% phosphate-buffered formaldehyde, pH 7.4) for 2–5 days. Transverse 40-μm sections were cut on a sliding microtome, mounted on gelatin-coated glass slides, and air dried. The sites of electrolytic lesions were identified microscopically.

Data presentation and statistical analysis

Data are expressed as means ± SE. All statistical analyses employed either a one-tailed Fisher’s exact test or a χ² test. Values of P < 0.05 were considered significant.

RESULTS

Sample of DHNs

In 56 spinally transected rats we simultaneously recorded the ongoing activity of the left renal sympathetic nerve and the activity of single DHNs, ipsilateral and contralateral to the renal nerve recording (Table 1). Spike-triggered averages were computed for all DHNs listed in Table 1. Affherent modalities of DHNs were based on the neurons’ responses to stimulation of their estimated primary dermatomes (METHODS). The fields from which unitary responses could be elicited by noxious stimuli were completely surveyed for a subset of these neurons (Table 1, Somatic fields fully surveyed). The excitatory and inhibitory somatic fields of this subset of DHNs were later compared with fields that, when stimulated by pinch, increased or decreased RSNA (Fig. 6A).

<table>
<thead>
<tr>
<th>Spinal Segments</th>
<th>Somatic fields fully surveyed</th>
<th>Somatic fields not fully surveyed</th>
<th>Not Tested for Responses to Stimulation</th>
<th>Total Number of Neurons Recorded</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₂ ipsi</td>
<td>21</td>
<td>0</td>
<td>0</td>
<td>21</td>
</tr>
<tr>
<td>T₃ ipsi</td>
<td>0</td>
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<td>25</td>
<td>25</td>
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<td>15</td>
<td>15</td>
</tr>
<tr>
<td>L₂ ipsi</td>
<td>25</td>
<td>1</td>
<td>0</td>
<td>26</td>
</tr>
<tr>
<td>T₁₀ contra</td>
<td>25</td>
<td>1</td>
<td>0</td>
<td>26</td>
</tr>
<tr>
<td>Totals</td>
<td>91</td>
<td>11</td>
<td>46</td>
<td>148</td>
</tr>
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</table>

Ipsi, ipsilateral; contra, contralateral.

After spinal cord transection, the ongoing activity of only those DHNs recorded in segments containing renal sympathetic preganglionic neurons (Schramm et al. 1993) was correlated to ongoing RSNA

When DHN-triggered averages were compared with averages triggered by frequency-matched dummy neurons, it was clear that the action potentials of a subset of these neurons regularly preceded bursts of RSNA (Fig. 1A). The spike-triggered averages of a much larger subset of DHNs (Fig. 1B) exhibited no relationship between the firing of the spinal neurons and RSNA. A correlation index was used to measure the degree of correlation between the activity of DHNs and RSNA (METHODS). A population of DHNs with an average correlation index of ~0.9 existed at each spinal level investigated (Fig. 2). Spike-triggered averages generated from nonoverlapping segments of the records from these neurons rarely exhibited a consistent peak at any latency. In the ipsilateral T₈ and T₁₀ segments, however, we observed DHNs with substantially higher correlation indexes. More neurons in the ipsilateral T₁₀ segment than in T₈ exhibited these higher indexes. Spike-triggered averages generated from nonoverlapping segments of the records from these neurons invariably exhibited a consistent peak ~60 ms after action potentials of the DHNs.

For the purpose of further analyses, DHNs that had correlation indexes ≥2 and that also exhibited peaks in their spike-triggered averages at repeatable latencies in nonoverlapping segments of their records were considered correlated. Using these criteria, 43% of the neurons recorded in the ipsilateral T₁₀ segment were sympathetically correlated, compared with 16% of the neurons recorded in the T₈ segment. The average latency between the action potentials of correlated DHNs and the peak of their spike-triggered averages of RSNA was 59 ± 8 (SE) ms. Because histologically identified, sympathetically correlated neurons were widely distributed across dorsal horn laminae, their numbers were too small to permit statistical comparisons of their laminar distributions (Fig. 3). However, the relative incidence of correlated neurons appeared to increase in the deeper laminae of ipsilateral T₁₀.

ISIHs were computed for all ipsilateral T₂, T₁₀, L₂, and contralateral T₁₀ neurons. All correlated T₁₀ ipsilateral neurons had similarly shaped ISIHs with modes that were <10
ms and degrees of skewness that were >0.5 (Fig. 4, top). Neurons with these ISIH properties exhibited high incidences of action potentials occurring in clusters (bursts). The absence of RSNA-correlated neurons at spinal levels other than T 8 and T 10 was not due to an absence of bursting neurons at those levels, for the relative distributions of neurons with ISIH modes <10 ms and degrees of skewness >0.5 were not significantly different between segments (P = 0.674, χ² test for independence).

RSNA-correlated T 10 ipsilateral DHNs (12 WDR, 1 HT) were significantly more likely to exhibit WDR properties than uncorrelated T 10 ipsilateral DHNs (1 LT, 9 WDR, 6 HT; P = 0.04, Fisher’s exact test). Uncorrelated ipsilateral T 10 DHNs (17 WDR, 2 HT, 2 other), L 2 DHNs (1 LT, 21 WDR, 1 HT), and contralateral T 10 DHNs (17 WDR, 8 HT, 1 other) were heterogenous with respect to modality. Although statistical tests were impossible, correlated and uncorrelated T 10 ipsilateral DHNs were sorted by modality and laminar location (Fig. 3). All correlated T 10 ipsilateral DHNs found in laminae III–V exhibited WDR properties. Uncorrelated T 10 ipsilateral DHNs found in laminae III–V were heterogeneous with respect to modality, and those found in lamina I all exhibited HT properties.

**Figure 1.** Representative recordings from sympathetically correlated (A) and sympathetically uncorrelated (B) dorsal horn neurons. Top: discriminator output showing action potential occurrences. Middle: renal sympathetic nerve activity (RSNA) recorded simultaneously with dorsal horn neuron recordings. Bottom: bold traces, spike-triggered averages of RSNA triggered with action potentials of dorsal horn neurons (METHODS); fine traces, spike-triggered averages of RSNA triggered with “action potentials” of “dummy neurons.” Occurrences of triggers: 0 s. Vertical scales for middle and bottom reflect amplification of 10,000 times (METHODS). Correlation indexes for these representative correlated and uncorrelated neurons: 2.5 and 0.6, respectively.

**Fields from which excitatory and inhibitory renal sympathetic nerve responses were evoked by noxious cutaneous stimulation were topographically organized.**

In all rats, pinch of the left flank, back, and abdominal regions increased RSNA (Figs. 5A, top and 6A, left). The magnitude of this increase in sympathetic activity was positively related to the applied pressure. Pinch of areas on the rostral and superior boundaries of the laparotomy elicited the greatest responses. Progressively smaller responses were elicited by pinch at increasing distance from the incision. Pinch of the left shoulder and forelimb and the left hip and hindlimb elicited transient reductions in RSNA (Figs. 5B, top and 6A, right). Pinch of contralateral dermatomes occasionally decreased RSNA. The largest reductions in RSNA elicited from the contralateral side followed pinch of the right abdomen and flank.

**Fields from which excitatory and inhibitory responses in the activity of DHNs evoked by noxious cutaneous stimulation were topographically organized.**

Most DHNs were excited when their primary dermatomes were stimulated (Fig. 6, B–F, left). These neurons were...
ipsilateral neurons had nociceptive excitatory and inhibitory fields circumscribed by fields which, when stimulated, increased and decreased RSNA, respectively (compare Fig. 6, A and C). The nociceptive excitatory and inhibitory fields of individual correlated T10 ipsilateral neurons were generally smaller than those for responses of RSNA. Collectively, however, they encompassed the respective excitatory and inhibitory fields for RSNA. Of the 20 fully surveyed T10 DHNs, 11 exhibited ongoing activity that was not correlated with ongoing RSNA. Of these 11 DHNs, 8 (72%) had nociceptive excitatory and inhibitory fields circumscribed by fields that, when stimulated, elicited similar effects in RSNA. Three uncorrelated T10 ipsilateral neurons had nociceptive excitatory fields extending into regions that, when pinched, reduced RSNA. Significantly, these three neurons had no nociceptive inhibitory fields. Noxious stimulation of the left shoulder and forelimb decreased RSNA but increased the activity of 90% (19 of 21) of T2 DHNs. Similarly, noxious stimulation of the left hip and hindlimb increased the activity of 92% (23 of 25) of L2 DHNs. Conversely, the inhibitory fields of many T2 and L2 DHNs, when present, extended into regions from which increases in RSNA could be elicited. Nociceptive excitatory fields of all (25 of 25) contralateral T10 DHNs overlapped regions that, when stimulated, decreased RSNA. Pinch of the left flank, back, and abdominal regions often reduced the activity of contralateral T10 DHNs.

DISCUSSION

Our major observations are 1) that the activity of DHNs in a limited range of thoracic segments is strongly correlated with bursts of simultaneously recorded RSNA and 2) that somatic stimuli that alter RSNA similarly alter the activity of these DHNs. Both of these observations support the hypothesis that a subset of DHNs consists of excitatory synaptic antecedents to renal sympathetic preganglionic neurons.

Correlations between ongoing activity in DHNs and ongoing RSNA

We began our search for sympathetically correlated neurons in the left T10 segment for two reasons. First, renal injections of pseudorabies virus (Schramm et al. 1993) indicated that this segment contained the largest number of renal sympathetic preganglionic neurons, and the distribution of renally infected DHNs correlated strongly with that of renal preganglionic neurons. Second, microinjection of D,L-homocysteic acid into the intermediolateral column at T10 in rats produces the largest increases in RSNA (Taylor and Weaver 1992). In many previous attempts, we failed to find renal sympathetically correlated DHNs at segments rostral to T8 and caudal to T13. These failures were explained when we reinvestigated a wide, longitudinal range of segments in the current study. We found that, rostral and caudal to T10, the incidence of sympathetically correlated DHNs diminished rapidly. At T8, a segment that contains a substantial number of renal sympathetic preganglionic neurons and presumptive renal interneurons (Schramm et al. 1993), the activity of only 16% of DHNs was correlated with RSNA. At T13, a segment that contains a small number of renal sympathetic preganglionic neurons (Schramm et al. 1993) and presump-
We were unable to find any sympathetically correlated DHNs on the contralateral side of the spinal cord at T_{10}. This observation is consistent with the results of three previous studies. First, Taylor and Weaver (1992) found that contralateral injections of D,L-homocysteic acid produced few increases in RSNA. Second, many fewer DHNs are infected contralateral to renal pseudorabies virus injections (Schramm et al. 1993). Third, Cabot et al. (1994) observed no retrograde, transynaptic transport of cholera toxin from sympathetic preganglionic neurons to neurons on the contralateral side of the spinal cord. All of these observations are consistent with the hypothesis that, in spina1y transected rats, the DHNs that are excitatory antecedents to renal sympathetic preganglionic neurons are both longitudinally and laterally restricted.

All sympathetically correlated DHNs exhibited bursting patterns of ongoing activity, and bursts of ongoing renal sympathetic activity were usually correlated with bursts of (rather than single) DHN action potentials. This observation is consistent with the hypothesis that temporal summation plays an important role in determining the strength of coupling between renal sympathetic preganglionic neurons and their synaptic antecedents. Nevertheless, this observation does not preclude a role for steady, nonbursting patterns of action potentials in the generation of sympathetic activity. Single action potentials may, on a longer time scale, affect the overall level of depolarization of sympathetic preganglionic neurons and thereby affect the average level of sympathetic nerve activity without being correlated with identifiable bursts of ongoing activity. Therefore we cannot yet exclude the possibility that apparently uncorrelated DHNs, in any segment or on either side of the spinal cord, could affect levels of ongoing RSNA.

These experiments were feasible only because anesthetized rats, unlike cats (and other mammals), exhibit ongoing RSNA immediately after acute spinal cord transection (Kimura et al. 1995; Osborn et al. 1987; Taylor and Weaver 1993). The potential sources of ongoing renal sympathetic activity after spinal transection in rats have been thoroughly reviewed by Taylor and Weaver (1993). Ongoing activity could be attributed to drive provided by primary afferents. Alternatively, the source of ongoing sympathetic activity could be spinal networks that are independent of afferent drive. After spinal transection, extensive dorsal rhizotomy decreases renal sympathetic activity by 25%, suggesting a role for tonic afferent drive in generating renal sympathetic activity (Taylor and Weaver 1993). On the other hand, the fact that 75% of RSNA (and 100% of mesenteric nerve sympathetic activity) survives these rhizotomies supports the existence of endogenous spinal sympathetic generators. Although the present experiments are the first to suggest a role for DHNs in either the generation or transmission of ongoing activity.
excitatory drive to sympathetic preganglionic neurons, we are not able to conclude which of these roles, generation or transmission, is manifested by the observed correlations. It should be noted, however, that some portion of the ongoing RSNA and some of the ongoing activity in DHNs observed in these acutely spinalized rats may have been generated by the surgery and anesthesia necessary to perform our experiments. Maiorov et al. (1997) have recently shown that, in the absence of anesthesia, acute surgery, or other somatic or visceral stimuli, chronically transected rats exhibit relatively little ongoing RSNA. The role of DHNs in generating this small, residual RSNA after chronic spinal transection is conjectural. On the basis of the data from present experiments, however, it is plausible that DHNs play a role in generating the temporal patterns of stimulus-driven RSNA in the chronic spinal mammal.

**Relationships between reflex responses in renal sympathetic activity and reflex responses of DHNs**

Our observation that noxious mechanical stimulation of the caudal flank, back, and abdomen of spinally transected rats increased RSNA confirms previous data from this and other laboratories (Kimura et al. 1995; Poree and Schramm 1992). In our earlier experiments, we showed for the first time that noxious thermal stimulation of these regions not only increased RSNA, but synchronously increased the activity of caudal thoracic DHNs (Poree and Schramm 1992). On the basis of those results, we tentatively hypothesized that thoracic DHNs may mediate homotopic somatosympathetic and viscerosympathetic reflex responses to noxious stimuli by increasing excitatory input to sympathetic preganglionic neurons.

The detailed mapping of the somatic fields of DHNs conducted in the present experiments further supports this hypothesis by showing that the excitatory and inhibitory somatic fields of sympathetically correlated T10 DHNs corresponded more closely to the excitatory and inhibitory fields for RSNA than did the excitatory and inhibitory fields of sympathetically uncorrelated T10 DHNs. The conjunction of the excitatory somatic fields of the sympathetically correlated T10 DHNs was nearly identical to the conjunction of the excitatory somatic fields for RSNA (compare Fig. 6, A, *left* with *C*, *left*). The excitatory fields of some sympathetically uncorrelated DHNs, however, extended further rostrally and caudally, well beyond the borders of the excitatory somatic fields for RSNA (compare Fig. 6, *A*, *left* with *D*, *left*). It is not surprising that the excitatory fields of some sympathetically uncorrelated DHNs were very similar in extent to excitatory fields for RSNA, because many DHNs that play no role in the generation or transmission of drive to renal sympathetic preganglionic neurons would be expected to be excited by stimulation of these fields.

Detailed mapping of inhibitory fields for RSNA and the activity of T10 DHNs provided additional support for a role of DHNs in sympathetic processing. Stimuli that transiently decreased RSNA, i.e., pinch of the ipsilateral fore- and hindlimbs and the contralateral flank, synchronously reduced the ongoing activity of ipsilateral sympathetically correlated T10 DHNs (Figs. 5*B* and 6*C*, *right*). With one exception, inhibitory fields of ipsilateral uncorrelated T10 DHNs also matched inhibitory fields for RSNA (Fig. 6*D*, *right*). Again, it is not surprising that the inhibitory fields of many sympathetically uncorrelated DHNs were nearly identical to inhibitory fields for RSNA because widespread inhibition of DHNs by heterotopic noxious stimulation, both before and after spinal cord transection, has been well documented (see, for example, Cadden et al. 1983). That separate propriospinal mechanisms might mediate inhibition of DHNs and inhibition of sympathetic preganglionic neurons is possible. It is more likely, however, that noxious stimulation of ipsilateral fore- and hindlimb dermatomes and the contralateral flank reduces the activity of many lower thoracic DHNs. Reduction of the activity of those DHNs that are excitatory synaptic antecedents to renal sympathetic preganglionic neurons would be expected to reduce RSNA.

Despite reports of significant intersegmental sympathetic reflexes (Laskey et al. 1979; Weaver et al. 1983), most authors emphasize the segmental character of sympathetic reflexes after
spinal cord transection (see Kimura et al. 1995 for review). The reduction of RSNA by noxious stimulation of the extremities and the inhibition of most DHNs at T2, T10, and L2 by heterosegmental, noxious stimulation are important, however, because they demonstrate the existence of functional, intersegmental regulation of the activity of DHNs and sympathetic activity after spinal transection.

The hypothesis that some DHNs are excitatory synaptic antecedents to renal sympathetic preganglionic neurons is supported by observations from previous experiments in this laboratory. Schramm and Livingstone (1987) found that, after C1 spinal cord transection in rats, electrical or chemical stimulation of the dorsolateral surface of the cervical spinal cord substantially reduced RSNA, very likely by exciting part of a descending brain stem inhibitory pathway or a propriospinal inhibitory pathway. Poree and Schramm (1992), recording simultaneously from thoracic DHNs and the renal sympathetic nerves, showed that electrical and chemical cervical stimulation, similar to that described by Schramm and Livingstone, synchronously reduced ongoing DHN activity and ongoing RSNA. Further, cervical stimulation reduced responses in both RSNA and DHNs to noxious stimuli applied to cutaneous excitatory fields on the left flank.

In summary, the activity of a subpopulation of thoracic DHNs is closely correlated with bursts of RSNA. Somatic stimulation that increases and decreases RSNA excites and
inhibits these DHNs. Finally, in previous experiments we showed that cervical stimulation that decreases RSNA decreases the activity of thoracic DHNs and reduces somatically elicited responses in both DHNs and renal nerves. Taken together, these observations suggest that a subpopulation of DHNs constitutes excitatory antecedents to renal sympathetic preganglionic neurons.

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