Sensory modalities conveyed in the hindlimb somatic afferent input to nucleus tractus solitarius

GLENN M. TONEY AND STEVEN W. MIFFLIN

Departments of Physiology and Pharmacology, The University of Texas Health Science Center at San Antonio, San Antonio, Texas 78229-3900

Toney, Glenn M., and Steven W. Mifflin. Sensory modalities conveyed in the hindlimb somatic afferent input to nucleus tractus solitarius. J Appl Physiol 88: 2062–2073, 2000.—To determine the somatic sensory modalities conveyed by hindlimb somatic afferent inputs, the discharge of neurons in the nucleus tractus solitarius (NTS) was recorded in anesthetized rats after electrical stimulation of either the contralateral sciatic nerve or L2 spinal nerve, which innervates the hindlimb. The discharge of seven of eight cells was increased (P < 0.05) by capsaicin injected into the arterial supply of the hindlimb. Discharge was unaltered in 19 neurons tested for sensitivity to nonnoxious (40°C) and noxious (47°C) heating of the hindlimb skin. In contrast, lightly stroking the skin elicited discharge in 2 of 14 cells, whereas noxious pinching increased activity in 4 other cells. Rhythmic (1- to 3-s) muscle contraction (MC) increased (P < 0.05) discharge in >60% of neurons tested (11 of 18). Static (10- to 30-s) MC significantly (P < 0.05) increased discharge in four cells, two of which were also responsive to rhythmic MC. Rhythmic and sustained muscle stretch increased discharge (P < 0.05) in three of eight neurons tested. These data indicate that nucleus tractus solitarius neurons receive input from low- and high-threshold cutaneous mechanoreceptors, respond to capsaicin delivered into the hindlimb arterial supply, lack thermal sensitivity, and respond to activation of mechanosensitive as well as metabosensitive endings in skeletal muscle.

skeletal muscle; skin; muscle contraction; thermal receptor; exercise

IN ADDITION TO INFLUENCING neuronal activity in the dorsal column nuclei, somatic afferent activation produces mostly excitatory responses among neurons located in autonomic nuclei of the brain stem, including those in the lateral reticular nucleus (11), rostral ventrolateral medulla (3, 6, 24), and the nucleus tractus solitarius (NTS) (26, 33, 34). Among neurons in the NTS, recent studies indicate that excitatory responses can be elicited after activation of both hindlimb cutaneous and skeletal muscle afferent inputs (33, 34). Of particular interest is the observation that these two sources of excitatory drive undergo significant temporal interaction within the NTS (33). This is evident in data showing that the NTS neuronal response to activation of either skeletal muscle or cutaneous afferents is attenuated when preceded by stimulation of the other source of hindlimb input. These inhibitory interactions are time dependent because they are observed only when the interval separating the two somatic nerve stimuli is <250 ms. Moreover, intracellular recordings indicate that these somatosomatic inhibitory interactions occur at a presynaptic site (34). In this regard, they are highly reminiscent of time-dependent inhibitory interactions reported among a number of different visceral nerve inputs (7, 8, 23). Indeed, NTS unit responses to hindlimb somatic afferent activation, although not directly affected by baroreceptor afferent inputs, do nevertheless undergo significant time-dependent inhibition after baroreceptor nerve stimulation (33). Studies performed so far indicate that time-dependent inhibition among somatic and visceral inputs to the NTS is mediated by similar presynaptic mechanisms (34).

On the basis of evidence from reflex studies, somatosomatic and viscerosomatic interactions could have important physiological consequences. It is interesting to note that increases in visceral afferent activity appear key to both the acute reduction in somatic pain associated with increasing arterial pressure (28, 39) and the long-term antinociception that accompanies arterial hypertension (9). Moreover, inhibitory time-dependent interactions among low- and high-threshold somatic inputs to NTS could provide an important mechanism underlying somatic afferent modulation of pain transmission (33).

In addition to a potentially important role in pain processing, viscerosomatic interactions may be key to the production of integrated cardiovascular and sympathetic nervous system effects of muscle exercise. For example, numerous studies have now demonstrated that cardiovascular adjustments to exercise depend on the level of arterial baroreceptor (1, 27, 31, 37) and cardiopulmonary (1, 18, 38) afferent activity. Indeed, Potts et al. (27) have recently provided evidence for an occlusive interaction between the carotid sinus baroreflex and the exercise pressor reflex. When combined with evidence that contraction-induced activation of skeletal muscle receptors can acutely increase the threshold pressures for baroreflex-mediated bradycardia and hypotension (5, 19), it is apparent that inhibitory somatovisceral interactions can have profound consequences for normal cardiovascular function. Despite evidence that visceral and somatic inputs to the central nervous system (CNS) interact to influence...
a variety of functions, little is known concerning the specific neuronal substrates involved in somatosomatic and somatovisceral interactions. To more fully understand the functional significance of hindlimb somatic afferent inputs to the NTS along with their interactions with other convergent somatic and visceral inputs, it is necessary to identify the specific types of sensory information being conveyed through the hindlimb sensory nerves. To achieve this goal, the present study was performed. The response of individual NTS neurons to hindlimb somatic afferent stimulation was determined by using extracellular recording techniques. Neuronal activity was evoked by electrically stimulating either the sciatic nerve (SCN) or the proximal cut end of the L₆ spinal nerve supplying the hindlimb on the side contralateral to the NTS recording site. Neurons were tested for cutaneous sensory input by 1) stroking and pinching the hindlimb skin and 2) heating the hindlimb to nonnoxious and noxious surface temperatures. In addition, the hindlimb gastrocnemius-soleus muscle was stretched and contracted both rhythmically and in a sustained manner to determine whether any or all of these stimuli were adequate to elicit an NTS neuronal response.

Results indicate that NTS neurons respond when hindlimb somatic afferents are chemically activated by intra-arterial injection of capsaicin, suggesting a prominent Aβ/C-fiber input (12). In response to physiological stimuli, mechanical activation of cutaneous endings was sufficient to evoke an excitatory neuronal response, whereas heating was without effect. In addition, discharge was increased significantly among some NTS neurons after rhythmic and sustained muscle contraction and stretch. Combined, these data demonstrate for the first time that NTS neurons receive and integrate information concerning a variety of specific somatic sensory modalities. Taken together with data from previous studies showing viscerosomatic and somatosomatic interactions among inputs to the NTS, it is apparent that NTS neurons do more than relay somatic afferent information to other brain stem regions controlling autonomic activity. In this regard, the present results are consistent with an NTS involvement in integrated autonomic responses to stimuli such as somatic pain and muscular exercise.

METHODS

General Procedures

Male Sprague-Dawley rats (275–375 g) were anesthetized with pentobarbital sodium (60 mg/kg bolus ip). Catheters were placed in the left femoral artery and vein to record arterial blood pressure and to administer drugs, respectively. After tracheal cannulation, rats were artificially ventilated with oxygen-enriched room air. Supplemental anesthetic was diluted in a solution of 0.45% saline and 2.5% dextrose in water and infused through a left jugular vein catheter at a rate of 10 mg·kg⁻¹·h⁻¹ to maintain a stable arterial pressure and an absence of hind paw withdrawal in response to noxious pinching. Each rat was then placed in a stereotaxic apparatus with the neck ventroflexed to an angle of ~35°, and an occipital craniotomy was performed to expose the dorsal surface of the medulla. Body temperature was maintained at 37 ± 1.0°C by using a ventrally placed water-circulating pad.

Activation of Cutaneous Endings

Afferent fibers innervating the skin of the hindlimb and sacrum were activated by electrical stimulation of the SCN. The right SCN was exposed, cleared of connective tissue, and mounted on a bipolar hook electrode. To prevent desiccation and to insulate the electrode from body fluids, the nerve preparation was covered with a flexible silicone impression material. To ensure that nearly all A and C fibers were activated, square-wave current pulses of 1.0-ms duration were delivered at a frequency of 1.0 Hz by using intensities of 500–750 µA (24).

Mechanical stimulation. Cutaneous receptors sensitive to light mechanical touch were stimulated by stroking/probing the skin on the right hindlimb, foot, and sacrum by using a fine bristle or forceps. Deep pressure was produced with a cotton-tipped applicator. Noxious mechanical stimulation was produced by pinching the skin with tissue forceps or hemostats in a manner perceived as moderately painful when applied to the investigators.

Thermal stimulation. Nonnoxious and noxious heating was produced by using an infrared lamp positioned 10 cm above and perpendicular to the surface of the left hindlimb. The lamp was fitted with a focusing adapter to avoid heating regions other than the hindlimb, and a small thermister was used to measure the skin surface temperature. For nonnoxious thermal stimulation, heating was continued until the surface temperature reached 40°C (~10 s). Thermal pain was produced by raising skin temperature to 47°C (~18–24 s).

Stretch and Contraction of Hindlimb Skeletal Muscle

Muscle contraction. As the ventral roots exit the spinal cord between lower thoracic and upper lumbar segments they join and, together with afferent fibers from the hindlimb, form spinal nerves L₂, L₅, and L₆. These spinal nerves course distally to form the SCN, which eventually separates into its sural (cutaneous afferents), tibial (skeletal muscle afferents), and peroneal (mixed afferents) branches. To activate hindlimb afferent fibers, the L₆ spinal nerve was transected, and square-wave-current pulses of 1.0-ms duration were delivered to its proximal cut end at a frequency of 1.0 Hz by using intensities of 250–500 µA. Motor fibers innervating hindlimb skeletal muscle were activated by stimulating the distal cut end of the L₆ spinal nerve. The gastrocnemius-soleus muscle was attached by the Achilles tendon to a force-displacement transducer. Motor fibers were stimulated with currents (~10–150 µA) sufficient to develop 100–500 g of force. By using this procedure, contraction-sensitive endings with afferent fibers traveling in the intact L₂ and L₅ spinal nerves were able to transmit afferent signals to the CNS. To minimize movements during hindlimb muscle contraction, vertebral clamps were placed above and below the isolated spinal segments (L₅–L₂). Another clamp was used to fix the femur to the surgical platform. To allow the gastrocnemius-soleus muscle complex to contract with little mechanical interference, the femur was clamped near the calcaneous bone, reflecting the quadratus femoris muscle just beneath the vastus lateralis. Finally, the distal end of the hindlimb was fixed in place by placing an additional clamp on the tibia next to the calcaneous bone.

Muscle stretch. To produce rhythmic and sustained stretch of the gastrocnemius-soleus muscle, the same hindlimb muscle preparation was used except that a small electric motor fitted with an eccentric cam was mounted adjacent to the tether
connecting the Achilles tendon to the force transducer. As the motor armature rotated, the cam would deflect the tether and thereby stretch the attached gastrocnemius-soleus muscle. The speed of rotation was controlled to vary the frequency of rhythmic stretch. By stopping the motor at various points during the deflection of the tether, graded levels of sustained stretch were produced.

Intra-Arterial Injection of Capsaicin

In some experiments, capsaicin was used to chemically activate unmyelinated afferent fibers in the hindlimb. This was accomplished by injecting capsaicin (1.0 µg in 100 µl) directly into the hindlimb arterial supply via a fine-diameter catheter inserted into the superficial epigastric artery. To ensure maintenance of hindlimb blood flow, the catheter was advanced so that its tip ended just before entering the femoral artery. Capsaicin was dissolved in 50% solution of ethanol in saline. Experiments involving the use of capsaicin were performed after testing for NTS neuronal responses to physiological stimuli. Capsaicin responsiveness was tested only once or twice in any given experiment to avoid complications associated with both functional and pharmacological desensitization of sensory endings (for review see Ref. 40).

Single-Unit Recording

Neuronal activity was recorded by using glass microelectrodes filled with a solution of 2 M NaCl and 2% Chicago sky blue dye. High-resistance (20- to 50-MΩ) electrodes were used to improve the likelihood of recording from individual NTS units. Electrodes were advanced vertically through the NTS in 2-µm steps by using a microdrive instrument. Signals were obtained via a direct-current amplifier and were passed through a differential alternating-current preamplifier equipped with half-amplitude filters set to exclude frequencies outside a 0.3- to 3.0-kHz band. The processed signal was led to a window discriminator, an audio monitor, an oscilloscope, and a videotape multiplex adapter and recorder. Analog signals were digitized by using a 1401plus analog-to-digital converter interfaced with an IBM-compatible computer. Each extracellular action potential that crossed the window discriminator (set to exclude background electrical noise) was recorded as a single discharge event. Discharge data were displayed on-line as peristimulus time histograms (PSTHs) constructed from 30 to 60 sweeps of 100- to 250-ms duration (bin width, 1.0 ms). Sweeps were triggered every second by a transistor-transistor-logic-compatible signal synchronized with the applied nerve stimuli. Spontaneous activity was tested for rhythmic and pulse-related discharge by using autocorrelation and systole-triggered cross-correlation analysis, respectively. Ratemeter records (bin width, 1.0 s) were used to observe changes in cell discharge during physiological activation of sensory endings. Data were analyzed by using Spike2 software.

Experimental Protocols

When NTS unit activity evoked by electrical stimulation of hindlimb afferents was recorded, threshold stimulus intensity was determined. The window discriminator level was adjusted to ensure that activity of only single NTS units was collected for analysis. Next, neuronal responses to suprathreshold electrical stimuli were recorded. The peak onset latency and the cumulative number of action potentials were determined from PSTH data. The hindlimb region was then explored to determine whether discharge could be evoked by light stroking/probing, nonnoxious pressure, or noxious pinching. Next, NTS unit activity was recorded during hindlimb exposure to nonnoxious and noxious heating.

In experiments to examine effects of muscle contraction and stretch, a PSTH was constructed first from NTS unit responses to stimulation of the proximal end of the cut L₆ spinal nerve; then a second PSTH was constructed from unit responses to stimulation of the distal end of L₆. This procedure was used to compare the onset latency of activity evoked by each stimulus. It was reasoned that, if responses to stimulation of the distal end of L₆ resulted from activation of contraction-sensitive endings in the gastrocnemius-soleus muscle, a longer onset latency response would be recorded compared with that evoked by stimulation of afferent fibers in the proximal cut end of the same nerve. Next, responses to rhythmic and sustained muscle contraction were determined from ratemeter records to further confirm the contraction-dependent nature of evoked NTS unit responses. This was accomplished by stimulating the distal end of L₆ before and again after muscle paralysis (galanthamine triethylidine, 25 mg/kg iv). Finally, discharge effects of rhythmic and static stretch were determined.

Histology

Recording sites for NTS units were marked by pressure ejecting 10–50 nl of Chicago sky blue dye (determined by viewing the dye meniscus through a dissecting microscope and measuring its movement with an eyepiece micrometer) via a sidearm of the microelectrode holder. After each experiment the brain stem was removed and placed in a 10% Formalin solution for several days. Then the brain stem region containing NTS was cut into 40-µm-thick coronal sections and stained with neutral red. The location of each recorded neuron was then determined by using a bright-field microscope.

Statistical Analysis

Changes in cell discharge in response to electrical stimulation of hindlimb afferents were analyzed from PSTHs. Effects of chemical and physiological stimulation of hindlimb sensory endings were determined from ratemeter records. Evoked NTS neuronal responses were determined by comparing the maximum discharge frequency recorded after stimulation with the discharge recorded over a similar time interval before stimulation. For rhythmic stretch and contraction, changes in unit activity were also determined by measuring the number of evoked spikes recorded in PSTHs triggered by the initiation of force development and by comparing the values with those obtained after an equal number of stimuli delivered after muscle paralysis. Among cells with irregularly occurring spontaneous discharge, changes in firing rate in response to physiological stimuli were determined for periods when firing increased to at least the baseline mean frequency ± 3 SDs. Recovery of basal firing rate was indicated by a return of cell discharge to the prestimulus mean ± 1 SD (26, 41). Statistical differences were determined by using analysis of variance for repeated measures. Mean differences in pairwise comparisons were determined by using a Tukey test. Changes in arterial pressure and heart rate were analyzed by using Student’s t-test. Mean differences were considered significant at a critical value of P < 0.05. Summary data throughout the text and figures are reported as means ± SE.

RESULTS

Responses to SCN Stimulation

Experiments were performed in 41 male Sprague-Dawley rats. In 21 animals, extracellular single-unit activity was recorded after electrical stimulation of the
SCN supplying the contralateral hindlimb. In these experiments, neuronal responses to stimulation of cutaneous endings were examined. In the remaining 20 animals, NTS neurons were identified by stimulating the proximal cut end of the L6 spinal nerve. Identified neurons were then tested for responses to stimulation of skeletal muscle endings. Before testing for neuronal responses to physiological stimuli, we examined spontaneous discharge for phasic activity and for correlation with the arterial pressure pulse. Among the population of cells studied (n = 36), none displayed phasic or pulse-rhythmic activity.

Among cells responsive to SCN stimulation (n = 28), nearly 60% (16 of 28) were spontaneously active and discharged at an average frequency of 1.2 ± 0.8 spikes/s. When SCN-evoked activity was displayed as a PSTH, two distinct patterns of activity were observed. The majority of cells (n = 18) displayed a unimodal pattern of activity that had a latency-to-peak discharge of 25.1 ± 4.1 ms (Fig. 1A). The remaining cells (n = 10) discharged with a bimodal pattern consisting of a short- and a long-latency response that averaged 28.3 ± 2.3 and 117.0 ± 21 ms, respectively (Fig. 1B). The onset latency for the early response was not statistically different from that of cells displaying only a unimodal response. In addition, the average threshold stimulus intensities for both unimodal and bimodal responses were not statistically different and together averaged 239.0 ± 31 µA. As previously reported for NTS unit responses to stimulation of the hindlimb tibial (skeletal muscle afferents) and sural (cutaneous afferents) nerves (33, 34), each suprathreshold stimulus typically evoked only one NTS unit spike (Fig. 1A, inset), although two to three action potentials per stimulus were observed among some cells (n = 4) that responded with a bimodal discharge pattern (Fig. 1B, inset).

Afferent Fiber Activation

To obtain a tentative classification of the afferent fiber type(s) mediating NTS neuronal responses to electrical nerve stimulation, compound action potentials were recorded proximal to the SCN stimulation site in five animals (Fig. 2). As stimulus intensity was gradually increased, a short-latency (5-20 ms) “peak” was first identified at a threshold intensity of 16 ± 7.3 µA (Fig. 2A) and became maximal between 75 and 100 µA (Fig. 2B and C). The estimated conduction velocity of fibers transmitting this activity was 31.7 ± 2.3 m/s. As stimulus intensity was further increased, a longer latency (6- to 8-ms) afferent response was recorded at a threshold intensity of 192 ± 31.5 µA and became maximal at ~400 µA (Fig. 2D). On the basis of these data and an estimated axonal conduction velocity of 8.3 ± 3.0 m/s, the late afferent response appeared to result from activity in lightly myelinated (Aδ-fiber) afferents and possibly unmyelinated (C-fiber) afferents as well. In contrast, the early afferent response appears to be due to activation of more heavily myelinated (A-fiber) afferent fibers (4, 26, 27). Given that NTS neuronal activity was evoked by SCN stimulation at a threshold intensity that was considerably larger than that required to produce a maximal A-fiber peak (251.8 ± 31 vs. 91 ± 19 µA; n = 6), it appears likely that NTS unit activity resulted from activation of lightly myelinated (Aδ-fiber) or unmyelinated (C-fiber) hindlimb afferents.

Effects of Intra-Arterial Capsaicin

To confirm an Aδ/C-fiber involvement, afferents from the hindlimb were activated chemically by injecting capsaicin into the hindlimb arterial supply, and changes in NTS unit activity were recorded (Fig. 3A). In seven of eight neurons tested, capsaicin increased cell discharge (P < 0.05) nearly threefold, from a baseline of 3.1 ± 1.2
to 8.9 ± 3.5 spikes/s (Fig. 3B). The increase in NTS unit activity preceded changes in arterial pressure (Fig. 3A), suggesting that changes in neuronal activity were produced by direct actions of capsaicin on hindlimb sensory endings and not from effects evoked after circulation to other tissues.

Effects of Cutaneous Mechanical Stimulation

Among 28 NTS neurons identified by their response to SCN stimulation, 13 spontaneously active (1.7 ± 0.5 spikes/s) and 8 quiescent neurons were tested for their sensitivity to light stroking/probing, deep pressure, and noxious pinching of the hindlimb skin. Only quiescent cells responded (n = 6). Two cells increased their discharge to 5.8 ± 1.9 spikes/s (P < 0.05) in response to light stroking of the skin, and four cells responded to noxious pinching (6.6 ± 0.5 spikes/s; P < 0.05). None of the cells responded to both low- and high-threshold stimulation. Mechanically sensitive units were all located superficially in the NTS at depths between 400 and 600 µm. They were distributed in a confined region between 0.5 and 1.5 mm caudal to the obex. In all cases, neurons receiving mechanosensitive cutaneous input were positioned just dorsal to the tractus, near the NTS border with fasciculus gracilus (Fig. 4).

Responses to Hindlimb Heating

NTS neuronal discharge among 13 quiescent and 6 spontaneously active (2.0 ± 1.2 spikes/s) neurons was unaltered by warming the hindlimb skin. No significant change in discharge was recorded during either nonnoxious heating to 40°C (Fig. 5A) or noxious heating to 47°C (Fig. 5B). In the latter case, neurons failed to respond to noxious heating despite evidence that nociceptive endings were activated, as indicated by the fact that arterial pressure increased significantly from 101 ± 12 to 138 ± 15 mmHg (P < 0.05) and heart rate increased from 357 ± 22 to 387 ± 33 beats/min.

Neuronal Responses to Muscle Contraction and Stretch

Before testing for NTS neuronal responses to muscle contraction or muscle stretch, cells were identified by
stimulation of the proximal cut end of the L₆ spinal nerve. Responsive neurons were located between 450 and 1,100 µm below the surface of the medulla in the medial and commissural subnuclei of NTS. The majority were located slightly medial to the tractus (Fig. 4). As a group, cells responsive to stimulation of the proximal cut end of the L₆ nerve discharged spontaneously at a frequency of 3.3 ± 1.4 spikes/s. In contrast to SCN stimulation, only 2 of 18 cells responsive to L₆ stimulation displayed a bimodal pattern of evoked activity, whereas 16 cells responded with a single, short-latency response (i.e., unimodal response). The onset latency for cells with a unimodal (n = 16) response averaged 26.6 ± 11 ms, whereas the latencies for early and late discharges among cells having a bimodal response (n = 2) averaged 29.1 ± 3 and 197 ± 29 ms, respectively.

After stimulation of the proximal cut end of L₆, stimuli were directed to the distal end of the same spinal nerve. Among the cells responding to the latter
stimulus \( (n = 8) \), spontaneous discharge averaged 2.1 ± 1.2 spikes/s. Of the two neurons exhibiting a bimodal response to \( L_6 \) afferent activation, neither responded to stimulation of the distal end of the nerve. To evoke an NTS neuronal response to stimulation of \( L_6 \) motor fibers, stimulus intensity was increased from an average motor threshold of 15.7 ± 3.6 µA, which was sufficient to generate 25 g of tension, to an average intensity of 76.7 ± 32 µA, which produced between 100 and 400 g of contractile force. The latter intensity was significantly less \( (P < 0.05) \) than the threshold intensity \( (491.0 ± 75 \mu A; n = 12) \) required to elicit an NTS neuronal response to activation of \( L_6 \) afferent fibers (Fig. 6). These results are consistent with the conclusion that hindlimb afferent inputs to the NTS are transmitted mostly through lightly myelinated or unmyelinated afferents that respond to muscle tension development subsequent to activation of large-diameter motor nerve fibers.

Compared with the response to stimulation of the proximal cut end of \( L_6 \), the onset latency for responses to stimulation of the distal end of \( L_6 \) was significantly \( (P < 0.05) \) increased from 28.9 ± 12 to 158.2 ± 26 ms in 8 of 11 neurons tested (compare PSTHs in Fig. 7A). The difference in onset latency \( (~130 \text{ ms}) \) reflects the time required for activation of low-threshold large-diameter motor fibers in the \( L_6 \) nerve to elicit a contractile response in the hindlimb muscle along with time for activation of contraction-sensitive sensory endings, the latter being the likely afferent mediator of the recorded NTS unit discharge.

In response to rhythmic contractions (1- to 3-s duration) of the hindlimb muscles (Fig. 7B), unit discharge increased \( (P < 0.05) \) from 2.1 ± 1.2 to 6.8 ± 2.2 spikes/s in 8 of 11 neurons tested. Discharge in the remaining cells was unaltered by rhythmic muscle contraction. During sustained muscle tetany, which produced 100–400 g of tension for 20–60 s, discharge increased \( (P < 0.05) \) in four neurons from 1.8 ± 0.8 to 10.9 ± 3.2 spikes/s (Fig. 8A). Among these cells, two also responded to rhythmic contraction. In three neurons responsive to rhythmic or sustained contraction, muscle paralysis abolished NTS neuronal responses to stimulation of motor fibers in the distal end of the \( L_6 \) spinal nerve (Fig. 8B). These results provide additional evidence that increases in NTS unit discharge were evoked by contraction-sensitive inputs and not by nociceptive inputs activated by mechanical stretch.

Rhythmic and sustained muscle stretch evoked activity in three of eight neurons tested. In response to rhythmic stretches of ~1.0-s duration, responsive cells increased their discharge from 1.3 ± 1.1 to 7.4 ± 3.1 spikes/s \( (P < 0.05) \). One neuron responded at a threshold tension between 90 and 100 g. The remaining two cells responded only when stretch produce tension in excess of 350 g. The latter two cells also responded \( (1.8 ± 0.9 to 5.1 ± 1.4 \text{ spikes/s}) \) throughout a 10-s period of sustained muscle stretch.

**DISCUSSION**

Significance of Unimodal and Bimodal Response Patterns

In the present study, neurons were identified in the NTS that exhibited two distinct patterns of discharge in response to electrical stimulation of hindlimb somatic nerves. One group displayed a short-latency unimodal response, whereas the other responded with a bimodal pattern consisting of a short- and long-latency response. These two patterns of discharge have been previously reported for somatic nerve-evoked neuronal responses in nearby regions of the dorsal medulla (30), ventrolateral medulla (3, 6, 24), and pontine nucleus locus coeruleus (10). Because our previous work demonstrates that both unimodal and bimodal responses can be elicited after stimulation of hindlimb cutaneous and skeletal muscle afferents (33, 34), it appears that the type of response pattern observed \( (i.e., \text{unimodal vs. bimodal}) \) for an individual NTS neuron is not sufficient to establish whether it receives a cutaneous or skeletal muscle afferent input.

That some cells responsive to low-threshold cutaneous stimulation had unimodal responses to SCN stimulation, whereas others exhibited a bimodal-response pattern, indicates that, within a class of afferent inputs, the pattern of evoked activity cannot be used to predict the sensory modality to which the neuron is responsive. This conclusion is supported by data showing that unimodal and bimodal responses were also observed among cells responsive to high-threshold cutaneous inputs. An exception is that none of the cells responsive to muscle contraction or muscle stretch displayed a bimodal response to afferent nerve stimulation. As mentioned above, however, electrical stimulation of skeletal muscle afferents from the rat hindlimb in a previous study (33) was shown to elicit bimodal responses in ~50% of the recorded NTS neurons. The most obvious conclusion, therefore, is that activation of certain skeletal muscle afferents can produce a bimodal response, but this pattern does not result from activity among afferents responsive to contraction or stretch. Evidence from Hirata and Aston-Jones (10) has demonstrated that among locus coeruleus neurons displaying
a unimodal response, longer latency discharge can be elicited by increasing the duration of percutaneous electrical stimuli from 0.5 to 5.0 ms. The longer latency response was shown to depend on recruitment of afferent C fibers because treatment of the SCN with capsaicin not only prevented the C-fiber component of afferent activity but also eliminated the long-latency neuronal response. Studies by Morrison and Reis (24) and Roy and co-workers (30) indicate that similar bimodal responses observed among other medullary neurons also result from combined effects of two convergent populations of afferents, one with a relatively slower average axonal conduction velocity than the other. Taken together, available evidence indicates that the bimodal pattern of activity is an integrated neuronal response to convergent inputs. On the basis of these data, it would seem that the bimodal pattern of discharge may not be consistently observed among any group of cells identified on the basis of their responsiveness to a specific physiological stimulus that activates a single class of primary afferent fibers.

Sensitivity to Cutaneous Stimuli

Among NTS neurons responsive to stimulation of the hindlimb SCN, both light stroking and forceful pinching elicited NTS neuronal responses. Although these results are consistent with a large body of functional (26, 33, 34) and anatomic (16, 21, 25, 42) evidence showing that the NTS is a key recipient of hindlimb somatic afferent input, the fact that neurons responsive to nonpainful (light stroking) and painful stimuli (pinching) were located in close proximity within the NTS was somewhat unexpected. One reason for this is that activity recorded in the present study appeared to be evoked by activation of Aδ- and/or C-fiber afferents, whereas low-threshold mechanoreceptors in the skin are innervated by large-diameter myelinated afferents (for review see Ref. 4). Moreover, afferent inputs arising from low-threshold mechanoreceptors, unlike nociceptive inputs, would be expected to travel in the dorsal column-medial lemniscus pathway that ascends in the spinal dorsolateral funiculus on the side ipsilateral, not
contralateral, to the site of activation (4). One possible explanation for the clustering of neurons responsive to low- and high-threshold cutaneous inputs may be that nociceptive endings in the present study were activated by light stroking as well as by noxious pinching. One mechanism whereby light stroking could have activated cutaneous nociceptors is if endings were exposed to tissue factors known to sensitize receptive endings. Accumulation of endogenous algesic factors such as histamine, bradykinin, potassium ions, and prostaglandins after surgical preparation of the hindlimb could explain the present findings (40).

On the other hand, if both low- and high-threshold afferents do indeed innervate the NTS, as shown for
neurons in the nearby nucleus reticularis dorsalis (36), the possibility should be considered that they undergo interaction. One possible implication for interactions among low- and high-threshold inputs is that they may participate in a form of supraspinal antinociception. In this regard, somatic inputs to the NTS could be organized in a manner similar to the gate-control circuit proposed by Melzack and Wall (20) to attenuate pain transmission within the spinal dorsal horn. Indeed, a similar organization between inputs arising from somatic nociceptors and visceral afferents may underlie the well-documented antinociception produced by arterial hypertension (9, 39) and by acute and chronic increases in arterial baroreceptor (9, 28, 39) and cardiopulmonary afferent activity (28). Accordingly, a local antinociceptive network within the NTS is consistent with data showing that both electrical (2) and chemical (29) stimulation of the NTS can produce significant analgesia in the pentobarbital-anesthetized rat. In the latter context, it is significant to consider our previous conditioning-test stimulation study (33) in which NTS neuronal responses to somatic nerve stimulation were found to be significantly inhibited when conditioned by prior stimulation of the aortic nerve.

Significance of Thermal Stimulation

Another interesting result of the present study is the apparent lack of sensitivity of NTS neurons to nonnoxious and noxious heating. It is well established that nonnoxious and noxious thermal information is conveyed to the CNS through both C and Aδ fibers (4). When combined with results from the present study showing that these same categories of afferents mediate NTS neuronal responses to somatic nerve stimulation, the lack of thermal responsiveness was surprising. Evidence showing that neuronal pathways conveying information to nearby regions of the dorsal medulla transmit both thermal and polymodal nociceptive information (36) makes the apparent lack of thermal input to NTS even more unexpected.

It may be argued that the heating protocol used in the present study was not sufficiently intense to evoke an NTS neuronal response. However, this appears unlikely because the thermal stimulation used raised skin temperature from a normal level of ~34 to 47°C. The latter temperature is known to activate thermal nociceptors (4). That a nociceptive level of skin temperature was reached is evident in the characteristic increases observed in both arterial pressure and heart rate at the end of the thermal stimulation procedure. It is important to note that, in the present study, heating was stopped as soon as the latter responses were observed to avoid tissue damage that can sensitize cutaneous endings to subsequent stimuli (15). On the basis of the present results, it seems reasonable that endings sensitive to nonnoxious temperatures would have been activated at some intermediate level during the gradual ramp increase in surface temperature. Thus data from the present study suggest that direct effects of heating, including pain-induced increases in sympathetic drive or autonomic adjustments to changes in skin temperature, may not depend on transmission through the NTS. The latter conclusion, however, should be tempered by evidence that thermoregulatory changes in autonomic activity elicited from heating the hindlimb to nonnoxious temperatures (<47°C) are typically small relative to those produced by raising skin temperature to noxious levels (14, 35). Moreover, studies indicate that sympathetic responses to heating are more pronounced when heat is applied to other regions of the body surface such as the chest and abdomen or thermoregulatory tissues such as the ear and tail (14, 35). Accordingly, NTS neuronal responses to nonnoxious heating may require inputs from regions of the body surface other than the hindlimb. Finally, it is possible that the heating protocol used in the present study may not have been adequate to recruit sufficient afferent activity to depolarize NTS neurons to action potential threshold. To determine whether this is the case, additional experiments using intracellular recording techniques will be required.

Significance of Skeletal Muscle Input to NTS

Perhaps the most significant finding from the present study is that NTS neurons receive excitatory input from skeletal muscle afferents responsive to changes in muscle tension. Tension resulting from both muscle contraction and muscle stretch evoked NTS neuronal responses, with contraction-induced excitation being more frequently observed. From previous studies it is clear that endings in skeletal muscle attached to fine-diameter (Aδ/C) afferents can be classified as responsive to muscle contraction per se, the so-called mechanosensitive endings, or to sustained muscle tetany accompanied by local accumulation of cellular metabolites, the metaboreceptors (13, 22). Thus, for some skeletal muscle receptors, even brief contractions appear adequate to evoke discharge, whereas others require a more sustained level of activity. Although considerable overlap exists, Aδ fibers appear more robustly mechanosensitive, whereas C-fiber endings are more sensitive to contraction accompanied by tissue ischemia (22). When results from the present study are considered, NTS neurons responsive to muscle contraction fell into one of three groups. In one group, discharge increased at the onset of contraction and diminished as contraction continued (Fig. 7B). Responses in this group may be mediated solely by the mechanically sensitive afferents, which have discharge that adapts within just a few seconds. In a second group, discharge increased gradually in proportion to the duration of contraction, a response consistent with receiving input from metabolically sensitive endings. Finally, a third group of cells responded both during the early phase of contraction and again as contraction was maintained (Fig. 8A). This pattern of discharge is consistent with receiving convergent input from both mechanically and metabolically sensitive afferent endings. To our knowledge these results provide the first electrophysiological evidence indicating that fine-diameter mechanosensitive afferents from
skeletal muscle are capable of influencing activity among individual NTS neurons.

Results from the present study have a number of potentially important functional implications. First, it is now well established that a significant increase in sympathetic nerve activity is required for generation of the exercise pressor reflex. This reflex pressor response is accompanied by activation of neurons in regions of the medullary reticular formation such as the rostral ventrolateral medulla (3, 6, 24) and lateral reticular nucleus (11), which have a demonstrated influence on sympathetic regulation. Evidence from the present study indicates that neurons in the NTS may be an additional site through which exercise-induced activation of skeletal muscle afferents elicits an increase in sympathetic activity.

Given that exercise pressor effects result from an integrated response that occurs in combination with a significant increase in baroreceptor afferent activity (5, 17, 18, 19, 27, 38), it has been proposed that muscle and baroreceptor afferent inputs undergo significant interaction within CNS regions controlling autonomic outflow. To date, however, evidence supporting this concept has principally come from reflex studies, with little direct evidence that such interactions actually occur within the NTS. In a recent study, Potts et al. (27) demonstrated that the increase in arterial pressure produced by simultaneously unloading the carotid sinus baroreceptors while producing static hindlimb muscle contraction was less than the algebraic sum of the individual responses. In addition, a number of studies have demonstrated that the magnitude of the exercise pressor reflex is enhanced in animals after transection of carotid sinus and aortic baroreceptor afferent nerves (18, 38). From these studies, investigators have concluded that an occlusive interaction occurs among baroreceptor and skeletal muscle inputs. Although the site(s) within the CNS where such interactions occur has yet to be determined, it has been postulated that interactions could occur within the NTS (27). Results from the present study provide the first direct functional evidence that contraction-sensitve skeletal muscle afferent inputs from the rat hindlimb are conveyed to the NTS and evoke significant increases in neuronal activity. Because the NTS is also the site where arterial baroreceptor afferents make their initial synapse, it is apparent that the NTS indeed could provide a substrate wherein skeletal muscle and baroreceptor afferent interactions could occur.

When the synaptic basis for interactions among skeletal muscle and baroreceptor afferents within the NTS is considered, our previous studies indicate that prior activation of baroreceptor inputs significantly attenuates unit discharge evoked by activation of skeletal muscle afferents (33). That this inhibitory effect was observed in the absence of a direct effect of baroreceptor inputs on the ongoing discharge of recorded neurons raised the possibility that baroreceptor inputs acted presynaptically to attenuate skeletal muscle afferent transmission. The magnitude of the inhibitory effect was further shown to be time depen-

dent. As a result, skeletal muscle afferent-evoked NTS unit discharge was only inhibited when baroreceptor nerve stimulation preceded skeletal muscle afferent activation by <250 ms. As a result, it is postulated that the signal transmitted beyond the NTS to neurons mediating the exercise pressor reflex is a high-frequency filtered version of the original afferent input. Whether downstream synapses diminish or amplify processing that occurs within the NTS remains unresolved.

In conclusion, the present study provides the first direct electrophysiological evidence describing the types of somatic sensory modalities conveyed to the NTS by somatic nerves innervating the rat hindlimb. Our results indicate that somatic afferent inputs from skin encode information concerning low- and high-threshold mechanical stimuli. Despite receiving input from finely myelinated and/or unmyelinated afferents, NTS unit activity was not altered by either noxious or nonnoxious heating. Skeletal muscle inputs to NTS were identified as transmitting information encoding rhythmic and static muscle contraction and stretch. Neuronal responses to prolonged static muscle contraction may indicate that NTS neurons receive excitatory input from receptors sensitive to metabolites of muscle contraction. At present, it is not clear how these various inputs elicit integrated reflex responses, nor is it clear how interactions identified among visceral and somatic nerve inputs to NTS influence autonomic responses such as those resulting from muscle exercise or pain.

The authors thank Melissa Vitela and Myrna Herrera-Rosales for excellent technical assistance while performing these experiments.

This work was supported by National Heart, Lung, and Blood Institute Grants HL-56637 (S. W. Mifflin) and HL-58834 (G. M. Toney).

A portion of these results has been published previously in abstract form (32).

Address for reprint requests and other correspondence: G. M. Toney, Univ. of Texas Health Science Center, Dept. of Physiology, 7756, 7703 Floyd Curl Dr., San Antonio, TX 78229-3900 (E-mail: Toney@uthscsa.edu).

Received 8 November 1999; accepted in final form 10 February 2000.

REFERENCES


7. Felder RB. Excitatory and inhibitory interactions among renal and cardiovascular afferent nerves in the dorsolateral medulla of...