Excitatory convergence of periaqueductal gray and somatic afferents in the solitary tract nucleus: role for neurokinin 1 receptors

Pedro Boscan and Julian F. R. Paton

Department of Physiology, School of Medical Sciences, University of Bristol, Bristol, United Kingdom

Submitted 18 May 2004; accepted in final form 28 August 2004

Boscan, Pedro, and Julian F. R. Paton. Excitatory convergence of periaqueductal gray and somatic afferents in the solitary tract nucleus: role for neurokinin 1 receptors. Am J Physiol Regul Integr Comp Physiol 288: R262–R269, 2005. First published September 2, 2004; doi:10.1152/ajpregu.00328.2004.—Our previous studies (Boscan P, Kasparov S, and Paton JF. Eur J Neurosci 16: 907–920, 2002) showed that activation of somatic afferents attenuated the baroreceptor reflex by neurokinin type 1 (NK1) and GABA_A receptors within the nucleus of the solitary tract (NTS). The periaqueductal gray matter (PAG) can also depress baroreceptor reflex function and project to the NTS. In the present study, we have tested the possibility that the dorsolateral (dl)-PAG projects to the NTS neurons that also respond to somatic afferent input. In an in situ, arterially perfused, unanesthetized rat preparation, somatic afferents (brachial plexus), cervical spinal cord, and dl-PAG were stimulated electrically, whereas NTS neurons were recorded extracellularly. From 45 NTS neurons excited by either brachial plexus or dl-PAG stimulation, 41 received convergent excitatory inputs from both afferents. Onset latency and evoked peak discharge frequency from brachial plexus afferents were 39.4 ± 4.7 ms and 10.7 ± 1.1 Hz, whereas this was 43.9 ± 6.4 ms and 7.9 ± 1 Hz, respectively, following dl-PAG stimulation. As revealed by using a paired pulse stimulation protocol, monosynaptic connections were found in 9 of 36 neurons tested from spinal and dl-PAG. We tested NK1-receptor sensitivity in 38 neurons that received convergent inputs from brachial plexus/PAG. Fifteen neurons were sensitive to selective antagonism of NK1 receptors. CP-99994, the NK1 antagonist, failed to alter ongoing firing activity but reduced the evoked peak discharge frequency following stimulation of both brachial plexus (from 12.3 ± 1.8 to 7.2 ± 1.3 Hz; \( P < 0.01 \)) and PAG (from 7.8 ± 1.5 to 4.5 ± 1 Hz; \( P < 0.01 \)). We conclude that 1) somatic brachial and PAG afferents can converge onto single NTS neurons; 2) this convergence occurs via either direct or indirect pathways; and 3) NK1 receptors are activated by some of these inputs.

METHODS

A working heart-brain stem preparation (WHBP; see Ref. 38) of Sprague-Dawley rats (75–150 g) of either sex was employed. This was prepared in a home office animal care and use approval. The preparation was performed by using this preparation; 1) stable cellular recordings can be maintained because there is minimal arterial pulse pressure and no respiratory related movements; and 2) there is good visual and pharmacological access to both the brain stem and midbrain for simultaneous single-unit recording and stimulation.

Preparation of the WHBP

Animals were anesthetized deeply with halothane until there was no sign of withdrawal reflexes following a pinch to a paw or the tail. Rats were decerebrated precollicularly, via a partial parietal bone craniotomy (so sparing part of the bregma suture line), bisected subdiaphragmatically, and exsanguinated. The halothane anesthesia was withdrawn at this time, and the head and thorax were immersed in ice-cold Ringer. The descending aorta and one phrenic nerve were surgically isolated. The brain stem and midbrain were exposed following a cerebelllectomy. The preparation was transferred to a recording chamber where the head was fixed with the use of ear bars and a

ALTERATIONS IN THE BEHAVIOR OF A MAMMAL IN RESPONSE TO SOMATICafferent activation are associated with the appropriate and concomitant cardiovascular responses. However, the central nervous mechanisms that underlie the coordination of somatic afferent inputs with the adjustment of cardiovascular autonomic motor outflows remain poorly defined.

Within the brain, two sites known to integrate somatic information and mediate powerful cardiovascular responses are the periaqueductal gray matter (PAG) and nucleus of the solitary tract (NTS). The PAG, especially its dorsolateral (dl) region, receives abundant somatic afferent information from the dorsal horn and also mediates profound cardiorespiratory changes comprising tachycardia and hypertension (3, 4, 26, 32). Turning to the NTS, although it is a major site for integrating multiple visceral afferent inputs and mediating their reflex responses (e.g., Refs. 7, 39), it also processes somatic afferent information (9, 41, 49–51) and receives direct and indirect projections from both the dorsal horn (13, 14, 26, 32) and dl-PAG (5, 15). Interestingly, there are regions of the NTS that can evoke tachycardia and a pressor response, a pattern mimicking that produced both from the dl-PAG and following somatic afferent stimulation, such as that observed during exercise or nociception (30). However, it remains unknown whether somatic afferent-evoked tachycardia-pressor responses depend on either dl-PAG and/or NTS or the pathways that exist between these structures.

With the similarity in cardiovascular responses that can be evoked from the dl-PAG, NTS, and somatic afferents, and their intimate connectivity, the present study was designed to examine whether single NTS neurons that receive somatic afferent inputs from the brachial plexus are also excited following stimulation of dl-PAG. Because our previous data support a role for neurokinin type 1 (NK1) receptors in transmitting somatic afferent input into the NTS (8, 42), we also tested the hypothesis that any convergent inputs from the dl-PAG and somatic afferents to NTS neurons may involve activation of NK1 receptors. A preliminary report of this work was communicated previously (10).

METHODS

A working heart-brain stem preparation (WHBP; see Ref. 38) of Sprague-Dawley rats (75–150 g) of either sex was employed with home office animal care and use approval. This preparation was chosen because 1) much of the background work that led to this project was performed by using this preparation; 2) the pattern of cardiorespiratory reflex response during somatic afferent stimulation is consistent and comparable to that reported in lightly anesthetized and conscious animals (2, 6, 9, 35); 3) stable cellular recordings can be maintained because there is minimal arterial pulse pressure and no respiratory related movements; and 4) there is good visual and pharmacological access to both the brain stem and midbrain for simultaneous single-unit recording and stimulation.

Preparation of the WHBP

Animals were anesthetized deeply with halothane until there was no sign of withdrawal reflexes following a pinch to a paw or the tail. Rats were decerebrated precollicularly, via a partial parietal bone craniotomy (so sparing part of the bregma suture line), bisected subdiaphragmatically, and exsanguinated. The halothane anesthesia was withdrawn at this time, and the head and thorax were immersed in ice-cold Ringer. The descending aorta and one phrenic nerve were surgically isolated. The brain stem and midbrain were exposed following a cerebelllectomy. The preparation was transferred to a recording chamber where the head was fixed with the use of ear bars and a
nasal clamp. The angle of the head was preset to a precise level in every experiment to allow for consistent positioning of the stimulating electrode (see below). The descending aorta was cannulated with a double-lumen catheter to perfuse the upper body with a Ringer solution that was prewarmed to 31°C, contained 1.25% ficoll, and was continually gassed with carbogen (i.e., 95% oxygen and 5% carbon dioxide). With the use of a roller pump, perfusion was maintained at a constant flow (range 28–32 ml/min; Watson Marlow 505S). The second lumen of the catheter was used to monitor aortic perfusion pressure via a transducer. The previously isolated phrenic nerve was drawn up into a suction electrode, and its activity was recorded as an index of central inspiratory activity. This returned 5–10 min after the onset of perfusion, and, once present, the preparation was paralyzed by the addition of vecuronium bromide (Norcuron; 1.5 μg/ml) to the perfusion solution. After adjustment of perfusion flow rate, the respiratory motor pattern consisted of an incrementing discharge indicative of adequate oxygenation of the brain stem and hence preparation viability. ECG was recorded simultaneously with the activity of the phrenic nerve as the suction electrode was within close vicinity of the left ventricle. The R wave of the ECG was discriminated by using a window discriminator to triggered transistor-transistor logic pulses used to determine heart rate (HR). Phrenic nerve activity and ECG signals were amplified and filtered (8 Hz to 3 kHz; Neurolog modules 104 and 125; Digitimer) and displayed on an oscilloscope.

Stimulation Methods

dl-PAG. A bipolar concentric stimulating electrode (Clarke; diameter 0.12 mm) was aimed toward the dl area of the left PAG. The coordinates used were −8.2 to −8.6 mm caudal to bregma, 0.5–1.0 mm lateral to the midline, and 2.3–2.9 mm down from the dorsal surface of the intercolliculi junction (see Fig. 1). The bipolar concentric electrode was connected to an isolated stimulator (Digitimer DS2A) controlled by a pulse generator (Digitimer 4030). All sites in the dl-PAG evoked an increase in respiratory drive, HR, and perfusion pressure during high-frequency stimulation (1–15 V, 0.1–0.2 ms, 50 Hz). At the end of each experiment, constant current was passed to mark stimulation sites (50 μA; 30 s). Brains were fixed (4% paraformaldehyde) and cryoprotected (20% sucrose), and histological sections (50 μm thick) were cut of the midbrain to anatomically identify electrode placement. During extracellular recording in the NTS, the dl-PAG was stimulated with the following parameters: 1–15 V, 0.1 ms, and 0.2–1 Hz.

Brachial plexus. The left brachial plexus was isolated close to the spine, and two silver wires (100 μm in diameter) were wrapped around the entire nerve bundle. Each wire was insulated except for their ends that were bare. Both the exposed silver wires and surrounding plexus were embedded in low-melting-point paraffin wax for electrical insulation. With the use of a second isolated stimulator (Digitimer DSA) driven by the same pulse generator, two types of stimuli were delivered. To evoke a systemic cardiorespiratory reflex response, 10–30 V, 0.2 ms, and 50 Hz were delivered for 3–6 s. In contrast, during extracellular recording, a stimulus consisting of 10–30 V, 0.5 ms, and 0.2–1 Hz was used.

Cervical spinal cord. To bypass any neuronal processing within the spinal cord and to assess monosynaptic connections from spinal afferents to the NTS, the cervical spinal cord was stimulated electri-

Fig. 1. A: photomicrograph of a coronal section through the periaqueductal gray matter (PAG). The photomicrograph shows the stimulation site in the dorsolateral (dl)-PAG (see asterisk). Aq, aqueduct sylvius; DR, dorsal raphe nucleus; DRVL, dorsal raphe nucleus ventrolateral part; IC, inferior colliculi. B: to determine the right placement of the stimulating electrode for each experiment, the dl-PAG was stimulated at high frequency to evoke a cardiorespiratory response. The electrical stimulation evoked an increase in respiratory drive, heart rate (HR), and perfusion pressure (PP) and was similar in pattern to that evoked from the brachial plexus. Brachial plexus stimulation was 20 V, 0.2 ms, and 50 Hz; PAG stimulation was 10 V, 0.2 ms, and 50 Hz. 8PNA, integrated phrenic nerve activity; bpm, beats per minute.

AJP-Regul Integr Comp Physiol • VOL 288 • JANUARY 2005 • www.ajpregu.org
cally. A bipolar electrode made from silver wire with tip diameters of 100 μm was positioned at the level of C3 (~5 mm caudal to calamus scriptorius). In neurons that did not receive monosynaptic spinal afferents from the C3 level but were activated, the latency of responses allowed some assessment of the degree of synaptic processing within the upper cord/NTS. Each pole of this electrode was in direct contact with the lateral edge of the spinal cord and connected to a third isolated electrical stimulator that was also driven by the pulse generator. The stimulation parameters were 3–10 V, 0.1 ms, and 0.2–1 Hz.

Orthodromic and Antidromic Stimulation Protocols

To identify direct monosynaptic connections between either the spinal cord and NTS neurons or the PAG and NTS neurons, a paired pulse protocol, in which pulses were separated by 10 ms, was adopted as described previously (33, 47, 54). Neurons responding to each stimulus of this paired pulse were considered to receive monosynaptic inputs.

The antidromic test consisted of observing a constant latency-evoked action potential followed by a collision cancellation test of the antidromic spike with a spontaneous spike, as described before (e.g., Refs. 27, 37). Successful collision was used to distinguish between antidromic vs. short-latency orthodromic-evoked spikes.

Extracellular Recording from the NTS

Neurons were recorded using multibarreled glass microelectrodes, with an external tip diameter of 2–4 μm and resistance of 15–30 MΩ. Microelectrodes were driven in 3-μm steps by using a pizelectric stepper motor (Burleigh inchworm), which provided z-axis positional feedback. NTS neurons were recorded bilaterally between 300 and 700 μm below the dorsal medullary surface. Under visual guidance of a binocular microscope, microelectrodes were aimed toward the NTS by placing the caudal region of the dorsal medulla relative to calamus scriptorius, a structure that is easily seen by the absence of the cerebellum. The rostrocaudal recording zone was between –13.7 and –14.4 mm relative to bregma. The microelectrodes were positioned between 0.4 and 1.5 mm lateral to the midline for more rostral sites and from midline to 1 mm lateral to midline more caudally. The cerebellectomy enhanced consistency of placement of recording microelectrodes within and between preparations. The three barrels of the microelectrodes were filled with 1) a mixture of 1.5 M NaCl and 100 mM glutamate (recording electrode); 2) a NK1 receptor antagonist, CP-99994 (10 mM; applied for 30–300 s); and 3) saline as control. The recording electrode signal was amplified and filtered (8 Hz to 3 kHz; Neurolog modules 104 and 125). The barrels were connected to a positive-pressure ejection system fitted with solenoids and valves that permitted fine control of applied pressure. The NaCl/glutamate recording solution was pressure ejected intermittently during cell searching to identify neurons that were either silent or had low-firing frequencies. This also maintained the patency of the microelectrode tip during cell searching. When a neuron was found, the positive pressure was nullled (or made slightly negative) to prevent NaCl/glutamate leakage. This allowed cells with ongoing firing to go back to baseline rates within seconds. The CP-99994 was pressure ejected onto neurons that responded to brachial plexus and/or PAG stimulation. As a pressure control, we applied equal pressure to the saline-filled barrel and assessed evoked activity responses. In all cases, saline affected neither ongoing firing nor the evoked firing responses.

Data Analysis

All data were relayed via a 1401 plus CED interface to a computer running Spike 2 software (Cambridge Electronic Design) with custom-written scripts for data acquisition and on- and off-line analysis. The ongoing activity of single units and the electrically evoked firing response frequency were both measured. The criteria used to consider an increase in firing frequency during stimulation was when neuronal activity increased by at least 25% relative to ongoing activity. Peri-stimulus time histograms (10-ms bin width) were plotted that showed the electrically evoked activity from single NTS neurons and allowed a measure of the onset latency. Changes in firing frequency and onset latencies after application of CP-99994 were compared by using a matched-paired nonparametric Wilcoxon signed-rank test. All values quoted are means ± SE, and n is the number of cells recorded. Differences were taken as significant at the 95% confidence limit.

RESULTS

Cardiovascular and Respiratory Data

The data were obtained from a total of 17 preparations. The mean phrenic nerve activity cycle length, HR, and perfusion pressure were 3.7 ± 0.4 s, 268 ± 8 beats/min, and 92.3 ± 3.3 mmHg, respectively. High-frequency electrical stimulation (trains) applied to either the brachial plexus or the dl-PAG exhibited a similar pattern of response, including tachypnea, tachycardia, and a pressor effect (Fig. 1). Brachial plexus and dl-PAG stimulation decreased phrenic nerve activity cycle length to 0.7 ± 0.2 and 0.9 ± 0.2 s, increased HR to 313 ± 18 and 295 ± 16 beats/min, and increased perfusion pressure to 99 ± 5.4 and 94 ± 4.4 mmHg, respectively (all P < 0.01 except for dl-PAG stimulation changes in perfusion pressure; P = 0.1).

Patterns of NTS Neuronal Responses

This study is based on recordings from 205 NTS neurons. Of these, ~20% responded to stimulation of the brachial plexus and/or PAG. Thus single-pulse stimulation of the brachial plexus and the dl-PAG resulted in 41 NTS neurons being excited by both brachial plexus and dl-PAG (Fig. 2). In addition to these 41 neurons exhibiting convergent inputs, we found an additional four neurons that received only one of these inputs; three neurons were excited by the brachial plexus, and one by dl-PAG only. In a further five cells with ongoing activity and excited by brachial plexus stimulation, dl-PAG activation showed a trend of reducing the spontaneous firing (ongoing, 1.04 ± 0.4 Hz; brachial, 5.4 ± 1.7 Hz; PAG, 0.2 ± 0.2 Hz). However, these data were not significantly different (P = 0.1 for ongoing activity vs. activity during dl-PAG stimulation).

The 41 neurons with excitatory convergent inputs (brachial plexus and dl-PAG) were considered further. All 41 neurons displayed ongoing activity with a mean of 2.6 ± 0.4 Hz. The onset latency evoked from the brachial plexus and dl-PAG was 39.4 ± 4.7 and 43.9 ± 6.4 ms (ranging from 9–100 and 4–120 ms), respectively. The firing response evoked from stimulation of either the brachial plexus or dl-PAG was a burst of spikes lasting up to 2 s in some neurons. The evoked firing frequency following stimulation of the brachial plexus and dl-PAG was 10.7 ± 1.1 and 7.9 ± 1 Hz, respectively, which was significantly higher than the ongoing activity during control (P < 0.01 in both cases).

Role of NK1 Receptors

Of the 41 neurons receiving convergent excitatory inputs from brachial plexus and dl-PAG, 38 were tested with the NK1 receptor antagonist CP-99994. In all 38 neurons, there was no change in ongoing activity, suggesting an absence of endogenous substance P release or NK1-receptor activity. Of
these 38 neurons, 15 exhibited a significant reduction in the firing response evoked from both brachial plexus and dl-PAG when CP-99994 was administered; in all cases, the effect was reversible (Fig. 3 and Table 1). Thus the evoked discharge frequency was attenuated from 12.3 ± 1.8 to 7.2 ± 1.3 Hz (P < 0.01) during brachial plexus stimulation and from 7.8 ± 1.5 to 4.5 ± 1 Hz (P < 0.01) during dl-PAG stimulation. There was a reversible trend for an increased onset latency in the presence of CP-99994 in these 15 neurons (brachial plexus: 46.2 ± 9.7 ms for control vs. 97.2 ± 37.7 ms during CP-99994, P = 0.011; dl-PAG: 73.5 ± 28.7 ms for control vs. 121.8 ± 43.1 ms during CP-99994, P = 0.001). Following a 3- to 10-min washout period, the effect of CP-99994 was reversed in the 15 neurons: the evoked response for brachial plexus stimulation was 11.6 ± 2.3 Hz and 66.7 ± 12.9 ms, and for dl-PAG stimulation was 7.2 ± 1.4 Hz and 87.5 ± 33.6 ms. These values in washout were not different to control.

To control for nonspecific effects of CP-99994, such as sodium channel inactivation (12), we coapplied glutamate (100 mM) from the adjoining barrel before and during CP-99994 application to 8 of the 15 neurons inhibited with CP-99994. The NK₁ antagonist did not change the glutamate-evoked firing frequency response (control: 19.8 ± 2.9 vs. 18.6 ± 2.6 Hz; P = 0.7). Additionally, this antagonist did not alter the shape of the action potentials recorded in all neurons tested.

**Paired Pulse Stimulation and a Role for NK₁ Receptors**

A paired pulse protocol (10-ms interpulse interval) from both the spinal cord and dl-PAG was tested in 36 of the 41 neurons exhibiting convergent inputs following stimulation of the brachial plexus and dl-PAG. It was noteworthy that spinal cord stimulation evoked a firing response pattern and peak frequency reminiscent of that evoked from the brachial plexus. This consisted of a peak firing frequency of 7.9 ± 1 Hz with an onset latency of 34 ± 6.8 ms (range 5–160 ms). Of the 36 neurons tested, 9 were capable of following the paired pulse stimuli such that firing responses were faithfully evoked without failures from both spinal cord and dl-PAG (Fig. 4). Thus convergence from spinal cord and dl-PAG in these NTS neurons was monosynaptic. In addition, five neurons were orthodromically activated only from the spinal cord and three only from dl-PAG. In all cells that followed the paired pulse protocol (17 neurons in total), the evoked response was sensitive to NK₁-receptor antagonist in six neurons (3 monosynaptic from both spinal cord and dl-PAG, 2 monosynaptic only from spinal cord, and 1 monosynaptic only from dl-PAG). The latter suggests a role for NK₁ receptors in mediating 33% of the direct afferent inputs from the spinal cord and/or the dl-PAG.

As a result, six neurons with monosynaptic inputs represent 40% of the total neurons sensitive to the NK₁ antagonist. The remaining 60% (9 of 15 neurons) received polysynaptic inputs.

**NTS Neuronal Antidromic Responses from the dl-PAG and Spinal Cord**

None of the 36 NTS neurons orthodromically excited from either the spinal cord or dl-PAG could be antidromically activated from the spinal cord. In contrast, 3 of these 36 NTS neurons were activated antidromically from dl-PAG (Fig. 5). An additional group of three NTS neurons orthodromically activated from spinal cord but not from dl-PAG were activated antidromically from dl-PAG.

**DISCUSSION**

To our knowledge, this is the first study showing convergence of somatic and dl-PAG afferents to single NTS neurons. The majority of the NTS neurons that were excited by brachial nerve stimulation were also excited by dl-PAG stimulation (i.e., 41 of 45 neurons). Thus a NTS neuron that responds to brachial nerve stimulation has a 91% chance of responding to...
dl-PAG. NK₁ receptors appear to mediate ~39% of the somatic and dl-PAG afferent inputs to NTS neurons. All neurons receiving brachial nerve inputs were also activated by ascending spinal afferents, with some of these being mediated by a monosynaptic pathway.

### Somatic Afferent Inputs to NTS

Previous studies have shown that stimulation of somatic nerves that include nociceptive fibers excite NTS neurons (11, 49, 50). Both the present study and a previous publication from

![Figure 3](http://apregu.physiology.org.org/)

**Fig. 3.** Convergent excitatory inputs from brachial plexus and dl-PAG to some NTS neurons is mediated by neurokinin type 1 (NK₁) receptors. Application of the selective NK₁-receptor antagonist CP-99994 reduced the firing frequency response evoked following stimulation of both brachial plexus and dl-PAG. This effect was reversible.

### Table 1. NTS neuron activity during NK₁-receptor blockade

<table>
<thead>
<tr>
<th></th>
<th>Firing Frequency, Hz</th>
<th>Onset Latency, ms</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>CP-99994</td>
</tr>
<tr>
<td>Brachial nerve plexus stimulation</td>
<td>12.3 ± 1.8</td>
<td>7.2 ± 1.3*</td>
</tr>
<tr>
<td>Dorolateral PAG stimulation</td>
<td>7.8 ± 1.5</td>
<td>4.5 ± 1.1*</td>
</tr>
</tbody>
</table>

Values are means ± SE. Nucleus of the solitary tract (NTS) neuron evoked firing frequency and onset latencies during stimulation of the brachial plexus and dorsolateral periaqueductal gray matter (PAG) before and after application of the neurokinin type 1 (NK₁)-receptor blocker (n = 15 neurons). CP-99994 attenuated the evoked activity during stimulation of both brachial plexus and dorsolateral PAG. *P < 0.01 Student’s t-test.
our laboratory (8) indicate that the brachial plexus-evoked NTS responses occur over a large range of onset latencies in a subpopulation of NTS neurons. These studies support the presence of both direct and polysynaptic pathways. One possibility is that brachial plexus inputs arrive via direct spinal projections from the dorsal horn. This is supported by both anatomic and electrophysiological evidence showing direct projections of dorsal horn neurons to caudal regions of the NTS that overlap with our recording sites (see Refs. 13, 14, 17, 24, 26, 32, 45). While we show monosynaptic spinal inputs to NTS neurons also activated from the brachial plexus, this does not necessarily imply that these direct spinal inputs mediated the inputs evoked from the brachial plexus. Indeed, indirect pathway(s) from somatic afferents to NTS-mediating nociceptive information cannot be ruled out and may include spinal, medullary, as well as midbrain relays. Certainly the latter would explain the longer latency-evoked responses from the brachial plexus and account for the failure of many spinal-evoked responses to follow the paired-pulse stimulation protocol.

There was no difference in the overall mean latencies between inputs from the brachial plexus and the dl-PAG to the NTS. However, with the finding that 51% of NTS neurons received longer latency inputs from the brachial plexus compared with dl-PAG, the possibility that this midbrain region acts as a relay for somatic afferents exists. This is supported by the following previously published evidence: first, the PAG receives dense projections from the spinal cord (4, 13, 20); second, the PAG projects directly to the NTS (5, 15); third, NTS neurons were shown to respond to stimulation of the dl-PAG (19); and, fourth, activation of either somatic nociceptive afferents or skeletal muscle contraction-sensitive afferents increases c-Fos expression in the lateral region of the PAG (21–23, 25).
The finding of only six neurons antidromically activated from the dl-PAG suggests that this is a minority target for NTS neurons recorded in the present study. Furthermore, no NTS neurons could be antidromically activated from the spinal cord. One potential pitfall in the present study regarding the antidromic activation is that the stimulus artifact may have camouflaged antidromic spikes of short latency. Thus the data may underrepresent the number of NTS neurons projecting to dl-PAG and/or spinal cord. Nevertheless, the data as presented suggest that the majority of NTS neurons excited from both the brachial plexus and dl-PAG may be either intrinsic interneurons or neurons with projections to brain sites that do not include the dl-PAG and spinal cord.

New Role for Substance P in the NTS

Our data support a role for substance P in mediating the firing responses of NTS neurons responding to either brachial plexus or dl-PAG inputs. In this study, we showed that a percentage of the neurons excited by somatic brachial afferents and dl-PAG were sensitive to NK1-receptor blockade. Axon terminals from dorsal horn into NTS have been shown to contain substance P-like immunoreactivity (16). Moreover, activation of skeletal muscle receptors from the hindlimb releases substance P in the NTS (44). We are unaware of studies that have specifically examined whether a direct substance P-containing pathway from the dl-PAG to NTS exists. The paired pulse stimulation protocol with CP-99994 administration is suggestive that a subset of directly projecting fibers from dl-PAG could contain substance P. Clearly, this needs to be substantiated, as electrical stimulation as used here does not ensure activation of fibers of passage that originate outside of dl-PAG. Indeed, we acknowledge this as a caveat of the present study. Alternatively, activation of the dl-PAG releases substance P in the NTS via indirect projections.

Roles for SP in the NTS remain controversial (see Ref. 46 for a review). Substance P has produced variable and inconsistent responses in respiration, HR, and arterial blood pressure. Substance P is reported to be released in the NTS during baroreceptor activation (34, 43). Some authors have indicated that substance P in NTS potentiates the vascular response of the baroreceptor reflex (48). In contrast, other data indicate that the peptide attenuates carotid sinus nerve-evoked responses (40) and mediates somatic nociception-induced inhibition of the cardiac baroreceptor reflex via an NK1-GABA<sub>A</sub> receptor mechanism in the NTS (8, 42).

Why Does the dl-PAG Input to the NTS?

The answer to the question posed is that, despite being well established as a major viscerosensory nucleus within the brain, the NTS also integrates somatic afferent activity. Our previous data have shown that 50% of the tachycardia evoked by somatic noxious stimulation in the rat are mediated by the NTS (9). Moreover, noxious stimulation attenuated the cardiac baroreceptor reflex that was dependent on the integrity of GABA<sub>B</sub>ergic synapses in the NTS (8). Thus the NTS plays a major role as an interface for somatic afferent-evoked modulation of autonomic motor activity as well as homeostatic reflexes. This is the first time that we show, in the WHBP of rat, activation of the dl-PAG region. The cardiorespiratory response from dl-PAG is tachypnea, tachycardia, and a pressor response, similar to that shown in anesthetized or decerebrated animals (1, 18, 28, 53). Thus the response evoked from dl-PAG is similar to those following somatic afferent stimulation (2, 6, 9, 31, 36). One possibility is that the NTS may mediate a component(s) of this common pattern of cardiovascular response. Additionally, the pathways from the dl-PAG to the rostral ventrolateral medulla, which are known to increase bulbospinal rostral ventrolateral medulla presympathetic monotoneur activity (29, 52), may also play a role. A caveat with the present study is that the NTS neurons studied were not identified as “cardiovascular,” and this is a limitation, with any speculation concerning the functional significance for the dl-PAG-NTS pathway identified here. Nevertheless, not all NTS neurons affecting central cardiovascular (or respiratory) function need to be activated by cardiovascular afferents. Whether the neurons recorded in this study fall within this subgroup remains an open question.

Conclusion

Somatic afferents from the brachial plexus and inputs from the dl-PAG can show excitatory convergence onto single NTS neurons. Furthermore, our data suggest that, if a NTS neuron were excited by dl-PAG stimulation, it is likely that it would also be excited by brachial nerve stimulation. This convergence appears to be mediated by both monosynaptic and indirect pathways and stimulates, in part, NK1 receptors in NTS. Our data support a role for the NTS as a major interface for somatic afferent integration with descending inputs from the midbrain. The possibility remains that these connections form the substrate for modulation of visceral function, whether it be cardiovascular, respiratory or gastrointestinal.

GRANTS

The work was supported by the British Heart Foundation (BS/93003 and PG/99055).

REFERENCES


50. Yardley CP and Hilton SM. The hypothalamic and brainstem areas from which the cardiovascular and behavioural components of the defence reaction are elicited in the rat. J Auton Nerv Syst 15: 227–244, 1986.