Modulation of Carotid Sinus Afferent Input to Nucleus Tractus Solitarius by Parabrachial Nucleus Stimulation

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There is increasing evidence that the parabrachial nucleus (PBN) may be integrally involved in cardiovascular reflex regulation. In cats in which anesthesia was induced with pentobarbital and maintained with α-chloralose, we studied the effects of PBN stimulation on cardiovascular afferent inputs to nucleus tractus solitarius (NTS), the site of first central termination for cardiovascular afferent fibers. Electrical stimulation of PBN resulted in an initial excitation followed by prolonged inhibition of the spontaneous activity of NTS neurons activated by ipsilateral carotid sinus nerve (CSN) stimulation. In 53 units recorded extracellularly in and around NTS, the number of action potential responses to ipsilateral CSN stimulation was reduced 73 ±3% by a prior conditioning stimulus to PBN at an interval of 30–60 msec. CSN input to 10 units excited by selective baroreceptor stimulation was inhibited by the PBN conditioning stimulus, as were convergent inputs from contralateral CSN, vagus, and renal nerves. The inhibitory influence of the PBN stimulus lasted as long as 450 msec. We examined the mechanism for these phenomena in additional intracellular recording experiments. In 57 units, PBN stimulation evoked a long lasting (65–359 msec) membrane potential hyperpolarization. In 42 cells, the PBN evoked inhibitory postsynaptic potential (IPSP) was preceded by an excitatory postsynaptic potential (EPSP). CSN and convergent inputs were inhibited when timed to occur during the PBN induced IPSP. Conversely, CSN and convergent afferent nerves inhibited PBN input to NTS neurons with no associated change in membrane potential (n = 9 of 14). These data demonstrate for the first time a potent modulatory influence of PBN on NTS neurons processing cardiovascular afferent input. (Circulation Research 1988;63:35–49)

Substantial evidence suggests that the parabrachial nucleus (PBN) may play an important role in cardiovascular regulation. The PBN is a major secondary projection nucleus for visceral afferent systems innervating nucleus tractus solitarius (NTS), the site of first central synapse for cardiovascular afferent fibers. Single units in PBN can be influenced by electrical stimulation of aortic nerves, carotid sinus nerves, or both. PBN, in turn, has direct interconnections with higher brain stem nuclei involved in cardiovascular reflex control. Although monosynaptic projections from PBN to NTS are few, electrical stimulation of PBN has been reported to evoke discharge in baroreceptor-related NTS units over oligosynaptic pathways. Finally, high frequency electrical stimulation in PBN causes increased arterial pressure and tachycardia (in cat) or bradycardia (in rabbit). Collectively, these observations suggest the potential for a close physiological interaction between PBN and NTS in cardiovascular reflex control.

We investigated the hypothesis that parabrachial neurons might modulate carotid sinus baroreceptor input to NTS neurons. We found that PBN neurons exert a potent postsynaptic influence (both excitatory and inhibitory) on the spontaneous discharge of NTS neurons and on carotid sinus afferent inputs to NTS, whether elicited by electrical stimulation of the carotid sinus nerves or by selective baroreceptor stimulation.

Materials and Methods

General Methods

Experiments were performed on 44 adult male or female cats, weighing 2.1–3.6 kg, anesthetized initially with sodium pentobarbital (40 mg/kg i.p.) followed by supplemental intravenous doses of α-
chloralose (10 mg/kg) as needed to maintain anesthesia. Experiments were carried out with animals paralyzed using gallamine triethiodide (initially 8 mg/kg i.v., supplemented as needed) and mechanically ventilated. Level of anesthesia following paralysis was gauged by the stability of arterial pressure and the degree of pupillary constriction. A femoral artery and vein were cannulated for measurement of arterial pressure and administration of drugs, respectively. Arterial blood gases were periodically sampled and the respirator settings adjusted or bicarbonate injected to maintain these values within normal limits.

Bilateral cervical incisions were made to expose the carotid sinus regions using techniques previously described. The animal was positioned in a stereotaxic head holder with the head flexed ventrally. Both carotid sinus nerves were isolated from surrounding tissue and mounted intact on bipolar silver—silver chloride electrodes for recording and stimulation. Nerves and electrodes were covered with a mixture (1:1) of mineral oil and vacuum grease to prevent drying. The right vagus and/or renal nerve was also isolated in some experiments and placed on bipolar silver wire stimulating electrodes as previously described. Balloon catheters (5F) were inserted into the external carotid arteries bilaterally and advanced to the sinus regions for selective baroreceptor stimulation by balloon inflation. Each balloon was positioned at a point at which inflation evoked a maximum barrage of baroreceptor activity recorded in the ipsilateral sinus nerve.

An incision was made over the occipital region, the muscles were retracted, and an occipital craniotomy was made to expose the brain stem at obex level. The cerebellum was removed rapidly by suction to expose the surface of the brain stem completely from obex to inferior collicular levels. The craniotomy was then extended rostrally through the right or left bony tentorium into parietal bone, and a small amount of caudal cerebrum was removed using fine cautery and suction to permit unobstructed access to the PBN region for placement of stimulating electrodes.

**Stimulation of PBN**

A pair of monopolar tungsten stimulating electrodes (impedance 100 kΩ) separated by 0.5—1.0 mm was placed in PBN using a combination of stereotaxic coordinates and visual landmarks. In five experiments, we used an assembly consisting of two angled glass microinjection pipettes with approximately 50—μm tips glued to the shank of a monopolar tungsten stimulating electrode (impedance 100 kΩ) so that the three tips converged on the same point. The pipettes were filled with 0.2 mM kainic acid and attached by rigid tubing to a remote pressure injection system (Neuro Phore BH 2-Medical Systems, Great Neck, New York). In both instances, electrodes were advanced simultaneously at 0.5 mm increments until a position was found at which a rise in arterial pressure occurred on continuous low threshold electrical stimulation from at least one electrode (33 Hz, 0.2 msec pulse duration, <100 μA). In experiments using the multibarrel pipette assembly, the electrode was positioned at a site at which injection of kainic acid (50—200 nl) reversibly blocked the pressor response evoked by 25—50 μA electrical stimulation.

**NTS Recording Techniques**

Single unit recordings were made using either extracellular or intracellular recording techniques. Extracellular recordings were obtained using glass microelectrodes filled with 0.5 M sodium acetate saturated with 2% Pontamine Blue (pH 7.6), impedance 5—10 MΩ. Action potentials were amplified with a Dagan 2400 extracellular preamplifier (Minneapolis, Minnesota). Intracellular recordings were made using glass microelectrodes filled with 3 M potassium chloride (impedance 60—100 mΩ) or 2 M potassium citrate (impedance 80—120 mΩ). Intracellular recordings were obtained using a Dagan 8100 preamplifier equipped with capacity compensation and a bridge circuit. Recorded signals were passed via a patch panel to a Nicolet digital oscilloscope (Madison, Wisconsin), a Tektronics storage oscilloscope, an FM tape recorder, a window discriminator, and a sound system.

**Protocols**

**Extracellular studies.** The recording electrode was lowered into the NTS region during simultaneous electrical stimulation (1.0—1.4 Hz, 0.2 msec, 3—30 V) of the two carotid sinus nerves. Single neurons excited by one or both sinus nerves were then tested for activation by stimulation of PBN. Units whose spontaneous and/or CSN evoked discharge was influenced by input from PBN were selected for study.

In some experiments, the neuron was tested for responses to selective baroreceptor stimulation (i.e., balloon inflation in the respective sinus) and to convergent input from other peripheral afferent sources (i.e., vagus or renal). The peak latency to activation by each nerve was determined using computer-generated poststimulus time histograms (PSTHs) of 50 sequential triggered sweeps.

The threshold for activating the unit from each PBN stimulating electrode was determined, and the electrode with the lowest threshold for activation of the unit was used in the protocols that follow. Similarly, thresholds for unit activation were determined for CSN and convergent afferent inputs. Summation tests were performed by simultaneously stimulating PBN and selected afferent nerves at threshold or subthreshold levels.

Conditioning tests were performed as previously described. The ipsilateral CSN was stimulated continuously at 1.0—1.4 Hz at an intensity just
above threshold. Following a control period, the CSN stimulus was preceded by single-pulse (0.2 msec) or double-pulse (0.2 msec, separated by 2 msec) stimulation of PBN at intensities ranging from 25 to 500 μA and at selected intervals ranging from 30 to 450 msec. PSTHs triggered by the CSN stimulus were obtained during control intervals before and after PBN stimulation and at each PBN stimulus interval (50 sweeps per histogram). The same protocol was used to test the effect of a PBN conditioning stimulus on convergent input to the neuron from other afferent sources. Finally, the sequence was reversed and the effect of a conditioning stimulus to peripheral afferent nerves on the PBN evoked response was also tested.

Kainate block studies. Kainic acid is an excitatory amino acid which, like L-glutamate, excites neurons in many areas of the vertebrate central nervous system. In large doses, kainic acid selectively lesions neurons without affecting axons of passage; this toxic effect of kainic acid is thought to be a consequence of prolonged depolarization mediated by kainic acid receptors, which are localized to the cell soma and dendrites. In lesser concentrations, kainic acid appears to selectively block neuronal function without inducing cellular injury. Recent reports have suggested that low concentrations of kainic acid may reversibly block neuronal function. Unpublished observations from this laboratory showing that microinjection of kainic acid into PBN in relatively low concentrations results in transient blockade of the arterial pressure responses to electrical stimulation at the same site are consistent with that view. Because of the known cellular depolarizing effects of both kainate and L-glutamate, the presumptive mechanism for this apparently reversible phenomenon is a "depolarizing block." In five additional experiments, we used the neuronal blocking properties of kainic acid to determine whether responses of NTS neurons to PBN stimulation were mediated by PBN neurons or by fibers originating elsewhere and passing through the PBN region. The PBN conditioning protocol described above was carried out before and after microinjection of 50–200 nl of 0.2 mM kainic acid in the vicinity of the PBN stimulating electrode tip. In these experiments, we first determined that the pressor response to electrical stimulation could be blocked by local microinjection of kainic acid. Following return to control of the pressor response to electrical stimulation, an NTS unit that responded to PBN and CSN was found, and the effects of the PBN stimulus on that unit were observed before and up to 1 hour after a second kainic acid injection. This protocol required maintenance of stable unit recording for a prolonged period of time. Since injection of kainic acid into PBN commonly
A. INHIBITION OF CSN INPUT BY PBN STIMULATION

![Graph showing inhibition of CSN input by PBN stimulation.]

B. INHIBITION OF PBN INPUT BY CSN STIMULATION

![Graph showing inhibition of PBN input by CSN stimulation.]

**FIGURE 2.** Grouped data for the extracellular recording studies, showing percent inhibition of unit discharge during the conditioning tests at selected intervals. A: Effects of PBN stimulation on CSN input. B: Effects of CSN stimulation on PBN input. See text for details.

Increased mean arterial pressure by up to 12 mm Hg, data were collected after arterial pressure had returned to pre-injection levels (approximately 5–15 minutes) to avoid the effects of movement artifact. We did not test these units for the immediate responses to the kainic acid injection.

**Intracellular studies.** Units with a sustained membrane potential greater than −45 mV were examined for synaptic responses (i.e., excitatory postsynaptic potential [EPSP] and inhibitory postsynaptic potential [IPSP]) to stimulation of one or both carotid sinus nerves, vagus nerve, renal nerve, and PBN. The effect of preceding the stimulus to CSN, vagus nerve, renal nerve and PBN was examined at intervals ranging from 30–450 msec. The reverse sequence, that is, preceding the PBN stimulus by a conditioning stimulus to a peripheral afferent nerve, was also tested.

**Histology**

Extracellular recording locations in NTS were marked by iontophoresis of Pontamine Sky Blue stain from the tip of the recording electrode. Stimulation sites in PBN were marked by passing 500 μA anodal direct current for 10 seconds through the stimulating electrode tip.

At the conclusion of each experiment, the brain stem was removed and placed in 10% formalin in saline for at least 3 days. The NTS and PBN regions were sliced (50-μm sections) on a freezing microtome, mounted, and stained with neutral red. Tissue was examined for marked stimulating and recording sites, which then were plotted on representative cross-sections of pons and medulla.

**Data Analysis**

Extracellular data were analyzed using post-stimulus time histograms to determine the peak latencies to electrical stimulation of CSNs, vagus nerve, renal nerve and PBN. The conditioning test data were analyzed by comparing the numbers of action potential responses to a test stimulus of the same intensity before, during and after a conditioning stimulus at each interval. Changes in numbers of action potential responses to 50 sequential test stimuli were expressed as a percent of the control value.

Intracellular data were analyzed off-line using a digital oscilloscope to determine the onset latency, amplitude, and duration of excitatory and inhibitory postsynaptic potentials and the extent to which postsynaptic potentials evoked from one stimulation site were influenced by prior stimulation from another site.

Significance of differences between means was determined using Student's t test (p<0.05). All values are expressed as mean ± SEM.

**Results**

**Extracellular Recording Data**

We studied a total population of 72 units in the NTS and surrounding region. Fifty-nine of these were excited by both PBN and ipsilateral CSN stimulation, 11 were excited by CSN with no obvious input from PBN, and two were excited only by PBN stimulation. This distribution reflects the bias of our selection process, not the relative frequency of NTS neuronal responses to the two stimuli.

Of the 59 units receiving an excitatory input from ipsilateral CSN and PBN stimulation (n=53 ipsilateral; n=6 contralateral), the peak latency to activation following stimulation of CSN ranged from 4.0–25.7 msec (10.9±0.6 msec). Peak latency to activation by ipsilateral PBN stimulation ranged from 2.3–17.1 msec (6.9±0.5 msec); peak latency to activation by contralateral PBN stimulation ranged from 8.5–17.9 msec (12.2±1.6 msec). The latency of the ipsilateral PBN excitatory input was significantly less than that of the ipsilateral CSN excitatory input (p<0.01). The peak latencies for input from other sources were 6.5–29.8 msec (16.8±2.7 msec) for contralateral CSN (n=19), 18.2–37.8
msec (29.9 ± 2.7 msec) for ipsilateral renal nerve (n = 8), and 7.2–18.9 msec (9.8 ± 1.1 msec) for ipsilateral vagus nerve (n = 12).

When the CSN and PBN were stimulated simultaneously there was summation of the excitatory inputs as previously shown for convergent peripheral inputs. Similar evidence for summation was found when excitatory inputs from vagus and renal nerves were tested.

Ten cells met previously described presumptive criteria for monosynaptic activation (the action potential response to stimulation does not vary in latency by more than 0.5 msec and addition of a second stimulus at a 5 msec interval consistently evokes a second action potential) by PBN stimulation. The latencies for these units ranged from 2.3–4.2 msec (3.2 ± 0.2 msec) and, with a conduction distance of 12–15 mm, the calculated conduction velocities for these descending inputs ranged from 2.9–5.6 m/sec.

Figure 1 illustrates the reciprocal inhibitory interactions between PBN and CSN inputs that were seen in 12 of 20 cells tested. The response of this unit to CSN stimulation alone and then preceded by a conditioning stimulus to PBN is shown in Figure 1A, and the response to PBN stimulation alone and then preceded by CSN stimulation is shown in Figure 1B. Figure 1C shows the time course for these interactions and demonstrates the usual finding that the influence of PBN stimulation on CSN input is more prolonged than the influence of CSN stimulation on PBN input to the same cell.

For the 11 cells which received an excitatory CSN input (one of six tested excited by inflation of intrasinus balloon) and no obvious excitation by PBN stimulation, conditioning tests revealed that the PBN stimulus was capable of inhibiting CSN evoked discharge in these cells as well. Finally, we encountered two units activated by PBN stimulation that received no obvious input from the CSN. However, conditioning tests revealed that CSN was
FIGURE 4. Effects of a conditioning stimulus to right PBN on right CSN input to an NTS neuron. A: Two panels on left show poststimulus time histograms illustrating the unit response to PBN stimulation (100 μA) and CSN stimulation (14 V) at time 0; right panel shows the effect of PBN stimulus (not shown) on the responses to the CSN stimulus. The PBN stimulus preceded the CSN stimulus by the intervals indicated. Each histogram shows unit responses during 50 sequential triggered sweeps. B: Top panels show burst of unit activity evoked by inflating a balloon in the right carotid sinus. Middle panels show the inhibitory influence on this response of continual PBN stimulation at a slow rate. Bottom panels show recovery of the unit response to balloon inflation immediately after the PBN stimulus has been turned off. Balloon inflations were performed at intervals of approximately 10 seconds. C: Summation of PBN and CSN input. Top trace, right CSN stimulation alone, 10 V. Middle trace, right PBN stimulation alone, 50 μA. Bottom trace, both together, same stimulus intensities. Dots over traces mark stimulus artifacts. D: Location (filled circle) of the recording site 1.25 mm rostral to obex and just dorsal to tractus solitarius (TS) in NTS. DMN, dorsal motor nucleus of vagus; XII, hypoglossal nucleus; IV, fourth ventricle.

capable of inhibiting PBN evoked discharge in both cells.

Figure 2 shows grouped extracellular data obtained using the conditioning test paradigm. In 53 of the 60 cells tested, there was substantial reduction (73 ± 3%; range 30–100%) in the number of action potential responses to electrical stimulation of the CSN by prior stimulation of PBN at the 30–60 msec conditioning interval (Figure 2A). Twenty-five cells were tested further for the duration of inhibition, which persisted as long as 450 msec in two cells. In all 14 units tested that also received a convergent input from the contralateral CSN and/or inputs from the vagus and/or renal nerves, these convergent inputs were similarly inhibited, as was spontaneous discharge when present. Figure 2B shows the data obtained when the stimulation sequence was reversed. Again, there was substantial inhibition of PBN input to these neurons by CSN stimulation at the 30–60 msec interval (78 ± 4%; range 34–100%), but the duration of inhibition was less. In six units tested receiving convergent peripheral afferent inputs (contralateral CSN; renal; vagus) if the ipsilateral CSN inhibited the PBN evoked discharge, the convergent input(s) did also.

The excitatory and inhibitory effects of PBN stimulation were graded with stimulus intensity. Increasing stimulus intensity increased the number of action potentials discharged during the initial excitation and prolonged the inhibition of spontaneous discharge (Figure 3). The PBN inhibition of CSN evoked discharge persisted for a time period equal to the duration of the inhibition of spontaneous discharge, approximately 175 msec for this unit.

Thirty-seven units excited by CSN and PBN stimulation were tested for specific baroreceptor inputs by inflation of an intrasinus balloon. Ten units were shown to receive an excitatory barore-
FIGURE 5. Reversible blockade of the effects of PBN stimulation induced by microinjection of kainic acid at the PBN stimulating electrode site (details in text). Each histogram shows unit activity during 50 sequential triggered sweeps. Top row, responses to right CSN stimulation alone (left panel), preceding the right CSN stimulus by a PBN stimulus (middle panel) and once again to right CSN stimulation alone after the PBN stimulus is turned off (right panel). The RCSN stimulus is indicated by the filled circle at 50 ms in all three panels; PBN stimulus (two pulses) indicated by an open circle at time 0 in the middle panel. The PBN stimulus (middle panel) evokes unit discharge, inhibits the response to the CSN stimulus, and inhibits spontaneous discharge. Middle row, tests are repeated 15 minutes after microinjection of kainic acid (0.2 mM, 100 nanoliter) from an attached pipette at the PBN stimulation site. The control response to CSN stimulation (right and left panels) is unchanged. The PBN stimulus (middle panel) now fails to evoke a unit response and no longer inhibits the response to CSN stimulation or the spontaneous unit activity. Bottom row, partial recovery 55 minutes after the kainic acid injection. The PBN stimulus again evokes unit discharge and inhibits the input from CSN at the 50 ms interval (middle panel). Numbers above middle panels in each row indicate counted responses to PBN (left) and CSN (right) stimuli.

FIGURE 6. PBN evoked EPSP/IPSP, demonstrating increase in amplitude and duration of the IPSP with pulse train stimulation. Top tracing, single pulse to PBN evokes an early EPSP with action potential (truncated) response followed by an IPSP which interrupts spontaneous discharge of the neuron. Dot indicates stimulus artifact. Bottom tracing, a train of five pulses evokes three action potentials, followed by an IPSP of greater amplitude and longer duration which inhibits spontaneous unit discharge for a longer time compared with a single stimulus. Dots indicate PBN stimulus artifacts.
FIGURE 7. Intracellular recording from a neuron with input from both PBN and CSN. The response to the CSN stimulus alone (A) and to the same CSN stimulus when preceded by a PBN pulse train (B) at 100 msec (left) and 200 msec (right) intervals. During the hyperpolarization of membrane potential, the CSN evoked action potential discharge is abolished but has returned to control levels at the 200 msec interval. The sweeps are superimposed in C to more clearly demonstrate this point. Action potentials truncated. ▲, CSN stimulus artifact; ●, PBN stimulus artifact.

Intracellular Recording Data

To determine whether the PBN inhibition of CSN input to NTS units was due to disfacilitation or postsynaptic inhibition, we recorded intracellularly from 61 units in which CSN stimulation evoked an EPSP (n = 26), an EPSP/IPSP (n = 28) or an IPSP (n = 7) with onset latencies ranging from 3.1-27.2 msec, 2.1-19.4 msec and 10.4-16.7 msec, respectively. In 57 cells, PBN stimulation evoked a long-lasting hyperpolarization of membrane potential, ranging from 65 to 359 msec (171±8 msec) in duration, and from 1.4 to 9.8 mV (3.6±0.2) in amplitude (Figure 6). In 42 of these 57 cells, the PBN evoked hyperpolarization of membrane potential was preceded by an EPSP whose onset latency ranged from 1.7 to 29.6 ms (7.2±1.1; <5 msec in 16 cells).
FIGURE 8. Inhibition of convergent inputs to the same neuron by PBN stimulation. Left: A, EPSP and action potential response evoked by right CSN stimulation (artifacts indicated by bar). B, a prior stimulus to PBN (artifacts indicated by dot) excites the cell and then causes a prolonged hyperpolarization, during which the EPSP evoked by the CSN stimulus is subthreshold to generate an action potential response. C, the two tracings (A and B) are superimposed to accentuate the hyperpolarization induced by the PBN stimulus. Right, an EPSP evoked by renal nerve stimulation (artifacts indicated by bar) is similarly inhibited by the PBN evoked IPSP. Format is the same as in left panel.

The PBN evoked EPSP generated action potentials when it preceded the IPSP. In 22 of 27 neurons tested, reductions in PBN stimulus intensity or number of stimuli revealed that the EPSP was the lower threshold input. In 13 cells the PBN evoked EPSP exhibited characteristics (described in the extracellular section) indicating it might be monosynaptic in origin (latency range 1.7–3.9 msec; 2.3 ± 0.2 msec). The calculated conduction velocity of the descending input ranged from 3.1–8.8 m/sec.

When CSN stimulation was preceded at a 50–100 msec interval by a PBN stimulus (1–4 pulses at 500 Hz) so that the postsynaptic potential evoked by CSN (EPSP, EPSP/IPSP, or IPSP) occurred during the hyperpolarized phase, the action potential responses resulting from EPSPs were reduced or eliminated. In 19 units tested, this inhibitory influence lasted for the duration of the PBN evoked IPSP (Figure 7). Convergent inputs, when present, were also inhibited (Figure 8).

In 14 neurons, the reverse stimulation sequence was tested. In seven neurons the PBN evoked EPSP was inhibited and action potential responses reduced or eliminated by a prior stimulus to CSN and convergent inputs over intervals ranging up to 200 msec, with no associated change in membrane potential (Figure 9). In two neurons the PBN evoked IPSP was reduced by a conditioning stimulus to the CSN and convergent inputs (Figure 10).

In eight cells the polarity of the PBN evoked hyperpolarization of membrane potential was reversed to a depolarization during injection of hyperpolarizing direct current or following injection of chloride (Figure 11), proving that it resulted from postsynaptic inhibition. The duration of the reversed, depolarizing IPSP was less than that of the control, hyperpolarizing IPSP, suggesting that the synapses generating the IPSP are distributed along the cell soma and proximal and peripheral dendrites.28

Further evidence that the PBN stimulus evoked a chloride dependent IPSP was obtained by comparing the PBN evoked IPSP obtained from cells in which the recording electrode was filled with potassium citrate with that from cells in which the electrode was filled with potassium chloride. Using potassium citrate filled electrodes, the amplitude of
Intracellular recording illustrating the inhibitory effects of a prior CSN stimulus (artifact indicated by triangle) on excitatory input to an NTS neuron from PBN (stimulus artifact indicated by dot). Same cell as in Figure 7. A and D show effects of the PBN stimulus alone. B and C show that the CSN stimulus also evokes an EPSP with action potential responses but inhibits the response to the PBN stimulus without altering membrane potential. One sweep per tracing.

FIGURE 9. Intracellular recording illustrating the inhibitory effects of a prior CSN stimulus (artifact indicated by triangle) on excitatory input to an NTS neuron from PBN (stimulus artifact indicated by dot). Same cell as in Figure 7. A and D show effects of the PBN stimulus alone. B and C show that the CSN stimulus also evokes an EPSP with action potential responses but inhibits the response to the PBN stimulus without altering membrane potential. One sweep per tracing.

the PBN evoked IPSP in 46 neurons ranged from 1.6–9.8 mV (3.9 ± 0.2 mV). The amplitude of the IPSP recorded with chloride filled electrodes in 11 cells was significantly less (p<0.01), ranging from 1.4–3.9 mV (2.3 ± 0.2 mV), presumably due to a decrease in the chloride equilibrium potential by diffusion out of the electrode. The duration of the PBN evoked IPSP recorded with citrate filled electrodes in 46 cells ranged from 76 to 350 msec (177 ± 10 msec). This was not significantly different from the duration recorded with chloride filled electrodes in 11 cells, which ranged from 65 to 224 msec (143 ± 13 msec).

The magnitude of both the EPSP and the IPSP evoked by PBN stimulation and the duration of inhibition of CSN evoked discharge could be increased by increasing the number of PBN stimuli in 12 of 20 cells tested (Figure 6). The amplitude of the EPSP and amplitude and duration of the IPSP were maximal with five to seven stimuli at 500 Hz. In six neurons in which a single PBN stimulus evoked only an EPSP, increasing the number of PBN stimuli evoked an IPSP following the EPSP (compare Figures 7 and 9). In eight cells the PBN IPSP was maximally developed with only one or two PBN stimuli.

Histology

Figures 12 and 13 illustrate the locations of the PBN stimulating sites and the NTS recording sites, respectively. Effective PBN stimulation sites were found both medial and lateral to brachium conjunctivum (Figure 12A). There was no correlation between the nature (excitatory-inhibitory versus solely inhibitory) of the PBN evoked NTS unit response and the location of the PBN stimulating sites; both types of response were elicited from a single PBN stimulation site in many instances (Figure 12B). No consistent PBN site was found from which monosynaptic excitatory inputs were evoked (Figure 12C). Units with polysynaptic responses were often evoked from the same PBN stimulating site as units having apparent monosynaptic input.

Units were recorded extracellularly in all subnuclei of NTS, in dorsal motor nucleus of vagus, in nucleus intercalatus, and in hypoglossal nucleus and surrounding reticular formation (Figure 13). There was no obvious subnuclear localization of NTS units characterized by a particular pattern of afferent input.

Discussion

These studies provide the first evidence that parabrachial nucleus may exert a powerful modulatory influence on the activity of NTS neurons processing cardiovascular afferent inputs. Previous work has demonstrated that electrical stimulation in the PBN results in short latency activation of NTS neurons over presumed oligosynaptic pathways, and that input from PBN and from baroreceptor afferent fibers (e.g., aortic nerve in rabbit) can influence single NTS neurons. We have extended this observation to demonstrate that PBN stimulation alters the membrane potential of the NTS neuron, rendering it more or less responsive to input from peripheral afferent sources. In most cases, we observed an initial early excitation (EPSP) by the PBN stimulus, followed by a prolonged IPSP that correlated in time with inhibition of both spontaneous unit discharge and of input from CSN, renal, or vagus nerves. The early excitation was generally observed at lower stimulus intensities than the subsequent IPSP, though an IPSP alone was observed in some instances. Thus, it seems likely that the PBN neurons stimulated in this study are capable of modulating input to brainstem neu-
Figure 10. Demonstration of inhibition of a PBN evoked EPSP/IPSP by convergent inputs to the neuron from peripheral afferent sources. PBN stimulus artifacts indicated by arrow. Top tracing, PBN stimulus evokes a prolonged IPSP response. Second tracing, a conditioning stimulus applied to right CSN 100 milliseconds prior to the PBN stimulus also evokes an IPSP but reduces the magnitude of the PBN evoked IPSP. Third tracing, when renal nerve stimulation precedes the PBN stimulus, both components of the PBN evoked PSP are reduced in amplitude. Bottom tracing, the response to PBN stimulation returns to control following the conditioning tests. One sweep per tracing. Vertical lines are stimulus artifacts.

Rons in a direction of either enhanced excitability or inhibition. The specific factors (i.e., central pathways activating the PBN neurons) that might determine the direction of the NTS unit response were not addressed in this study.

A prominent feature of the PBN modulation of NTS unit activity was its lack of selectivity for specific inputs. PBN inhibition of CSN and baroreceptor excitation of these units was associated with like inhibition of convergent input from other sources—opposite CSN, vagus, or renal nerve—and with inhibition of spontaneous unit discharge. In addition, PBN stimulation influenced those NTS units that were inhibited by peripheral afferent input (i.e., responded with an IPSP) as well as those that were excited. Thus, the PBN evoked IPSP exemplifies a common characteristic of postsynaptic inhibition, that is, all inputs arriving at the cell during the IPSP are inhibited.

Further insight into the mechanisms by which afferent inputs are processed within NTS was gained by reversing the sequence of stimulation, that is, by preceding the PBN stimulus with input from a peripheral afferent source. We observed inhibition of PBN input to the neuron by prior stimulation of CSN beyond the duration of any CSN evoked change in membrane potential, suggesting disfacilitation rather than a postsynaptic inhibitory mechanism to explain this interaction. This observation is consistent with data from this laboratory suggesting that interactions between the two CSNs at single neurons in NTS may be mediated by presynaptic rather than postsynaptic mechanisms. In any case, the present data demonstrate that the same neuron may be subject to different modulatory mechanisms depending on the origin and temporal sequence of convergent input from different sources.

An important consideration in these studies is the possibility that our electrical stimulus in PBN may have influenced NTS neurons after spread to other areas of the brain stem or through activation of fibers passing through PBN rather than stimulation of the PBN cellular mass per se. Several lines of evidence suggest that the electrical stimulus was limited in spread and likely evoked NTS unit responses via direct activation of PBN neurons. These include: 1) the NTS activity was usually evoked by only one of our two stimulating elec-
trodos, which were separated by less than 1 mm, or, alternatively, the threshold for evoking unit activity from the second stimulating electrode was at least twofold greater than that used to obtain data from the stimulation site; 2) the anatomical distribution of our stimulation sites spares the brachium area, suggesting that involvement of cerebellar pathways is unlikely; and 3) microinjection of kainic acid, used in these studies in low doses to cause a transient depolarizing block of neurons while sparing axons of passage (see references 20 and 21 for brief discussion), abolished both the excitatory and inhibitory influences of PBN stimulation on NTS units.

Although efferent projections from Kolliker-Fuse to NTS are well described,7 there is little evidence for direct projections from medial or lateral PBN nuclei to cardiovascular regions of NTS. Our data support the suggestion12 that neurons in medial and lateral PBN may influence NTS neurons over pathways composed of relatively few synapses. In a substantial number of cells, the early onset and minimal latency variability to PBN stimulation was consistent with monosynaptic transmission. We found no evidence for a specific subnuclear distribution of PBN stimulation sites having such characteristics, and, in fact, no subnuclear localization within PBN as a whole for sites from which excitation and inhibition of NTS neurons could be demonstrated. In some instances, both polysynaptic and apparent monosynaptic responses in NTS were elicited from the same PBN stimulation site. A similar lack of subnuclear organization has been found for electrical stimulation sites in PBN which evoke pressor responses.12,13 These observations contrast sharply with anatomical1-3 and immunocytochemical31-33 studies that suggest a preferential innervation of lateral PBN subnuclei by projection neurons from caudal NTS and a substantial degree of overlap between the afferent- efferent interconnections of PBN with other central nuclei (see discussion in reference 7). The explanation for this discrepancy may lie in the method used for stimulating electrode placement. We initially positioned the PBN electrode at a site from which low threshold stimulation elicited a pressor response. Because of the need for recording stability, it was not possible to subsequently reposition the PBN electrode at the lowest threshold position for modifying the sinus nerve input to particular NTS neurons (i.e., to "map" the response we observed at the single unit level). In addition, to avoid confusion in the interpretation of marked locations, we often used the same PBN stimulation site to study more
than one NTS neuron; the stimulation intensity requirements differed from unit to unit. Thus, the apparent anatomical dispersion of stimulation sites may simply represent an unavoidable artifact of our stimulation technique.

These data regarding modulation of cardiovascular inputs into the NTS region are best viewed in the context of other descending influences on cardiovascular reflex control. Perhaps the best defined center having a comparable effect is the hypothalamic defense area (HDA), which, when electrically stimulated, blocks the arterial baroreflex. Recent studies have demonstrated that HDA stimulation, like PBN stimulation, causes a postsynaptic inhibition of CSN inputs to NTS neurons activated by baroreceptors. However, the response to HDA stimulation differs from the response to PBN stimulation in several important respects. First, HDA stimulation is rarely associated with an initial excitation, which was routinely observed with PBN stimulation. Second, the inhibitory influence of HDA stimulation appears to be confined to neurons having excitatory CSN input, while PBN stimulation affects neurons which are either excited or inhibited by CSN stimulation. Third, the nature of the stimulus required to demonstrate postsynaptic inhibition by HDA stimulation is quite different from that required for PBN inhibition. The IPSP induced by PBN stimulation is commonly demonstrated with a single PBN stimulus, increasing somewhat in duration as pulse trains of up to five pulses at 500 Hz are employed; comparable IPSPs resulting from HDA stimulation require longer trains of up to 15 pulses. Thus, the PBN influence appears to be rather potent when compared with the influence of another classical center considered to have a prominent role in cardiovascular control.

A closer parallel might be drawn with the effects of fastigial nucleus stimulation on neurons in nucleus parasilicatarius. Fastigial stimulation causes similar excitation followed by inhibition of neurons which also receive convergent input from somatic afferents. While fastigial projections could conceivably synapse in PBN, we feel we have excluded direct stimulation of cerebellar pathways in this study in that our stimulation sites are strikingly absent from within the brachium. A similar distribution of stimulation sites was found by others who demonstrated PBN inhibition of thoracic sympathetic input to spinothalamic tract neurons.

The present data suggests a role for the parabrachial nucleus in the modulation of cardiovascular reflexes at NTS level. The anatomical relations of PBN with surrounding nuclei lend some credence to this speculation. The PBN is a major projection nucleus for neurons leaving NTS and may function to modulate these efferents.
FIGURE 13. Histologically recovered NTS recording sites plotted on representative transverse sections of medulla. Sites are plotted according to inputs: filled circles, units excited by CSN and both excited by and inhibited by PBN; filled squares, units excited by CSN, inhibited but not excited by PBN; open triangles, units excited by CSN and excited but not inhibited by PBN; open circles, units excited by CSN and PBN, not tested for inhibition by PBN; open squares, units excited by PBN, inhibited by CSN. T, tractus solitarius; DMN, dorsal motor nucleus; C, central canal; IV, fourth ventricle; AP, area postrema; XII, hypoglossal nucleus. Numbers (left) refer to distance in mm rostral (+) and caudal (−) to obex. Calibration bar, 1 mm.

central nucleus of amygdala, bed nucleus of the striae terminalis, paraventricular nucleus, and median preoptic nucleus.7-10 Some of these nuclei have connections back to PBN.7-10 Although there are additional projections directly from NTS to these higher cardiovascular nuclei, the major efferent projection from NTS to PBN suggests that PBN may be involved in the early processing of cardiovascular afferent input. Both anatomical31-33 and electrophysiological3 data have suggested that cardiovascular inputs secondarily project to PBN, where they might well be influenced by convergent input from higher brain stem nuclei. Our data would suggest that PBN neurons might then modulate additional afferent input to NTS neurons, either by a direct local feedback mechanism or by relaying signals from higher brain stem centers to NTS. With respect to the latter possibility, it is of interest that the nuclei involved in the caudal pathway subserving the defense reaction have not been identified. It is conceivable that PBN may play a role in mediating HDA inhibition of baroreflexes at NTS level. At present, we can suggest no specific descending influence that might be expressed as an augmented cardiovascular response mediated by PBN excitation of NTS neurons.

In summary, we have demonstrated a potent mechanism for excitation and inhibition of cardiovascular afferent input to NTS neurons that has not previously been described. Although the physiological significance of this observation is as yet unknown, the anatomical interconnections of PBN with other nuclei prominently involved in cardiovascular control would suggest that this nucleus may have an important functional role in that process. The particular physiological conditions that might result in enhancement or inhibition of peripheral cardiovascular afferent input to NTS neurons by the PBN remain to be determined.

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References


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