Stimulation of the paraventricular nucleus modulates firing of neurons in the nucleus of the solitary tract

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Duan, Yu-Fei, Irwin J. Kopin, and David S. Goldstein. Stimulation of the paraventricular nucleus modulates firing of neurons in the nucleus of the solitary tract. Am. J. Physiol. 277 (Regulatory Integrative Comp. Physiol. 46): R403–R411, 1999.—The present study assessed whether the baroreflex inhibition elicited by electrical stimulation of the hypothalamic paraventricular nucleus (PVN) involves altered activity in the nucleus of the solitary tract (NTS). Unit recordings were made from 107 neurons in the NTS in anesthetized rabbits. Intravenous phenylephrine was used to induce a pressor response and to activate baroreflexes. Of the neurons that responded to pressor responses, two-thirds were excited and one-third was inhibited. Stimulation of the PVN inhibited 70% of the phenylephrine-responsive NTS neurons, with or without concurrent baroreceptor stimulation. When PVN stimulation was delivered concurrently with phenylephrine injection, more NTS neuronal inhibition and less excitation occurred than with phenylephrine alone. Usually PVN stimulation inhibited NTS neurons that were excited by pressor responses; less commonly, PVN stimulation excited NTS neurons that were inhibited by pressor responses. The findings are consistent with the view that PVN activation during the defense reaction inhibits baroreflexes by altering firing of NTS neurons.

hypothalamus; medulla; electrophysiology; baroreflex; cardiovascular function

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ipsilateral to the stimulated PVN. The signals from the four conductors were fed through high-impedence cables (Grass H1P5) and then connected to AC preamplifiers (Grass p511). The tetrode recording technique, based on a stereotrode recording technique, was developed to enhance the quality of unit recording and separation (12). Digitized analog signal acquisition and storage were accomplished using an analog-to-digital converter (DT 2821-G-SE, Data Translation, Marlboro, MA) and DataWave System Software (Longmont, CO) installed on a Deli XMT/120 computer. The signals were monitored continuously on software oscilloscopes and a standard oscilloscope (Ramsey 2200) with an audio monitor (Grass 8).

Electrical stimulation (cathodal currents) of the PVN was delivered through a Grass S88 stimulator coupled with a stimulus isolation unit (Grass PSIU 6). Delivery of various PVN stimuli were triggered by computer keyboard commands. Time markers for PVN stimuli and for other events, such as phenylephrine injection, were recorded with the unit signals. Offline peri-event time histogram analysis was based on these markers.

Unit Recording Protocols

Protocol A. NTS recording was first made when a series of electrical stimuli (250–300 μA, 0.5-ms pulse, 100 Hz) was delivered to the PVN. These stimuli were relatively brief (0.1, 0.5, and 1 s), and at least several seconds elapsed between stimuli. After completion of recording with PVN stimulation, recording was then made continuously on the same neuron when an intravenous bolus injection of phenylephrine (50 μg/kg) was administered. In other words, once a stable baseline recording was obtained with a neuron, the neuronal firing recording began in the context of PVN stimulation and continued subsequently in the setting of phenylephrine injection and finally ended after the phenylephrine test.

Protocol B. The temporal relationship between phenylephrine injection and PVN stimulation was reversed from protocol A. NTS recordings were obtained when two intravenous bolus injections of phenylephrine at the same dose (50 μg/kg) were given consecutively, with a minimum of 5 min between injections. One phenylephrine injection was coupled with brief, intermittent PVN stimulation (100 μA, 0.5-ms pulse, 100 Hz, 0.5 s, × 30–40, 2-s interval), which began immediately after completion of the injection, so that the PVN stimuli were superimposed on the rising phase of blood pressure. To control for order effects, PVN stimulation was coupled with either the first or the second phenylephrine injection.

The PVN stimuli parameters were chosen to keep peripheral cardiovascular effects at a minimum, so as to avoid confounding influences of peripheral pressor feedback. This was accomplished by using short, intermittent stimuli in protocols A and B and by using reduced current intensity in protocol B. Stimulation of the PVN using these parameters did not alter blood pressure and heart rate.

Histology

At the end of each experiment, the animal was killed with an overdose of pentobarbital sodium. Electroclytic lesions induced by anodal currents were made at the sites of PVN stimulation and NTS recording (6). Preserved brains were subsequently examined for histological verification of the locations of the electrode tips (30).

Data Analysis

Offline analysis of the recorded signals was done using DataWave System common processing software. Cluster cutting and analysis using multidimensional waveform parameters were first performed for each recorded file. This ensured good quality of separation of a single unit from other recorded single units and from stimulus artifact in a multiunit recording file. Customized histogram display and quantification were used to analyze each separated single unit. A 50% or larger change in firing rate in response to phenylephrine or PVN stimulation was considered to be a positive response. Some single-unit data were grouped together later and subjected to contingency analysis, with the statistical significance level P < 0.05. The contingency analysis is used to determine whether two variables, NTS neuronal responses to PVN stimulation and baroreceptor activation in the present study, are independent. A significant result indicates that the two variables are not independent or they are associated.

The main criteria for choosing the time windows of recorded signals used in offline analysis were periods of stable baseline recording and the peak effects before and immediately after experimental manipulations. The basic experimental design for the present study was to compare the effects of PVN stimulation and phenylephrine injection. Tables and figures were constructed accordingly to contrast the rapidly occurring, stimulus-locked central effects of PVN stimulation versus the slowly recruiting, gradually peaked peripheral effects induced by phenylephrine injection.

Effects of PVN stimulation on NTS neuronal firing were assessed by comparing the baseline firing rate before PVN stimulation with the firing rate during PVN stimulation (see Tables 2 and 3 and Figs. 4A, 5A, 6B, and 7B). Effects of phenylephrine injection alone and phenylephrine injection coupled with PVN stimulation were assessed by comparing the firing rate before phenylephrine injection with the firing rate after phenylephrine injection (see Figs. 4B, 5B, 6A, 7A, and 8–10). Changes in magnitudes and directions of NTS neuronal responses to phenylephrine injection alone and to phenylephrine coupled with PVN stimulation constituted the basis for final classification of the NTS neuronal responses (i.e., inhibition or excitation) to phenylephrine injection coupled with PVN stimulation (see Tables 4 and 5).

The inhibition and excitation classified in Tables 4 and 5 are based on the following definitions. Inhibition included loss of firing or decreased firing rate. When phenylephrine injection was coupled with PVN stimulation, “complete” inhibition was defined by an inhibitory neuronal response in the NTS neurons that had an excitatory response when phenylephrine alone was given. “Partial” inhibition was defined by an attenuated excitatory response in neurons that had larger excitatory responses when phenylephrine alone was given. “Increased” firing was defined by an increased rate of firing or by augmented responses. “Increased” firing was defined by an increased rate of NTS neuronal unit activity both when PVN stimulation was coupled with phenylephrine injection and when phenylephrine was given alone. An “augmented” response was defined by a larger increase in firing when PVN stimulation was coupled with phenylephrine injection than when phenylephrine injection was given alone.

RESULTS

Cardiovascular Effects

In protocol A, a series of intermittent short trains (0.1, 0.5, and 1 s, 250–300 μA) of PVN stimuli did not
cause any changes in blood pressure or heart rate. This is consistent with our previous findings when similar stimulus parameters were used to stimulate the dorsomedial hypothalamus while unit recordings were made in the NTS in rabbits (6). Similarly, a series of intermittent short (0.5 s) trains of stimuli with reduced current intensity (80–100 µA) used in protocol B did not lead to any noticeable blood pressure or heart rate changes.

Phenylephrine bolus injections (50 µg/kg) induced an average of 35 mmHg increase in mean arterial pressure with an 30 beat/min bradycardia (Table 1). The pressor responses lasted 5–7 min. The time windows of the rising phase and peak changes of blood pressure induced by phenylephrine were usually within 2 min after a bolus injection and were associated with the maximum changes in neuronal firing in the NTS (Fig. 1).

### Table 1. Baseline levels and changes induced by intravenous phenylephrine in blood pressure and heart rate

<table>
<thead>
<tr>
<th></th>
<th>Systolic Blood Pressure, mmHg</th>
<th>Diastolic Blood Pressure, mmHg</th>
<th>Heart Rate, beats/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>113 ± 12</td>
<td>86 ± 11</td>
<td>133 ± 25</td>
</tr>
<tr>
<td>Peak or nadir</td>
<td>150 ± 15</td>
<td>122 ± 20</td>
<td>101 ± 17</td>
</tr>
</tbody>
</table>

Values are means ± SD (n = 16). Dose of phenylephrine was 50 µg/kg.

Neuronal Responses in the NTS

Unit recordings were made in a total of 107 NTS neurons in the intermediate NTS near the obex (Fig. 2). Recording protocol A was used in 27 neurons. Recording protocol B was used in 86 neurons. The recordings were mostly made in the medial and commissural NTS.

Protocol A. Stimulating sites in the PVN (Fig. 3) led most commonly to an inhibitory response. Among the 21 NTS neurons that responded to PVN stimulation, 16 (76%) were inhibited. Phenylephrine injection caused more excitatory NTS responses (17 neurons) than inhibitory responses (7 neurons) among the 24 neurons that responded to phenylephrine injection (Table 2). Neurons excited by phenylephrine were usually also inhibited by PVN stimulation (Fig. 4). Less commonly, PVN stimulation excited NTS neurons that were inhibited by phenylephrine (Fig. 5).

Protocol B. PVN stimulation usually led to an inhibitory response in the NTS neurons during concurrent phenylephrine injection (Table 3). The pattern of excitatory responses to phenylephrine alone and inhibitory effects of PVN stimulation (Fig. 6) replicated the pattern in protocol A; however, excitation by PVN stimulation and inhibition by phenylephrine injection were
seen more often, from a larger neuronal pool, in protocol B than A (Fig. 7).

The inhibitory effects of PVN stimulation occurred at similar amounts of stimulus summation (Figs. 4A and 6B), despite different current intensities (300 μA in protocol A vs. 100 μA in protocol B). Results using different orders of PVN stimulation and phenylephrine were similar (Table 2, Figs. 4 and 5 vs. Table 3, Figs. 6 and 7).

When phenylephrine injection was coupled with PVN stimulation, more NTS neuronal inhibition and less excitation occurred than with phenylephrine alone (Table 4). Similar patterns of response were evident regardless of the order of phenylephrine injection (Table 5). Note that Tables 4 and 5 describe effects of phenylephrine injections in contrast to Tables 2 and 3, which summarize effects of PVN stimulation. Both the excitatory (Fig. 8) and the inhibitory (Fig. 9) effects of phenylephrine injection on NTS neuronal firing were antagonized by PVN stimulation. Contingency analysis confirmed that among the possible combined effects of baroreceptor activation and PVN stimulation on an NTS neuron, a baroreceptor-mediated excitatory response is usually associated with an inhibitory response due to PVN stimulation. The reverse is true, though less often, for a baroreceptor-mediated inhibitory response in an NTS neuron.

Control experiments showed that neither the peripheral cardiovascular effects nor NTS neuronal responses

<table>
<thead>
<tr>
<th>Phenylephrine alone</th>
<th>Excitation</th>
<th>Inhibition</th>
<th>No effects</th>
<th>Sum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Excitation</td>
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<td>9</td>
<td>4</td>
<td>27</td>
</tr>
<tr>
<td>Inhibition</td>
<td>1</td>
<td>5</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>No effects</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Sum</td>
<td>5</td>
<td>16</td>
<td>6</td>
<td>27</td>
</tr>
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</table>

Values are numbers of neurons. Excitation or inhibition is determined by a 50% or larger change in firing rate. PVN, hypothalamic paraventricular nucleus; NTS, nucleus of the solitary tract.
DISCUSSION

The NTS is a highly complex structure that integrates a variety of visceral sensory inputs and involves many putative neurotransmitters and modulators (2). The sources of descending innervation to the NTS are also multiple, including structures in the hypothalamus, amygdala, pons, and midbrain (22, 27, 28). NTS neurons are also neurophysiologically heterogeneous (25). Therefore, it is not surprising that various patterns of interaction between PVN stimulation and baroreceptor activation can be seen.

In general, the present findings indicate that NTS neurons react in opposite directions to barosensory activation and to descending hypothalamic input from the PVN. PVN stimulation usually yields inhibitory effects on firing of NTS barosensitive neurons, both in the resting state (protocol A) and during phenylephrine-induced baroreceptor activation (protocol B).

In both NTS recording protocols, baroreceptor activation elicited by phenylephrine injection led to a primarily excitatory neuronal response in the NTS, and PVN stimulation superimposed on phenylephrine injection superimposed on phenylephrine injection on NTS neuronal firing.

Table 3. Protocol B: effects of PVN stimulation superimposed on phenylephrine injection on NTS neuronal firing

<table>
<thead>
<tr>
<th>PVN Stimulation Superimposed on Phenylephrine</th>
<th>Excitation</th>
<th>Inhibition</th>
<th>No effects</th>
<th>Sum</th>
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</thead>
<tbody>
<tr>
<td>Phenylephrine alone</td>
<td>7</td>
<td>29</td>
<td>4</td>
<td>40</td>
</tr>
<tr>
<td>Excitation</td>
<td>8</td>
<td>12</td>
<td>6</td>
<td>26</td>
</tr>
<tr>
<td>Inhibition</td>
<td>9</td>
<td>8</td>
<td>3</td>
<td>20</td>
</tr>
<tr>
<td>No effects</td>
<td>24</td>
<td>49</td>
<td>13</td>
<td>86</td>
</tr>
</tbody>
</table>

Values are numbers of neurons. Excitation or inhibition is determined by a 50% or larger change in firing rate.
stimulation suppressed these responses. Less commonly, NTS neurons that were inhibited by baroreceptor stimulation were excited by PVN stimulation. These inhibitory interactions are consistent with the hypothesis that the suppression of baroreflex-cardiac responses during stimulation of the PVN can occur via modulation of neuronal firing within the NTS.

Phasic suppression of the baroreflex maintains elevated heart rate and cardiac output in behavioral states such as “fight or flight” reactions and isotonic exercise. The findings in the present study are consistent with our previous study, in which suppression of the cardiac component of baroreflex was observed when the aortic depressor nerve and the PVN were stimulated simultaneously (9). The present neurophysiological findings indicating NTS involvement in PVN-mediated baroreflex suppression are in agreement with recent neurochemical studies (3, 24).

Functional characterization of PVN-NTS pathways in modulation of the baroreflex has been limited. An NTS extracellular recording study in rats by Kannan and Yamashita (18) reported a predominantly excitatory response to electrical stimulation of the PVN; PVN stimulation led to a depressor response in rats (31). Kannan et al. (17) reported increased sympathetic outflow elicited by PVN stimulation in a later study.

Kannan and Yamashita (18) reported that 20 (7 excitation and 13 inhibition) barosensitive neurons out of 81 tested neurons responded to PVN stimulation in rats, whereas in the present study of rabbits the majority of the NTS neurons was barosensitive (about two-thirds excited, one-third inhibited). The higher percentage of barosensitive neurons in the present study made it possible to conduct a contingency analysis to assess the interaction between PVN stimulation and baroreceptor activation.

Fig. 6. Peri-event time histogram of firing activity of an NTS neuron that was excited by an intravenous bolus injection of phenylephrine (A) and inhibited by electrical stimulation of PVN with reduced current intensity (B). Summation is 7 times (100 µA, 0.5-ms pulse, 100 Hz, 0.5-s train) in B. Bin width is 90 ms in A and 10 ms in B.

Fig. 7. Peri-event time histogram of firing activity of an NTS neuron that was inhibited by an intravenous bolus injection of phenylephrine (A) and excited by electrical stimulation of PVN with reduced current intensity (B). Summation is 40 times (100 µA, 0.5-ms pulse, 100 Hz, 0.5-s train) in B. Bin width is 90 ms in A and 3 ms in B.
The percentage of NTS neurons inhibited by intravenous phenylephrine injection in the present study was found to be similar to our previous NTS recording study involving stimulation of the hypothalamic defense area (the posterior dorsomedial hypothalamus) (6). There were more neurons that were inhibited by phenylephrine injection and excited by PVN stimulation in the present study (Table 3). Recording in the NTS with superimposed hypothalamic stimulation during peripheral barosensory activation was a novel attempt in the present study. It revealed that in addition to those neurons that were excited by barosensory activation and inhibited by PVN stimulation, there were also neurons in the NTS that were inhibited by barosensory activation and excited by PVN stimulation. This latter pattern of interaction between hypothalamic defense area stimulation and barosensory activation has not been clearly identified previously. Although this does not appear to be a dominant pattern of interaction, it indicates that there are multiple mechanisms mediating the integration of descending and ascending inputs at the barosensitive neurons in the NTS.

Table 4. Comparison of effects of phenylephrine injection alone and phenylephrine injection coupled with PVN stimulation on neuronal firing in the NTS

<table>
<thead>
<tr>
<th>Phenylephrine Followed by PVN Stimulation</th>
<th>Excitation</th>
<th>Inhibition</th>
<th>No effects</th>
<th>Sum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Excitation</td>
<td>12</td>
<td>28*</td>
<td>0</td>
<td>40</td>
</tr>
<tr>
<td>Inhibition</td>
<td>19*</td>
<td>7</td>
<td>0</td>
<td>26</td>
</tr>
<tr>
<td>No effects</td>
<td>11</td>
<td>5</td>
<td>4</td>
<td>20</td>
</tr>
<tr>
<td>Sum</td>
<td>42</td>
<td>40</td>
<td>4</td>
<td>86</td>
</tr>
</tbody>
</table>

Values are numbers of neurons. Excitation or inhibition under phenylephrine alone is determined by a 50% or larger change in firing rate compared with baseline. Excitation or inhibition under phenylephrine injection followed by PVN stimulation is determined by comparison of the firing rate changes with those under phenylephrine alone. *P < 0.05, contingency analysis for barosensitive neurons.

The percentage of NTS neurons inhibited by intravenous phenylephrine injection in the present study was found to be similar to our previous NTS recording study involving stimulation of the hypothalamic defense area (the posterior dorsomedial hypothalamus) (6). There were more neurons that were inhibited by phenylephrine injection and excited by PVN stimulation in the present study (Table 3). Recording in the NTS with superimposed hypothalamic stimulation during peripheral barosensory activation was a novel attempt in the present study. It revealed that in addition to those neurons that were excited by barosensory activation and inhibited by PVN stimulation, there were also neurons in the NTS that were inhibited by barosensory activation and excited by PVN stimulation. This latter pattern of interaction between hypothalamic defense area stimulation and barosensory activation has not been clearly identified previously. Although this does not appear to be a dominant pattern of interaction, it indicates that there are multiple mechanisms mediating the integration of descending and ascending inputs at the barosensitive neurons in the NTS.

Table 5. Order effects (group A vs. group B): comparison of the effects of phenylephrine injection alone and phenylephrine injection coupled with PVN stimulation in barosensitive NTS neurons

<table>
<thead>
<tr>
<th>Phenylephrine Followed by PVN Stimulation</th>
<th>Excitation</th>
<th>Inhibition</th>
<th>No effects</th>
<th>Sum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Excitation</td>
<td>12</td>
<td>28*</td>
<td>0</td>
<td>40</td>
</tr>
<tr>
<td>Inhibition</td>
<td>19*</td>
<td>7</td>
<td>0</td>
<td>26</td>
</tr>
<tr>
<td>No effects</td>
<td>11</td>
<td>5</td>
<td>4</td>
<td>20</td>
</tr>
<tr>
<td>Sum</td>
<td>42</td>
<td>40</td>
<td>4</td>
<td>86</td>
</tr>
</tbody>
</table>

Values are numbers of neurons. Group A: phenylephrine injection coupled with PVN stimulation (before phenylephrine injection alone). Group B: phenylephrine injection coupled with PVN stimulation (after phenylephrine injection alone). Group A: 24 neurons, group B: 42 neurons. *P < 0.05, contingency analysis for barosensitive neurons.

Electrical stimulation of the PVN in rabbits elicits a pressor response, tachycardia, and suppression of the cardiac component of arterial baroreflex (9). Numerous other studies in rats and cats, using electrical or chemical stimulation of the PVN, have reported increases in blood pressure, heart rate, or both (4, 11, 16, 19, 26), as well as suppression of the heart rate component of the arterial baroreflex (3, 24).

Perspectives

Suppression of the baroreflex is considered a cardiovascular hallmark of the defense reaction (5, 14). The present findings therefore are consistent with the hy-
hypothesis that the PVN contributes to the neurocirculatory components of the defense reaction (9). The findings in the present study, along with those from a previous PVN study (9), reveal a cardiovascular profile that resembles the one found in studies of the perifornical area, an established hypothalamic defense area in cats (1) and rats (15), and the dorsomedial hypothalamus, a recently identified hypothalamic defense area in rabbits (7, 8, 10). Our hypothesis that the PVN is involved with the defense reaction contradicts the earlier view that excluded the role of the PVN (1, 15). We propose that the PVN may participate in a serial or parallel manner with the perifornical structures in mediating the defense reaction. The present study, along with others (3, 24), provides evidence that the increased sympathetic outflow associated with activation of the PVN involves suppressed baroreflexes. This suppression is mediated, in part, via mechanisms in the NTS. The cardiovascular outcome as a result of PVN activation appears to be in line with some of the other functions associated with the PVN. For example, PVN-mediated vasopressin release into the periphery may act in concert with its central effects. These peripheral and central effects contribute to the maintenance of a cardiovascular profile that supports a wide range of motor activities such as eating, exercise, and the defense reaction.

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REFERENCES


