Metabotropic glutamate receptors depress vagal and aortic baroreceptor signal transmission in the NTS

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Liu, Zhi, Chao-Yin Chen, and Ann C. Bonham. Metabotropic glutamate receptors depress vagal and aortic baroreceptor signal transmission in the NTS. Am. J. Physiol. 275 (Heart Circ. Physiol. 44): H1682–H1694, 1998.—We sought to determine whether metabotropic glutamate receptors contribute to frequency-dependent depression of vagal and aortic baroreceptor signal transmission in the nucleus of the solitary tract (NTS) in vivo. In α-chloralose-anesthetized rabbits, we determined the number of extracellular action potentials synapticly evoked by low (1 Hz)- or high-frequency vagal (3–20 Hz) or aortic depressor nerve (ADN) (6–80 Hz) stimulation and postsynaptically evoked by the ionotropic glutamate receptor agonist α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA). The metabotropic glutamate receptor agonist 2S,3R-2-(carboxycyclopropyl)glycine (L-CCG-I) attenuated NTS responses monosynaptically evoked by 1-Hz vagus stimulation by 34% (n = 25; P = 0.011), while augmenting AMPA-evoked responses by 64% (n = 17; P = 0.026). The metabotropic glutamate receptor antagonist α-methyl-4-phosphonophenylglycine (MPPG) did not affect NTS responses to low-frequency vagal stimulation (n = 11) or AMPA (n = 10) but augmented responses to high-frequency stimulation by 50% (n = 25; P = 0.0001). MPPG also augmented NTS responses to high-frequency ADN stimulation by 35% (n = 9; P = 0.048) but did not affect responses to low-frequency stimulation (n = 9) or AMPA (n = 7). The results suggest that metabotropic glutamate receptors, presumably at presynaptic sites, contribute to frequency-dependent depression of vagal and aortic baroreceptor signal transmission in NTS.

THERE IS COMPELLING evidence that glutamate mediates visceral afferent signal transmission in the nucleus of the solitary tract (NTS) via activation of the fast ligand-gated ionotropic glutamate receptors (1, 3). Since the mid-1980s widespread evidence has been accumulated throughout the central nervous system that fast glutamatergic transmission can be modulated by coincident activation of the G protein-coupled metabotropic glutamate receptors (7, 8). Whether the modulation augments or decreases the fast transmission may vary with the prevalence of metabotropic glutamate receptors at presynaptic vs. postsynaptic sites, the frequency of afferent activity, and the contribution of specific metabotropic glutamate receptor subtypes. In general, presynaptic metabotropic glutamate receptors serve as autoreceptors to inhibit glutamate release and hence reduce synaptic transmission, and postsynaptic metabo-

tropic glutamate receptors increase the excitability of postsynaptic cells within glutamatergic circuits (7, 8), perhaps augmenting their responsiveness to afferent signals. Recent work on mossy fiber synapses in the hippocampus has suggested a use (frequency)-dependent activation of presynaptic metabotropic glutamate receptors, whereby at increased levels of afferent activity the presynaptic receptors are activated to inhibit glutamate release and hence reduce synaptic transmission (20).

The first suggestion that metabotropic glutamate receptors were functionally significant in the NTS came from work by Powloski-Dahm and Gordon (19), who found that stimulating metabotropic glutamate receptors by injecting the broad-spectrum agonist 1S,3R-1-amino-1,3-cyclopentane-dicarboxylate (ACPD) in the caudomedial NTS produced a kynurenate-insensitive decrease in arterial blood pressure, perhaps mimicking the effect of baroreceptor activation. Subsequent studies performed in the transverse rat medullary slice have provided evidence for functional presynaptic and postsynaptic metabotropic glutamate receptors in the intermediate NTS medial to the solitary tract (10–13). Specifically, the broad-spectrum agonist ACPD has been shown to evoke small excitatory postsynaptic currents (EPSCs) in NTS neurons and, in related studies, to transiently augment EPSCs mediated by the ionotropic glutamate receptor agonist α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) (10, 11). In other in vitro studies, bath application of various metabotropic glutamate receptor agonists has been shown to reduce the amplitude of solitary tract-evoked excitatory responses (13). Finally, synaptic depression of evoked NTS responses following a single high-frequency solitary tract stimulation (20 Hz for 2 s) has been shown to be prevented or converted into a postsynaptic excitation by bath application of the broad-spectrum metabotropic glutamate receptor antagonist α-methyl-4-carboxyphenylglycine (MCPG) (12).

Still lacking, however, is an understanding of the physiological roles of presynaptic and postsynaptic metabotropic glutamate receptors in shaping the transmission of visceral afferent signals at NTS synapses in vivo. The present study was undertaken to test the central hypothesis that at low frequencies of visceral afferent input, glutamate released by the central terminals in the NTS is insufficient to act at the inhibitory presynaptic metabotropic glutamate receptors, and hence synaptic transmission is not reduced. At increasing frequencies of visceral afferent activity, however, sufficient glutamate is released to activate the presynaptic receptors, which inhibits further glutamate release; hence, synaptic transmission is reduced. If the...
hypothesis is true, then 1) metabotropic glutamate receptor agonists should reduce visceral afferent signal transmission to NTS neurons via presynaptic actions and 2) metabotropic glutamate receptor antagonists should augment visceral afferent transmission to NTS neurons at high- but not at low-frequency stimulation of the visceral afferent fibers, while having no effect on postsynaptic responses of the neurons to direct activation by ionotropic glutamate receptor agonists. We obtained extracellular recordings in the whole animal to determine the action potential responses of NTS neurons to synaptic activation by stimulation of the vagus nerve and to postsynaptic activation by the ionotropic glutamate receptor agonist AMPA. Responses were determined before and in the presence of a metabotropic glutamate receptor agonist or antagonist. In addition, to determine whether metabotropic glutamate receptors contribute specifically to the depression of aortic baroreceptor afferent signal transmission at increased frequencies of afferent activity, we determined the action potential responses of NTS neurons to synaptic activation by stimulation of the aortic depressor nerve (ADN) at low and high stimulation frequencies and to postsynaptic activation by AMPA. On the basis of preliminary findings, we used the relatively selective group II metabotropic glutamate receptor agonist (2S,2′S)-2-(carboxycyclopropyl)glycine (L-CCG-I) and antagonist α-methyl-4-phosphonophenylglycine (MPPG).

METHODS

Experimental protocols followed were approved by the Institutional Animal Care and Use Committee in compliance with the Animal Welfare Act and in accordance with Public Health Service Policy on Humane Care and Use of Laboratory Animals. Experiments were performed in New Zealand White rabbits (2.7–3.9 kg). The rabbits received an initial intramuscular injection of a mixture of ketamine (50 mg/kg) and xylazine (2.5 mg/kg). A catheter was then advanced through the femoral vein into the inferior vena cava, and the rabbit was anesthetized with an initial intravenous injection of α-chloralose (20–30 mg/kg). Each rabbit was placed on a servo-controlled water blanket, and body temperature was monitored via a rectal temperature probe and maintained at 37 °C. A catheter was advanced through the femoral artery for measuring mean arterial blood pressure (MABP) and withdrawing blood for blood gases. The vagus nerve or the ADN was verified histologically. Recording sites were marked by passing current (10 µA for 7 s every 14 s for 15 min; electrode negative) through the recording electrode to deposit the Pontamine dye. At the end of an experiment, the brain stem was removed and fixed in 4% paraformaldehyde and 10% sucrose. The brain stems were cut in 40-µm coronal sections, and the recording sites were verified histologically.

Recordings were made on either side of the NTS but always ipsilateral to the afferent nerve stimulated. To determine the onset latencies of the evoked responses, the vagus nerve or ADN was stimulated at intensities of at least three times threshold to evoke the maximal number of action potential responses and to minimize latency variability. Recognizing the difficulties in establishing criteria for determining whether a cell is activated over monosynaptic vs. polysynaptic pathways, we presumed that a neuron was monosynaptically activated if it discharged an action potential to each of two stimuli separated by 5 ms (17, 22). Figure 1 shows an example of a neuron that met the criterion (Fig. 1A) and an example of one that did not (Fig. 1B). In Fig. 1A, the cell discharged an action potential to a single stimulus (top) and then to each of two stimuli when a second one was applied 5 ms after the first (bottom). In Fig. 1B, the cell discharged an action potential to a single stimulus (top) but failed to discharge an action potential in response to the second of the two stimuli (bottom).

On the basis of our previous experience in searching for ADN- and vagus nerve-responsive neurons in the NTS (4, 31), we limited the search to the region of the NTS extending in the rostrocaudal plane from 1 mm rostral to the rostral tip of the area postrema caudally to calamus scriptorius. This region corresponds to the intermediate and caudal (commissural) NTS, where cardiovascular and respiratory afferent
action potentials evoked. After the vagally evoked action potentials were determined, AMPA was locally ejected and the number of action potentials was determined. In some cells, AMPA was ejected with two currents to establish a dose (current) response. Once the responses to vagus nerve stimulation and AMPA were determined under control conditions, we iontophoretically applied L-CCG-I, and 3 min into the iontophoresis period we repeated the vagus nerve stimulation and AMPA ejections. L-CCG-I was applied in amounts that did not change baseline activity. Starting at 5 min after the L-CCG-I was stopped, we repeated the protocol to establish a final control (recovery) response. In protocol 2, to determine first that vagal afferent signal transmission to NTS cells displayed frequency-dependent inhibition, we examined the number of action potential responses of cells to a constant number of stimuli (either 100 or 120 stimuli) at low frequency (1 Hz) and then at increasing frequencies (3–20 Hz) of vagus nerve stimulation. The strength of the stimulating current was set such that an action potential was evoked ~100% of the time with 1-Hz stimulation to optimize the detection of depression of the responses evoked at high-frequency stimulation. From these studies we established the lowest frequency at which the number of action potentials evoked by vagal stimulation decreased by at least 20%; this frequency was used in subsequent experiments with the metabotropic glutamate receptor antagonist.

To determine the effect of the metabotropic glutamate receptor antagonist on synaptically evoked and postsynaptically evoked responses of NTS neurons, we stimulated the vagus nerve at either low frequency (50 stimuli at 1 Hz) or higher frequencies (100 or 120 stimuli at 3–20 Hz) and then iontophoretically applied AMPA. Once the numbers of action potentials evoked by vagal stimulation and by AMPA were established under control conditions, we applied the antagonist, MPPG, and 3 min into the iontophoresis period we repeated the vagus nerve stimulation and AMPA ejections. Starting at 5 min after the MPPG was stopped, the protocol was repeated to establish a final control (recovery) response.

In protocol 3, to determine whether metabotropic glutamate receptors contributed to the depression of aortic baroreceptor signal transmission, we tested the effect of MPPG on NTS responses to low-frequency (120 stimuli at 1 Hz) and high-frequency (120 stimuli at 6–80 Hz) ADN stimulation and to AMPA.

In protocol 4, to determine whether MPPG could prevent the effects of L-CCG-I on visceral afferent signal transmission, vagally evoked responses were examined under control conditions and during iontophoresis of the agonist L-CCG-I. After the L-CCG-I was stopped, a second control (recovery) response was established, and then MPPG was applied. At 1 min into the iontophoresis of MPPG, L-CCG-I was commenced; 3 min later, during a background of L-CCG-I and MPPG, the vagus nerve was stimulated and the number of action potentials was determined.

Data analysis. Data are expressed as means ± SE unless otherwise indicated. Significance was claimed at P < 0.05. To determine frequency-dependent synaptic depression of NTS neuronal responses, the numbers of action potentials evoked by vagus nerve or ADN stimulation were compared at three stimulation frequencies by use of a one-way ANOVA. To determine the effect of the metabotropic glutamate receptor agonist or antagonist on synaptically evoked responses, the number of action potentials evoked by vagus nerve stimulation was compared under control conditions, during the metabotropic glutamate receptor agonist or antagonist, and during recovery by use of a one-way ANOVA with repeated measures, followed by Scheffé's post hoc test when appropri-
The arterial blood pH was 7.41 ± 0.08, PCO₂ was 38 ± 4, and PO₂ was 236 ± 62 mmHg; resting MABP was 80 ± 14 mmHg and HR was 197 ± 23 beats/min (means ± SD). The neurons were concentrated in the intermediate and caudal NTS (15) from approximate depths of 700–1,500 µm ventral to the dorsal surface, and medial to the solitary tract. There were no detectable differences in the distribution of cells presumed to be monosynaptically or polysynaptically activated, and, in some instances, both were encountered in the same dorsoventral track.

Effect of the metabotropic glutamate receptor agonist L-CCG-I on NTS neuronal responses evoked by vagal afferent nerve stimulation and by AMPA. The metabotropic glutamate receptor agonist L-CCG-I, ejected with currents that did not change neuronal baseline activity (1.24 ± 0.6 Hz before and 1.42 ± 0.7 Hz during L-CCG-I; P > 0.05), decreased the action potential responses by at least 15% in 17 of 25 neurons monosynaptically activated by low-frequency (1 Hz) vagus nerve stimulation. In 17 neurons the effect of L-CCG-I was also tested on the responses to direct activation of postsynaptic ionotropic glutamate receptors with at least one dose of AMPA (ejecting current of −31 ± 18 nA; mean ± SD). In 5 of the 17 neurons, the effect of L-CCG-I was tested on an additional high dose of AMPA (ejecting current of −60 ± 34 nA; mean ± SD).

An example of the effect of L-CCG-I on vagally and AMPA-evoked responses in the same neuron is shown in Fig. 2. The vagally evoked synaptic responses, shown in the peristimulus time histograms (Fig. 2A), were markedly attenuated during L-CCG-I and then recovered. Iontophoresis of AMPA evoked a dose (current)-dependent increase in firing activity that was augmented during L-CCG-I and then recovered (ratemeter histograms in Fig. 2B).

The grouped data for the responses synaptically evoked by vagal stimulation and postsynaptically evoked by AMPA are presented in Fig. 3. The vagally evoked responses, which include data from all 25 neurons, were significantly attenuated during L-CCG-I and then partially recovered (Fig. 3A) (P = 0.011, ANOVA; *P < 0.05 Scheffe’s F test). Neither the mean baseline activity nor the onset latency or variability of the onset latency of the evoked responses was different from control.

To determine whether changes in MABP and HR contributed to the synaptically evoked responses during vagus nerve or ADN stimulation and whether there were differences when the responses were evoked in the control condition and during agonist or antagonist, we averaged the MABP and HR for 30 s immediately before and during the nerve stimulation. During the nerve stimulation we averaged the MABP and HR over the first and last seconds of the stimulation period, determined that the values were not different, and then averaged them. The MABP was compared by use of a one-way, repeated-measures ANOVA for four conditions (before and during vagal stimulation under control conditions and during agonist or antagonist). HR was analyzed in the same way.

Onset latency and variability of the onset latency of evoked responses, spontaneous activity, and magnitude of AMPA-evoked responses were compared for monosynaptically and polysynaptically activated cells by use of an unpaired Student’s t-test to compare the agonist-induced decrease in the vagally evoked responses before and in the presence of the agonist.

RESULTS

The results are based on 109 neurons recorded in 80 rabbits, in which the arterial blood pH was 7.41 ± 0.08, PCO₂ was 38 ± 4, and PO₂ was 236 ± 62 mmHg; resting MABP was 80 ± 14 mmHg and HR was 197 ± 23 beats/min (means ± SD). The neurons were concentrated in the intermediate and caudal NTS (15) from ~100 µm rostral to 1,000 µm caudal to the obex, at approximate depths of 700–1,500 µm ventral to the dorsal surface, and medial to the solitary tract. There were no detectable differences in the distribution of cells presumed to be monosynaptically or polysynaptically activated, and, in some instances, both were encountered in the same dorsoventral track.

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in the 17 cells in which L-CCG-I depressed vagal afferent signal transmission and in the 8 cells in which it did not (data not shown, \( P < 0.05 \), unpaired \( t \)-test).

By contrast to the synaptically evoked responses, the low-dose AMPA-evoked responses were significantly enhanced during L-CCG-I and partially recovered (Fig. 3B) (\( n = 17; P = 0.026 \), ANOVA; \( * P < 0.05 \), Scheffé's \( F \) test). The trend toward an augmentation of the high-dose AMPA responses did not reach statistical significance (Fig. 3C) (\( n = 5; P > 0.05 \), ANOVA). The extent to which L-CCG-I augmented the AMPA-evoked responses was not different in the monosynaptically activated neurons in which L-CCG-I inhibited the vagally evoked responses and in those in which L-CCG-I had no effect on the vagally evoked responses (\( P > 0.05 \); data not shown).

L-CCG-I was only effective in inhibiting vagal afferent signal transmission in 5 of 16 cells polysynaptically activated by vagus nerve stimulation; this was in contrast to the agonist's greater efficacy in inhibiting synaptic transmission in monosynaptically activated cells. For all 16 polysynaptically activated cells, the action potential response rate was 57 ± 4% before and 58 ± 7% during L-CCG-I (\( P > 0.05 \); paired \( t \)-test). Neither the mean baseline activity nor the onset latency or variability of the onset latency of the evoked responses was different in the 5 cells in which L-CCG-I depressed vagal afferent signal transmission and in the 11 in which it did not (data not shown, \( P > 0.05 \), unpaired \( t \)-test).

The mean onset latency and the variability of the onset latency of the vagally evoked responses were significantly greater in the polysynaptically activated than in the monosynaptically activated neurons (Table 1). On the other hand, the baseline activity was not different in the two groups (Table 1), nor was the extent to which L-CCG-I augmented the AMPA-evoked responses (\( P > 0.05 \); unpaired \( t \)-test; data not shown). For both monosynaptically and polysynaptically activated neurons, there was no change in the MABP or HR during the period of vagus nerve stimulation in the control period or during L-CCG-I (\( P > 0.05 \), ANOVA).

Frequency-dependent decay in vagally evoked responses. When a constant number of vagal stimuli were delivered at frequencies of 1, 3, 6, 10, and 20 Hz, there was a frequency-dependent decay in the number of action potentials evoked (\( n = 9; P = 0.0001 \), ANOVA). An example and the grouped data showing the fre-

### Table 1. Baseline activity and onset latency and onset latency variability of evoked responses of NTS neurons

<table>
<thead>
<tr>
<th></th>
<th>Baseline Activity, Hz</th>
<th>Onset Latency, ms</th>
<th>Onset Latency Variability, ms</th>
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<tbody>
<tr>
<td><strong>Vagal cells</strong></td>
<td></td>
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<tr>
<td>Monosynaptic responses (n = 53)</td>
<td>0.7 ± 0.3</td>
<td>32.4 ± 2.6*</td>
<td>2.1 ± 0.2*</td>
</tr>
<tr>
<td>Polysynaptic responses (n = 24)</td>
<td>1.5 ± 0.6</td>
<td>46.7 ± 4.2</td>
<td>9.2 ± 1.9</td>
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<tr>
<td><strong>ADN cells</strong></td>
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<tr>
<td>Monosynaptic responses (n = 2)</td>
<td>2.1 ± 0.7</td>
<td>4.0 ± 0.8</td>
<td>3.2 ± 1.6</td>
</tr>
<tr>
<td>Polysynaptic responses (n = 7)</td>
<td>4.2 ± 2.3</td>
<td>37.2 ± 12.2</td>
<td>9.0 ± 2.7</td>
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Values are means ± SE. NTS, nucleus of the solitary tract; ADN, aortic depressor nerve. \( * P < 0.05 \), monosynaptically vs. polysynaptically evoked responses.
frequency-dependent decay are shown in Fig. 4. In the example (Fig. 4A), the response rate of the synaptically evoked action potentials began to decay when 100 vagal stimuli were delivered at 6 Hz. For the grouped data (Fig. 4B), the decay became statistically significant at 6 Hz. Four cells were monosynaptically activated and five were polysynaptically activated.

Effect of the metabotropic glutamate receptor antagonist MPPG on NTS neuronal responses evoked by vagal afferent nerve stimulation and by AMPA. The decay in the action potential responses during high-frequency vagal nerve stimulation (3–20 Hz) was prevented by the metabotropic glutamate receptor antagonist MPPG, ejected with currents that did not change neuronal baseline activity (0.31 ± 0.10 Hz before and 0.37 ± 0.10 Hz during MPPG; P > 0.05, paired t-test). An example is shown in Fig. 5; the traces in Fig. 5A show the action potentials evoked during vagus nerve stimulation at 3 Hz. In the corresponding graphs in Fig. 5B, each dot represents the number of action potentials evoked by 10 stimuli. The vagally evoked responses of the cell decayed after ~30 stimuli under control conditions (Fig. 5A, top); the decay was prevented during MPPG (Fig. 5A, middle) and then recovered (Fig. 5A, bottom).

Figure 6 illustrates the ability of MPPG to augment synaptic transmission during high-frequency vagus nerve stimulation while in the same neuron having no remarkable effect on the neuronal response to postsynaptic activation of AMPA receptors. The synaptically evoked response nearly doubled during MPPG as shown in the peristimulus time histograms (Fig. 6A). In the same neuron, the AMPA-evoked responses at both low and high ejecting currents were not markedly changed during MPPG (ratemeter histogram in Fig. 6B). By contrast to the antagonist’s effect on higher-frequency vagally evoked responses, MPPG had no significant effect on the synaptically evoked responses during low-frequency vagus nerve stimulation or on the AMPA-evoked responses (Fig. 7).

The grouped data summarizing the effect of MPPG on the responses of NTS neurons to vagal stimulation at high (3–20 Hz) or low frequencies (1 Hz) and to AMPA are shown in Fig. 8. MPPG significantly augmented the number of action potentials synthetically evoked by vagus nerve stimulation at 3–20 Hz (Fig. 8A) (P = 0.0001, ANOVA; *P < 0.05, Scheffé’s F test). The augmentation was at least 15% in 20 of the 25 cells. Data from all 25 cells were included in the analysis. MPPG was equally effective in augmenting vagal transmission in neurons that met the criterion for monosynaptic activation (n = 20) and those presumed to be polysynaptically activated (n = 5; P > 0.05, unpaired t-test; data not shown). MPPG had no significant effect on the action potential responses evoked by low-frequency (1 Hz) vagus stimulation (Fig. 8B) (n = 11; P = 0.59, paired t-test) or on the responses evoked by AMPA (Fig. 8C) (n = 10; P = 0.53, ANOVA; data not shown).

For low-frequency vagus nerve stimulation, there was no change in the MABP or HR during the period of vagus nerve stimulation in the control period and during MPPG (P > 0.05, ANOVA). During high-frequency vagus nerve stimulation, there was no change in the MABP (P > 0.05, ANOVA); there was a small but statistically significant fall in HR, but the decrease was not statistically different in the control condition and during MPPG (−13 ± 2 vs. −14 ± 3 beats/min, respectively; P > 0.05, paired t-test).

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Fig. 4. Example (A) and grouped data (B) showing frequency-dependent decreases in number of action potentials evoked by vagus nerve stimulation. One hundred vagus nerve stimuli were delivered at each stimulation frequency. In A, each dot represents number of action potentials evoked by 10 vagus nerve stimuli. In B, number of action potentials evoked was expressed as a percentage of number of vagal stimuli delivered.

Fig. 5. Example of an NTS cell in which α-methyl-4-phosphonophenylglycine (MPPG) prevented the decay in number of action potentials evoked by vagus nerve stimulation at 3 Hz. A: action potential evoked during vagal stimulation. B: graphs corresponding to action potential responses. Each dot represents number of action potentials evoked by 10 stimuli.

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Fig. 6. The ability of MPPG to augment synaptic transmission during high-frequency vagus nerve stimulation while in the same neuron having no remarkable effect on the neuronal response to postsynaptic activation of AMPA receptors. The synaptically evoked response nearly doubled during MPPG as shown in the peristimulus time histograms (Fig. 6A). In the same neuron, the AMPA-evoked responses at both low and high ejecting currents were not markedly changed during MPPG (ratemeter histogram in Fig. 6B). By contrast to the antagonist’s effect on higher-frequency vagally evoked responses, MPPG had no significant effect on the synaptically evoked responses during low-frequency vagus nerve stimulation or on the AMPA-evoked responses (Fig. 7).
Frequency-dependent decay in ADN-evoked responses. When a constant number of ADN stimuli were delivered at frequencies between 1 and 80 Hz, there was a frequency-dependent decay in the number of action potentials evoked in 20 of 28 cells tested (P = 0.001, ANOVA; data not shown). Seven of eleven monosynaptically activated cells showed a decay, and thirteen of seventeen polysynaptically activated cells showed a decay. Two examples of this frequency-dependent decay are shown in Fig. 9. In Fig. 9A, the response rate of the ADN-evoked action potentials did not decay until ADN stimuli were delivered at 40 Hz. In another example (Fig. 9B), the decay began at 10 Hz and became more prominent at 20 Hz.

Effect of the metabotropic glutamate receptor antagonist MPPG on NTS neuronal responses evoked by ADN stimulation and by AMPA. The metabotropic glutamate receptor antagonist MPPG attenuated the decrease in the action potential responses of NTS neurons to high-frequency (6–80 Hz) ADN stimulation but had no effect on the responses to low-frequency (1 Hz) ADN stimulation. An example is shown in Fig. 10. MPPG had no effect on the synaptic responses to ADN stimulation at 1 Hz, as shown in the peristimulus time histograms (Fig. 10A), but prevented the decay in the response rate to ADN stimulation at 40 Hz (Fig. 10B).

The grouped data summarizing the effect of MPPG on the responses of NTS neurons to ADN stimulation at high frequencies (6–80 Hz) or low frequency (1 Hz) and to AMPA are summarized in Fig. 11. MPPG, ejected with currents that did not change neuronal baseline activity (3.8 ± 1.9 vs. 4.2 ± 2.2 Hz during MPPG; P > 0.05), significantly augmented the number of action potentials evoked by ADN stimulation at the high frequencies (Fig. 11A) (n = 9; *P = 0.048, paired t-test). For individual cells, the increase was at least 15% in six of the nine cells. All nine cells were included in the data analysis. Two cells met the criterion for monosynaptic activation by ADN stimulation (Table 1). MPPG had no effect on the number of action potentials evoked by
1-Hz ADN stimulation (Fig. 11B) (n = 9; P = 0.40) or on the AMPA-evoked responses in the seven of the nine neurons tested (P = 0.23, paired t-test).

There was no change in the MABP or HR during the period of ADN stimulation in the control period or during MPPG (P > 0.05, ANOVA).

MPPG blockade of L-CCG-I effects. To confirm that L-CCG-I effects were mediated by activation of metabotropic glutamate receptors, we tested the effect of the antagonist MPPG on the L-CCG-I-induced effects in three cells. An example and the summary are shown in Fig. 12. In the example (Fig. 12A), the vagally evoked response was decreased by L-CCG-I and then recovered after the L-CCG-I was stopped. Iontophoresis of MPPG, starting 1 min before L-CCG-I, prevented the L-CCG-I-induced diminution of the vagally evoked response. In the three cells tested (Fig. 12B), the L-CCG-I-induced diminution of the vagally evoked responses was abolished in the presence of MPPG (*P = 0.036, paired t-test).

DISCUSSION

The principal findings of this study were that in the intact animal, 1) the relatively selective group II metabotropic glutamate receptor agonist L-CCG-I de-
pressed the responses of NTS neurons to synaptic activation by vagus nerve stimulation while in the same cells augmenting the responses to activation of postsynaptic ionotropic AMPA receptors, 2) the relatively selective group II metabotropic glutamate receptor antagonist MPPG augmented the synaptic transmission of vagal afferent input at high-frequency vagal transmission (3–20 Hz) but had no effect on low-frequency (1 Hz) transmission or on AMPA-evoked responses, and 3) MPPG augmented the synaptic transmission of aortic baroreceptor afferent input at high-frequency ADN transmission (6–80 Hz) but had no consistent effect on low-frequency (1 Hz) transmission or on AMPA-evoked responses.

With iontophoretic application, the metabotropic glutamate receptor agonists and antagonists had access to both presynaptic and postsynaptic receptors, and thus the neuronal responses were composite responses to activation of receptors at both sites. Importantly, the agonist, L-CCG-I, reduced the neuronal responses to synaptic activation by vagal afferent input and in the same cells augmented the responses to activation of postsynaptic ionotropic glutamate receptors with AMPA. Glaum and Miller (13) similarly observed in the NTS slice that metabotropic glutamate receptor agonists reduced the amplitude of neuronal responses synthetically evoked by solitary tract-evoked stimulation. Together, the in vivo and in vitro observations suggest that actions of metabotropic glutamate receptor agonists at presynaptic sites predominate over actions at postsynaptic sites to reduce synaptic transmission of visceral afferent signals in the NTS.

In the present study the metabotropic glutamate receptor agonist L-CCG-I reduced synaptic transmission at a larger proportion of second-order neurons than at more distal neurons in the visceral afferent pathway. At first glance, the data raise the possibility

![Fig. 10. Examples of effect of MPPG on responses of an NTS neuron to 1-Hz and 40-Hz ADN stimulation. Peristimulus time histograms showing action potential responses to 120 ADN stimuli at 1 Hz (A) and at 40 Hz (B) during a control period and MPPG. Dots indicate stimuli. Bin width is 1.6 ms.](image)

![Fig. 11. Grouped data showing effect of MPPG on responses of NTS neurons to ADN stimulation at high frequency (6–80 Hz) (A) or low frequency (1 Hz) (B) and to AMPA (C). A: MPPG augmented response rate to highfrequency ADN stimulation (n = 9; *P = 0.048, paired t-test). B: in the same cells, MPPG had no significant effect on responses to ADN stimulation at 1 Hz (n = 9; P = 0.40, paired t-test). C: MPPG had no significant effect on AMPA-evoked responses (n = 7; P = 0.23, paired t-test).](image)
that metabotropic glutamate receptors located presynaptically on central vagal terminals are greater in number or have a greater affinity for or are more accessible to L-CCG-I compared with the receptors located presynaptically at more distal synapses in the NTS. However, the presumptive nature of the criteria for classifying cells as monosynaptically activated must be considered. In an NTS slice, Miles (17) used two criteria for presuming a cell was monosynaptically activated: 1) the ability of the cell to follow two stimuli separated by 5 ms and 2) an onset variability of <0.5 ms for solitary tract-evoked excitatory postsynaptic potentials (EPSPs). In an NTS slice, we too found that solitary tract-evoked EPSCs in neurons that followed two stimuli separated by 5 ms also had an onset variability of <0.5 ms (3). In the present study, for cells that followed two stimuli, the onset variability for the evoked action potentials was 1.6 ± 0.4 ms in cells studied with L-CCG-I (2.1 ± 0.2 ms for all vagally evoked responses), higher than the variability accepted by Miles (17) in the slice and lower than that reported by Scheuer et al. (22) for ADN-evoked action potentials in the rat (4 ± 1 ms). The method of measuring the postsynaptic response may also explain differences in onset variabilities, in that the EPSP onset may vary less than the rate of rise of the EPSP initiating the action potentials. Thus estimates of latency variability that rely on action potential onsets may exceed those made relying on EPSP or EPSC onsets. It may also be the case that the greater variability observed in vivo may be explained by ongoing synaptic inputs originating from neurons outside the confines of the slice that can have a fluctuating influence on the rate of rise of the EPSP and hence the appearance of the action potential. Of related interest are findings in a preliminary report by Scheuer and Mifflin (21) that in second-order NTS neurons identified anatomically by antero-
only if the paired stimuli were delivered at 20 Hz. We observed frequency-dependent synaptic depression of aortic baroreceptor transmission in both monosynaptically and polysynaptically activated NTS neurons at ADN stimulation frequencies of 3–80 Hz. Although there was no statistically significant difference in the frequencies at which the decay began in polysynaptically activated neurons compared with those monosynaptically activated, there was a trend for polysynaptically activated neurons to show a decay in the response rate at lower frequencies compared with the monosynaptically activated neurons. In both the Scheuer study (22) and the current study, the stimulus intensities used during low-frequency stimulation of 1 Hz were sufficient to evoke action potentials ~100% of the time; however, in our study, in general, the depression did not occur until after several stimuli were delivered, suggesting that the decay requires a critical number of afferent volleys. In both studies, the ADN stimulation frequencies associated with the decay in synaptic transmission were within the physiological range of baroreceptor afferent activity. Myelinated aortic baroreceptor activity can increase up to ~80 Hz for arterial blood pressure increases of ~28 mmHg (5), whereas unmyelinated baroreceptor activity can increase up to ~5 Hz with pressure increases of 40 mmHg (26). Further experiments will be required to determine whether presynaptic glutamate receptors similarly modulate baroreceptor afferent signal transmission that occurs with the natural stimulus of increasing levels of arterial blood pressure. The pattern of afferent input may be different, but the frequencies of ADN stimulation at which the metabotropic glutamate receptor antagonist was effective were within the physiologically relevant ranges of baroreceptor afferent activity. Thus it seems reasonable to infer that presynaptic metabotropic glutamate receptors would also modulate glutamate release associated with increasing afferent activity initiated by increases in arterial blood pressure.

Time-dependent inhibition of synaptic transmission in the NTS has also been observed with lesser stimulus intensities than were used in the current or Scheuer et al. (22) study. Felder and Heesch (9) found that a train of conditioning stimuli applied to the carotid sinus nerve (CSN) caused a prolonged inhibition of NTS responses to a test stimulus applied at threshold levels for initiating action potentials. Interestingly, the duration of the inhibition depended on the intensity of the conditioning stimuli, suggesting that increased glutamate release may contribute to synaptic depression. Mifflin and Felder (16) found that single or pulse trains of conditioning stimuli applied to the CSN produced time-dependent depression of the amplitude of postsynaptic potentials evoked by a test stimulus applied to the CSN. The depression of the neuronal response was directly related to the number of conditioning stimuli, suggesting that the amount of glutamate released at the synapse may contribute to the depression.

The observation that blockade of metabotropic glutamate receptors attenuated the decay in synaptic transmission associated with high-frequency vagal and ADN stimulation but had no effect on low-frequency synaptic transmission is consistent with the hypothesis that during higher frequencies of visceral afferent activity, the increased amounts of glutamate released can activate presynaptic metabotropic glutamate receptors to diminish further glutamate release and hence reduce synaptic transmission. Furthermore, the metabotropic glutamate receptor antagonist had no significant effect on the neuronal responses to activation of postsynaptic AMPA receptors in the same cells in which it attenuated the frequency-dependent synaptic depression. These data suggest that glutamate actions at presynaptic metabotropic glutamate receptors were predominant over its actions at metabotropic receptors located postsynaptically. Relevant to the current findings in the intact animal is the observation by Glaum and Miller (12) in the NTS slice that the broad-spectrum antagonist MCPG prevented posttetanic depression of postsynaptic excitatory currents evoked by solitary tract stimulation at 20 Hz for 2 s. Taken together, the in vivo and in vitro data suggest a frequency-dependent activation of presynaptic metabotropic glutamate receptors to limit high-frequency vagal and aortic baroreceptor afferent signal transmission in the NTS.

Frequency-dependent activation of presynaptic metabotropic glutamate receptors also occurs at mossy fiber synapses in the hippocampus; blockade of metabotropic glutamate receptors with the broad-spectrum antagonist MCPG has been shown to have no effect on synaptically evoked responses at low stimulation rates of the mossy fibers but to augment the responses at higher stimulation rates (20). A prolonged presence of glutamate in the synaptic cleft at these hippocampal synapses has been supported by anatomic studies indicating that group II metabotropic glutamate receptors are located at the preterminal zone of the mossy fiber synapses away from the release sites (30). Of direct relevance to the current findings is the recent observation of slow decay times for EPSCs evoked by solitary tract stimulation in the NTS slice, which are consistent with a prolonged resident time of glutamate in the synaptic cleft (27).

The current observations do not rule out the contribution of other mechanisms to frequency-dependent decay in synaptic transmission, including rapid AMPA receptor desensitization (18), depletion of synaptic vesicles at active zones (14), presynaptic calcium current inactivation (29), or activation of inhibitory interneurons. Indeed, multiple mechanisms may operate at NTS synapses to regulate sensory afferent signal transmission. Of related interest were the findings that the metabotropic glutamate receptor antagonist was equally effective in augmenting vagal afferent signal transmission to presumed second- and higher-order neurons in contrast to the actions of the agonist. The reasons for this difference in efficacy of the antagonist and agonist with regard to whether the synapses were second or higher order are not readily apparent. In any event, the findings raise the possibility that activation of presynaptic metabotropic glutamate receptors by endogenously released glutamate may not be limited to
synapses between primary visceral afferent fibers and the second-order NTS neurons.

The metabotropic glutamate receptors divide into three groups based on cloning studies and pharmacological profiles (8). The agonist used in this study, L-CCG-I, is relatively selective for group II receptors, which have been shown in expression systems to couple to inhibition of adenyl cyclase, but the agonist also has weak affinity for group I and group III receptors. The antagonist MPPG is also relatively selective for group II metabotropic glutamate receptors to vagal or aortic baroreceptor afferent signal transmission. In that regard, in vitro studies have determined that a broad-spectrum agonist, ACPD, and the selective group III agonist -(+)-2-amino-4-phosphonobutyrate similarly depress solitary tract-evoked responses of NTS neurons (13). Finally, it should also be noted that, although not addressed in this study, there is evidence obtained in the transverse medullary slice that metabotropic glutamate receptors not only inhibit glutamate release from visceral afferent fibers but may also depress GABA release from inhibitory neurons in the NTS (13).

In conclusion, the findings in this study are consistent with a model for vagal and aortic baroreceptor afferent signal transmission in the NTS, wherein at an afferent fiber activity of ~1 Hz, glutamate released at the central terminal is sufficient to activate postsynaptic ionotropic glutamate receptors to mediate the fast synaptic transmission and possibly postsynaptic metabotropic glutamate receptors to evoke a small postsynaptic excitation but is insufficient to activate presynaptic metabotropic glutamate receptors. At increased frequencies of afferent fiber activity, glutamate released at the central terminal is sufficient to activate presynaptic metabotropic glutamate receptors to inhibit further glutamate release and thereby reduce synaptic transmission.

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