Non-NMDA and NMDA receptors transmit area postrema input to aortic baroreceptor neurons in NTS

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Chen, Chao-Yin, and Ann C. Bonham. Non-NMDA and NMDA receptors transmit area postrema input to aortic baroreceptor neurons in NTS. Am. J. Physiol. 275 (Heart Circ. Physiol. 44): H1695–H1706, 1998.—We sought to determine whether glutamate acting at both N-methyl-D-aspartate (NMDA) and non-NMDA receptors transmits area postrema (AP) excitatory inputs to nucleus tractus solitarii (NTS) neurons in the aortic baroreceptor or vagal afferent pathways in vivo. In α-chloralose-anesthetized rabbits, we recorded extracellular NTS neuronal responses to low-frequency aortic depressor nerve (ADN), vagus nerve, and AP stimulation and to iontophoresis of α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid and NMDA during control, iontophoresis of 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo(\(f\))quinoxaline (NBQX), dl-2-amino-5-phosphonovaleric acid (AP5), or both, and recovery conditions. In neurons receiving ADN and AP inputs, NBQX attenuated AP- and ADN-evoked responses by 46 (P = 0.0206) and 49% (P = 0.0042). AP5 attenuated AP- and ADN-evoked responses by 39 (P = 0.0270) and 40% (P = 0.0157). NBQX + AP5 attenuated AP- and ADN-evoked responses by 74 (P = 0.0040) and 75% (P = 0.0028). In neurons receiving AP and vagal inputs, AP transmission was attenuated by 58, 60, and 98%; vagal transmission was attenuated by 62, 35, and 83% during NBQX, AP5, and both antagonists, respectively. These data suggest that both non-NMDA and NMDA receptors transmit AP input to NTS neurons in aortic baroreceptor or vagal afferent pathways.

There is considerable evidence that the area postrema (AP) modulates baroreflex control of arterial blood pressure (8, 15). Located on the dorsal surface of the medulla above the fourth ventricle, the AP is well suited for this modulatory function: it lacks a complete blood-brain barrier, making it accessible to circulating substances with cardiovascular-related actions, including angiotensin II, vasopressin, and endothelin (9, 10, 19, 34), and it sends prominent projections to other central nervous system (CNS) regions important in cardiovascular regulation, including the nucleus tractus solitarii (NTS) (26, 33, 35). Evidence for AP modulation of baroreflex function first emerged from studies examining the consequences of either stimulation or lesions in the AP on baroreflex control of sympathetic nerve activity. For example, either electrical or chemical stimulation in the AP was shown to augment baroreflex inhibition of sympathetic nerve activity (6, 16, 40), and lesions in the AP were shown to abolish the ability of circulating vasopressin to augment baroreflex-mediated sympathoinhibition (6, 34).

A likely target in the baroreflex central network for AP augmentation of baroreflex function is the NTS, where baroreceptor afferent signals are first integrated in the CNS (22). This proposal is supported by data obtained from extracellular recordings in the rabbit showing that AP neurons make excitatory synapses on NTS neurons in the aortic baroreceptor afferent pathway (5). Moreover, when the inputs from the AP and aortic depressor nerve (ADN) were optimally timed, they summed in a facilitative manner, suggesting an augmentation of the neuronal responses to combined AP and baroreceptor inputs (5). Facilitative interactions at NTS synapses have also been demonstrated for AP and vagal afferent inputs (5) in the intact rabbit and for AP and solitary tract inputs in a rabbit brain stem slice (18).

One important step in delineating the nature of AP modulation of baroreceptor signaling in the NTS is to identify the primary neurotransmitter(s) in the AP-NTS-baroreceptor synaptic pathway. Immunohistochemical studies have localized glutamate, norepinephrine, substance P, serotonin, and aspartate in the cell bodies of AP neurons (23, 27, 37). Whereas there are no data on the physiological role of the above putative neurotransmitter(s), one study has suggested that norepinephrine may be important in modulating AP augmentation of baroreflex function. Hassar and Bishop (14) found that microinjection of the α-2-adrenergic receptor antagonist yohimbine in the NTS diminished AP-mediated augmentation of baroreflex sympathoinhibition. In further support of a role for norepinephrine, extracellular recordings in the rabbit brain stem slice showed that yohimbine reduced the ability of AP stimulation to generate action potentials in NTS neurons (17) and facilitated solitary tract-evoked action potentials (18).

Glutamate must be considered as a likely neurotransmitter in the AP-NTS-baroreceptor synaptic pathway, in large part because of its ubiquitous distribution at excitatory synapses throughout the CNS. A more specific neurotransmitter role for glutamate in the AP-NTS pathway is suggested by its localization in AP nerve terminals (37) and by previous studies in the medullary slice documenting that AP stimulation synaptically activates both N-methyl-D-aspartate (NMDA) and non-NMDA receptors to evoke excitatory postsynaptic currents (EPSC) in NTS neurons that also receive excitatory input from the solitary tract (4). Still, we have no information on whether glutamate specifically mediates AP activation of NTS neurons in the aortic baroreceptor afferent pathway.
In the present study our major goal was to determine whether glutamate acting at either non-NMDA and NMDA receptors transmits AP excitatory inputs to NTS neurons in the aortic baroreceptor afferent pathway in the intact animal. Because AP and vagal afferent inputs also converge at NTS synapses, a secondary goal was to determine the extent to which non-NMDA and NMDA receptors may transmit AP excitatory inputs to NTS neurons that also receive general visceral inputs via the vagus nerve.

METHODS

General animal preparation. Experimental protocols 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 male New Zealand White rabbits (2.4–4.0 kg). Rabbits were preanesthetized with an intramuscular injection of a mixture of ketamine (50 mg/kg) and xylazine (2.5 mg/kg). A catheter was advanced into the inferior vena cava through the femoral vein for administration of drugs, sodium bicarbonate, and fluids. A catheter was advanced into the abdominal aorta through the femoral artery for measuring arterial blood pressure and for withdrawing blood for blood gases. Rabbits were then anesthetized with an initial intravenous injection of α-chloralose (20–30 mg/kg). The level of anesthesia was maintained by intravenous administration of α-chloralose (7–10 mg/kg) every 30 min before the animal was placed in a stereotaxic frame and every hour after.

Rabbits were tracheotomized through a midline cervical incision, ventilated with oxygen-enriched air at 25–30 breaths/min, and placed on 1-cmH2O positive end-expiratory pressure to prevent atelectasis. Arterial blood gases were maintained within normal limits (PO2, > 100 mmHg; PCO2, 35–45 mmHg, and pH = 7.35–7.45) by adjustment of the ventilation rate or intravenous infusion of sodium bicarbonate. Rectal temperature was maintained within 37 ± 1°C with a servo-controlled water blanket and a heat lamp. The electrocardiogram was recorded with subcutaneous electrodes for measuring heart rate and generating post-R wave histograms.

Each rabbit was placed in a stereotaxic head frame and suspended by a thoracic vertebral clamp. An occipital craniotomy was performed. The caudal portion of the fourth ventricle was exposed by removing the dura and arachnoid membranes and then covered with warm mineral oil. The ADN and vagus nerve were approached via a cervical lateral incision, placed on bipolar silver hook electrodes, covered with a mixture of warm petroleum jelly and mineral oil, and connected to constant current stimulus isolation units driven with a mixture of warm petroleum jelly and mineral oil, and incision, placed on bipolar silver hook electrodes, covered membranes and then covered with warm mineral oil. The ventricle was exposed by removing the dura and arachnoid ing heart rate and generating post-R wave histograms. gram was recorded with subcutaneous electrodes for measur-

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Before neuromuscular blockade, adequacy of anesthesia was determined every half hour by pinching the hindlimb paw and monitoring for hindlimb flinch or withdrawal or sudden fluctuation of arterial blood pressure (>5 mmHg) or heart rate (>10%). During neuromuscular blockade, adequacy of anesthesia was tested every half hour by determining whether there was a spontaneous or paw pinch-evoked fluctuation or increase in arterial blood pressure (>5 mmHg) or increase in heart rate (>10%). When any one of such responses was observed, a supplemental dose of pentobarbital sodium (10 mg iv) was given. A few minutes after the administration of pentobarbital sodium, the paw pinch test was reapplied to assure adequacy of anesthesia.

Extracellular single unit recording and iontophoresis. Extracellular recordings of single-unit activity were made through glass electrodes that extended ∼25 µm below the barrels of a five-barreled pipette (39). The recording barrel was filled with 2% Pontamine sky blue dye in 0.5 M sodium acetate. Of the remaining barrels, one contained normal saline for balancing ejection currents. The rest of the barrels contained aequous solutions of the NMDA receptor agonist NMDA (100 mM), the non-NMDA receptor agonist α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA, 20 mM), the NMDA receptor antagonist DL-2-amino-5-phosphonovale- ric acid (AP5, 100 mM), and the non-NMDA receptor antago-
ist 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo-(f)quinolin oxide (NBQX, 2 mM). The pH of all drugs was 6.0–8.0. Ejection and balancing currents were produced by a constant current source (Neuro Phore BH 2). All drugs were negatively charged and were ejected with anionic currents that ranged from 3 to 40 nA. Action potentials were fed via a high-impedance source follower to a second-stage amplifier, filtered (0.3–3 kHz), and fed in parallel to an oscilloscope and a computer. All data were recorded on a computer with data acquisition analysis software (RC Electronics) with a sampling rate of 10 kHz. Action potentials were presumed to arise from postsynaptic neurons if they exhibited variable onset latencies at low stimulus intensity and shorter onset latencies as the stimulus intensity was increased (11). Mean onset latencies and variability of the onset latencies were determined from poststimulus time histograms using stimulus intensities that evoked > 100% response rate.

All neurons were subjected to the presumptive criteria for monosynaptic versus polysynaptic activation (24, 32). Neurons were categorized as monosynaptically activated if they 1) discharged an action potential to each of two stimuli separated by 5 ms (24), and 2) the onset latency of the evoked response was varied by <2 ms. Neurons failing to meet these two criteria were categorized as polysynaptically activated.

At the beginning of each protocol and about every hour throughout the protocol, we stimulated the ADN at 30–100 µA, 0.7 ms, and 40 Hz. We presumed that a depressor response indicated viability of the nerve and of aortic baroreflex function. For screening the NTS for responsive neurons, the ADN or the vagus nerve was continuously stimulated at 0.5 Hz while the recording electrode was lowered slowly through the NTS. The current applied to the ADN was 5–10 times the current that decreased blood pressure 20–25 mmHg (<5 mmHg, 0.7 ms, 40 Hz). This stimulus intensity has been shown to activate both myelinated and unmyelinated fibers (29, 32). The searched NTS region extended from the rostral tip of the AP to calamus scriptorius in the rostrocaudal plane, 0–1,500 µm dorsal to midline (ipsilateral to the nerve), and 0–1,500 µm ventral to the dorsal surface. This region corresponds to the intermediate NTS, where cardiovascular and respiratory terminal afferent fibers are concentrated (22).

Protocols. To determine the NTS neuron responses to synaptic activation, we measured the number of evoked responses to 50 stimuli delivered at 1 Hz to the ADN, vagus nerve, or AP. In either AP/ADN- or AP/vagus-responsive neurons, AMPA and NMDA were locally ejected, and the increases in the number of action potentials were determined. All neurons were tested with at least one dose (current) of
AMP A and one dose of NMDA. In most neurons, AMPA and NMDA were ejected with two currents to establish dose (ejection current)-response curves. Once the responses to synaptic activation and the non-NMDA and NMDA receptor agonists were determined under the condition, we iontophoretically applied glutamate receptor antagonist (NBQX, AP5, or both). Three minutes into the iontophoresis period, the neuronal responses to synaptic activation and to AMPA and NMDA ejections were again measured. Starting 5 min after the antagonist injection was terminated, the neuronal responses to AMPA and NMDA ejections were tested for recovery. Once the AMPA- and NMDA-evoked responses recovered, the ADN- and AP-evoked or the vagal- and AP-evoked action potential responses were measured. In a pilot study, we performed a time control (same time interval as the protocol for antagonist except no antagonist was applied) of the neuronal responses to AP and ADN in two neurons. The neuronal response rates (the number of action potentials per second) were 46 ± 6 and 47 ± 10% for AP-evoked responses and 111 ± 21 and 107 ± 10% for ADN-evoked responses (means ± SD, n = 2).

Histology. 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 2% Pontamine sky blue 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 counterstained. Recording sites were reconstructed from dye spots and electrode tracks and plotted on coronal sections, with each section representing 400-µm rostrocaudal intervals with respect to the obex.

Data analysis. All data are expressed as means ± SE unless otherwise indicated. Significance was claimed at P < 0.05. Neuronal baseline activities were subtracted from the ADN-, vagal-, and AP-evoked responses for data analysis. The number of action potentials evoked by either ADN, vagal, or AP stimulation was expressed as a percentage of the number of stimuli delivered. The AMPA- and NMDA-evoked responses were expressed as the increase in the number of action potentials over baseline. For the effect of NBQX or AP5 on neuronal responses, ADN-, vagus nerve-, and AP-evoked responses were compared during a control period, antagonist, and a recovery period using a one-way ANOVA and followed by Fisher’s least-significant-difference (LSD) test when appropriate. For the effect of combined blockade (NBQX + AP5) on neuronal responses, synaptically evoked responses were compared between control and during combined blockade using a paired t-test. The spontaneous activity and the agonist-evoked responses were analyzed in the same way.

A chi-square test was used to determine whether the NTS neurons that receive monosynaptic or polysynaptic input from ADN is independent of whether the neurons also receive area postrema inputs.

RESULTS

The results are based on experiments performed in 71 rabbits. Resting mean arterial blood pressure was 86 ± 11 mmHg, and heart rate was 225 ± 22 beats/min. The arterial blood pH was 7.41 ± 0.03, Pco2 was 39 ± 2 mmHg, and PO2 was 307 ± 47 mmHg (means ± SD).

Neurons receiving convergent AP and ADN inputs. Seventy-four neurons received convergent excitatory inputs from the AP and the ADN. With the use of the criteria described in METHODS, of these 74 neurons only 9 (12%) were classified as monosynaptically activated by ADN stimulation, whereas 65 (88%) were classified as polysynaptically activated. By contrast, in an additional 36 neurons that received ADN but not AP input, 19 (53%) were monosynaptically activated by ADN stimulation, and 17 (47%) were polysynaptically activated. The ratio of NTS neurons monosynaptically activated to those polysynaptically activated by ADN stimulation was significantly less in the neurons that also received an excitatory AP input compared with those that received no excitatory AP input (P = 0.0001, chi-square test).

Of the 74 NTS neurons that received both AP and ADN inputs, 23 were further tested with non-NMDA and NMDA receptor agonists and antagonists. Of the 23 neurons, 2 neurons were first tested with AP5 followed by NBQX and 1 neuron was first tested with NBQX followed by AP5 + NBQX. All subsequent data are based on those 23 neurons. Seven of the twenty-three neurons exhibited a cardiac rhythm as shown by post-R wave histograms. From the presumptive criteria for monosynaptic activation, all 23 neurons were polysynaptically activated by AP stimulation, and all but one were polysynaptically activated by ADN stimulation. The mean onset latency for AP-evoked action potentials was 10.1 ± 3.4 (mean ± SD) ms. As we have previously shown for NTS neurons receiving combined AP and ADN inputs (5), ADN stimulation evoked action potentials having either a long-onset latency (71.5 ± 17.3 ms) in 20 neurons or a short-onset latency (5.3 ± 0.9 ms) in 3 neurons (means ± SD).

NBQX on AP/ADN neurons. Eleven neurons that received both AP and ADN inputs were tested with the non-NMDA receptor antagonist NBQX. NBQX, ejected in amounts that abolished AMPA-evoked responses and spared NMDA-evoked responses, significantly attenuated both AP- and ADN-evoked responses. An example is shown in Fig. 1. NBQX abolished the AMPA-evoked responses and spared NMDA-evoked responses at both low and high ejection currents (low dose and high dose), as shown in the ratemeter histograms (Fig. 1B). In the same neuron, NBQX markedly attenuated the AP- and ADN-evoked responses (peristimulus time histograms in Fig. 1A). All responses recovered. There was no statistically significant difference in the ability of NBQX to attenuate ADN-evoked action potentials having long-onset latencies compared with those having short-onset latencies (unpaired t-test, P > 0.05).

The grouped data are presented in Fig. 2. All 11 neurons were tested with at least one dose of AMPA and one dose of NMDA; 8 neurons were tested with two doses of AMPA and NMDA. As shown in Fig. 2B, NBQX abolished the neuronal responses to both low (P = 0.0008, ANOVA, *P < 0.05, Fisher’s LSD test) and high (P < 0.0001, ANOVA, *P < 0.05, Fisher’s LSD) AMPA doses. By contrast, NBQX had no effect on the responses to either low (P = 0.6637, ANOVA) or high (P = 0.9951, ANOVA) NMDA doses. As illustrated in Fig. 2A, NBQX significantly reduced the AP-evoked responses
Fig. 1. Example of effect of 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo(f)quinoxaline (NBQX) on area postrema (AP), aortic depressor nerve (ADN), α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA)-, and N-methyl-D-aspartate (NMDA)-evoked responses of one nucleus tractus solitarii (NTS) neuron. A: peri-stimulus time histograms of action potential responses of the neuron to 50 AP stimuli (1 Hz, 90 µA, 0.7 ms, top panels) and 50 ADN stimuli (1 Hz, 400 µA, 0.7 ms, bottom panels) during a control period, iontophoresis of NBQX (−3 nA), and a recovery period. Closed circle indicates stimulus. Bin width is 3.2 ms. Inset: single stimulus-triggered sweeps showing waveshape of an action potential evoked from each stimulus. Inset bar, 40 ms. B: ratemeter histograms of responses of same neuron to AMPA ejected with low (−5 nA) and high (−10 nA) currents (top panels) and NMDA ejected with low (−5 nA) and high (−8 nA) currents (bottom panels). Bar indicates ejection period.

Fig. 2. Group data summarizing effect of NBQX on AP-, ADN-, AMPA-, and NMDA-evoked responses of NTS neurons. A: number of action potentials evoked was expressed as a percentage of the number of stimuli delivered. NBQX significantly reduced the AP- [P = 0.0206, ANOVA, *P < 0.05 Fishers least significant difference (LSD) test] and ADN (P = 0.0042, ANOVA, *P < 0.05 Fishers LSD test)-evoked responses. B: increases in number of action potentials evoked by AMPA and NMDA ejection. NBQX abolished AMPA-evoked responses at both low (P = 0.0008, ANOVA, *P < 0.05, Fishers LSD test) and high (P < 0.0001, ANOVA, *P < 0.05, Fishers LSD test) ejection currents and spared NMDA-evoked responses at both low and high ejection currents. Numbers in parentheses indicate number of neurons.
The spontaneous activity was 5.2 ± 1.2, 3.5 ± 1.0, and 5.9 ± 2.8 Hz during the control, NBQX, and recovery periods. Although not statistically different (P = 0.6141, ANOVA), there was a trend toward a lower baseline activity during NBQX. In all experiments, to obviate the effect of any changes in baseline activity, baseline activities were subtracted from the neuronal responses. NBQX had no significant effect on mean arterial pressure or heart rate. Mean arterial pressure was 91 ± 3 mmHg before and 91 ± 3 mmHg during NBQX (P = 0.88, paired t-test), and heart rate was 227 ± 5 beats/min before and 228 ± 5 beats/min during NBQX (P = 0.79, paired t-test).

AP5 on AP/ADN neurons. Ten neurons that received both AP and ADN inputs were tested with the NMDA receptor antagonist AP5. AP5, ejected in amounts that abolished NMDA-evoked responses and had no effect on the AMPA-evoked responses, also significantly attenuated both AP- and ADN-evoked responses. An example is shown in Fig. 3. AP5 abolished the NMDA-evoked responses but did not decrease the AMPA-evoked responses, as shown in the ratemeter histograms (Fig. 3B). In the same neuron, AP5 also markedly attenuated the AP- and ADN-evoked responses (peristimulus time histograms in Fig. 3A). All responses recovered.

The grouped data are presented in Fig. 4. All neurons were tested with at least one dose of AMPA and one dose of NMDA, 7 neurons were tested with two doses of AMPA and 8 neurons were tested with two doses of NMDA. As illustrated in Fig. 4B, AP5 abolished neuronal responses to both low (P < 0.0001, ANOVA, *P < 0.05, Fishers LSD test) and high (P = 0.0065, ANOVA, *P < 0.05, Fishers LSD test) NMDA doses. By contrast, AP5 had no effect on the neuronal responses to either low (P = 0.8079, ANOVA) or high (P = 0.5747, ANOVA) AMPA doses. AP5 significantly reduced the AP-evoked responses (39% decrease; P = 0.0270, ANOVA, *P < 0.05 Fishers LSD test) and ADN-evoked responses (40% decrease; P = 0.0157, ANOVA, *P < 0.05 Fishers LSD test).

The spontaneous activity was 6.7 ± 1.4, 3.6 ± 1.0, and 5.6 ± 3.2 Hz during the control, AP5, and recovery periods, respectively. The values were not significantly different (P = 0.2840, ANOVA), although there was a trend for spontaneous activity to be lower during AP5. AP5 had no significant effect on mean arterial pressure or heart rate. Mean arterial pressure was 88 ± 4 mmHg before and 87 ± 4 mmHg during AP5 (P = 0.30, paired t-test).
t-test), and heart rate was 230 ± 6 beats/min before and
231 ± 6 beats/min during AP5 (P = 0.44, paired t-test).
NBQX + AP5 on AP/ADN neurons. The effect of
combined application of NBQX and AP5 was tested in
five neurons. NBQX + AP5, ejected in amounts that
abolished both AMPA- and NMDA-evoked responses,
significantly attenuated both AP- and ADN-evoked
responses. An example is shown in Fig. 5. NBQX
abolished both AMPA- and NMDA-evoked responses,
as shown in ratemeter histograms (Fig. 5B). In the
same neuron, NBQX + AP5 markedly attenuated the
AP- and ADN-evoked responses (peristimulus time
histograms in Fig. 5A). All responses partially recov-
ered.

The grouped data are presented in Fig. 6. All but one
neuron were tested with two doses of AMPA and two
doses of NMDA. As shown in Fig. 6B, NBQX + AP5
abolished neuronal responses to both low (P = 0.0347,
paired t-test) and high (P = 0.0453, paired t-test) AMPA
doses. NBQX + AP5 also abolished the responses to
both low (P = 0.0107, paired t-test) and high (P = 0.0211,
paired t-test) NMDA doses (Fig. 6B). NBQX + AP5
significantly reduced the AP-evoked responses
(74% decrease; P = 0.0040, paired t-test) and ADN-
evoked responses (75% decrease; P = 0.0028, paired
t-test).

During iontophoresis of both non-NMDA and NMDA
receptor antagonists, the spontaneous activity was
significantly reduced from 7.0 ± 0.7 to 2.7 ± 0.7 Hz (P
= 0.0136, paired t-test). NBQX + AP5 had no significant
effect on mean arterial pressure or heart rate. Mean
arterial pressure was 87 ± 8 mmHg before and 86 ± 8
mmHg during the antagonists (P = 0.51, paired t-test),
and heart rate was 213 ± 5 beats/min before and 212 ±
5 beats/min during the antagonists (P = 0.55, paired
t-test).

Neurons receiving convergent AP and vagal inputs.
Twenty-one neurons that received both AP and vagal
input were tested with the non-NMDA and NMDA
receptor agonists and antagonists. Of the 21 neurons, 2
neurons were first tested with AP5 followed by NBQX,
and 1 was tested with AP5, NBQX, and AP5 + NBQX.
No neurons were monosynaptically activated by AP
stimulation, and only one was monosynaptically acti-
vated by vagus nerve stimulation. The mean onset
latency for AP-evoked action potentials was 15.0 ± 8.2
ms. The mean onset latencies for vagal-evoked action
potentials were bimodally distributed as previously
reported (5), dividing into a short latency of 10.4 ± 1.1
ms (n = 2) and a long latency of 68.7 ± 13.0 ms
(means ± SD).

NBQX on AP/ vagus neurons. Ten NTS neurons that
received both AP and vagal input were tested with the
non-NMDA receptor antagonist. NBQX, ejected in
amounts that abolished AMPA-evoked responses and
spared NMDA-evoked responses, significantly attenu-
ated both AP- and vagal-evoked responses.

The grouped data are presented in Fig. 7. All neurons
were tested with two doses of AMPA and two doses of
NMDA. As illustrated in Fig. 7B, NBQX abolished the
neuronal responses to both low (P = 0.0036, ANOVA,
*P < 0.05, Fishers LSD test) and high (P = 0.0001,
ANOVA, *P < 0.05, Fishers LSD test) AMPA doses
and spared the neuronal responses to both low (P = 0.9489,
ANOVA) and high (P = 0.8476, ANOVA) NMDA doses.
As shown in Fig. 7A, NBQX significantly reduced both
AP-evoked responses (58% decrease; P = 0.0047,
ANOVA, *P < 0.05, Fishers LSD test) and vagal-
evoked responses (62% decrease; P = 0.0001, ANOVA,
*P < 0.05, Fishers LSD test).
Fig. 5. Example of the effect of NBQX + AP5 on AP-, ADN-, AMPA-, and NMDA-evoked responses of one NTS neuron. A: peristimulus time histograms of action potential responses of neuron to 50 AP stimuli (1 Hz, 150 µA, 0.7 ms, top panels) and 50 ADN stimuli (1 Hz, 1000 µA, 0.7 ms, bottom panels) during a control period, iontophoresis of NBQX (−15 nA) + AP5 (−15 nA), and a recovery period. Closed circle indicates stimulus. Bin width is 3.2 ms. Inset: single stimulus-triggered sweeps showing waveshape of an action potential evoked from each stimulus. Inset bar, 40 ms. B: ratemeter histograms of responses of the same neuron to AMPA ejected at −30 nA (top panels) and NMDA ejected at −15 nA (bottom panels). Bar indicates ejection period.

Fig. 6. Group data summarizing the effect of NBQX + AP5 on AP-, ADN-, AMPA-, and NMDA-evoked responses of NTS neurons. A: number of action potentials evoked was expressed as a percentage of number of stimuli delivered. NBQX + AP5 significantly reduced the AP- (*P = 0.0040, paired t-test) and ADN- (*P = 0.0028, paired t-test) evoked responses. B: increases in number of action potentials evoked by AMPA and NMDA ejections. NBQX + AP5 abolished AMPA-evoked responses at both low (*P = 0.0347, paired t-test) and high (*P = 0.0453, paired t-test) ejection currents, and NMDA-evoked responses at both low (*P = 0.0107, paired t-test) and high (*P = 0.0211, paired t-test) ejection currents. Numbers in parentheses indicate number of neurons.
The spontaneous activity averaged 1.0 ± 0.4, 0.8 ± 0.3, and 0.3 ± 0.3 Hz during the control, NBQX, and recovery periods, respectively, and was not significantly different (P = 0.5932, ANOVA).

AP5 on AP/vagus neurons. Twelve NTS neurons that received both AP and vagal inputs were tested with NMDA receptor antagonist. AP5, ejected in amounts that abolished NMDA-evoked responses and spared AMPA-evoked responses, significantly attenuated both AP- and vagus-evoked responses.

The grouped data are presented in Fig. 8. All neurons were tested with two doses of NMDA. In 5 of 12 neurons, only one dose of AMPA was tested. As shown in Fig. 8B, AP5 abolished the neuronal responses to both low (P = 0.0076, ANOVA, *P < 0.05, Fishers LSD test) and high (P = 0.0086, ANOVA, *P < 0.05, Fishers LSD test) NMDA doses and spared the responses to both low (P = 0.5966, ANOVA) and high (P = 0.9859, ANOVA) AMPA doses. As shown in Fig. 8A, AP5 significantly reduced both AP-evoked responses (60% decrease; P = 0.0378, ANOVA, *P < 0.05, Fishers LSD test) and vagal-evoked responses (35% decrease; P = 0.0043, ANOVA, *P < 0.05, Fishers LSD test).

The spontaneous activity was 2.6 ± 0.9, 0.9 ± 0.4, and 2.6 ± 2.2 Hz during the control, AP5, and recovery periods, respectively. Whereas there was a trend for

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**Fig. 7.** Group data summarizing the effect of NBQX on AP-, vagal-, AMPA-, and NMDA-evoked responses of NTS neurons. A: number of action potentials evoked was expressed as a percentage of number of stimuli delivered. NBQX significantly reduced AP- (P = 0.0047, ANOVA, *P < 0.05 Fishers LSD test) and vagal (P = 0.0001, ANOVA, *P < 0.05 Fishers LSD test)-evoked responses. B: increases in number of action potentials evoked by AMPA and NMDA ejection. NBQX abolished AMPA-evoked responses at both low (P = 0.0036, ANOVA, *P < 0.05, Fishers LSD test) and high (P = 0.0001, ANOVA, *P < 0.05, Fishers LSD test) ejection currents and spared NMDA-evoked responses at both low and high ejection currents. Numbers in parentheses indicate number of neurons.

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**Fig. 8.** Group data summarizing the effect of AP5 on AP-, vagal-, NMDA-, and AMPA-evoked responses of NTS neurons. A: number of action potentials evoked was expressed as a percentage of number of stimuli delivered. AP5 significantly reduced the AP- (P = 0.0378, ANOVA, *P < 0.05 Fishers LSD test) and vagal (P = 0.0043, ANOVA, *P < 0.05 Fishers LSD test)-evoked responses. B: increases in number of action potentials evoked by NMDA and AMPA ejections. AP5 abolished NMDA-evoked responses at both low (P = 0.0076, ANOVA, *P < 0.05 Fishers LSD test) and high (P = 0.0086, ANOVA, *P < 0.05 Fishers LSD test) ejection currents and spared AMPA-evoked responses at both low and high ejection currents. Numbers in parentheses indicate number of neurons.
spontaneous activity to be lower during AP5, the difference did not reach statistical significance (P = 0.3430, ANOVA).

NBQX + AP5 on AP/vagus neurons. Four NTS neurons that received both AP and vagal input were tested with combined application of NBQX and AP5. Similar to the findings of AP and ADN neurons, NBQX + AP5, ejected in amounts that abolished both AMPA and NMDA-evoked responses, significantly attenuated both AP- and vagal-evoked responses.

All four neurons were tested with two doses of AMPA and two doses of NMDA. NBQX + AP5 abolished the neuronal responses to both low (from 65 ± 31 to 0 ± 0 spikes/s) and high (177 ± 37 to 16 ± 10 spikes/s, P = 0.0159, paired t-test) AMPA doses as well as the responses to both low (from 51 ± 22 to 0 ± 0 spikes/s) and high (from 125 ± 41 to 0 ± 0 spikes/s) NMDA doses. NBQX + AP5 significantly reduced the AP transmission by 98% (from 65 ± 13 to 1 ± 1%, P = 0.0158, paired t-test) and vagal transmission by 83% (from 108 ± 14 to 18 ± 17%, P = 0.0003, paired t-test).

During iontophoresis of both non-NMDA and NMDA receptor antagonists, the spontaneous activity tended to be lower compared with the control period (1.5 ± 1.2 vs. 0.03 ± 0.03 Hz, respectively), but the difference did not reach statistical significance (P = 0.3262, paired t-test).

Location of recording sites. Neurons receiving convergent inputs from the AP and ADN or from the AP and vagus nerve were located medial to the tract in the intermediate and caudal NTS. This region, which spans the AP in the rostrocaudal plane, is where baroreceptor and cardiopulmonary afferent nerve terminals are concentrated (22, 36). An example of a recording site of a neuron receiving convergent inputs from the AP and ADN is shown in the photomicrograph in Fig. 9A. Figure 9B shows the composite of recording sites for neurons receiving combined excitatory inputs from the AP and ADN (closed circles) and neurons receiving convergent excitatory inputs from the AP and vagus nerve (open circles). The recording site shown in Fig. 9A is indicated by an asterisk in the composite within Fig. 9B. There were no detectable differences between the distribution of neurons presumed to be monosynaptically or polysynaptically activated by ADN stimulation. There were no detectable differences in the location of neurons receiving ADN input alone, convergent AP and ADN inputs, or convergent AP and vagus inputs. In some instances, they were encountered in the same dorsoventral track.

DISCUSSION

The major finding of the present study was that in the intact rabbit, AP activation of NTS neurons in the aortic baroreceptor afferent pathway was largely mediated by glutamate acting at both non-NMDA and NMDA receptors. In addition, the majority of aortic baroreceptor neurons that received a convergent excitatory input from the AP were higher-order neurons in the baroreceptor afferent pathway, being activated over polysynaptic pathways not only by ADN but also by AP input. Finally, AP activation of vagal afferent neurons in the NTS was also mediated by both non-NMDA and NMDA receptors.

To isolate the contributions of non-NMDA and NMDA receptors in the AP-NTS synaptic pathway, we used concentrations of NBQX that nearly abolished the neuronal responses to AMPA while sparing the responses to NMDA; the same strategy was used for AP5. Under these experimental conditions, low-frequency AP activation of NTS aortic baroreceptor neurons was diminished 46% by blockade of non-NMDA receptors, 39% by blockade of NMDA receptors, and 74% by combined blockade of both non-NMDA and NMDA receptors. The extent of diminution was similar, though slightly higher for AP activation of NTS vagal afferent neurons with individual and combined blockade of non-NMDA and NMDA receptors. Together the data support the hypothesis that glutamate is the primary neurotransmitter mediating low-frequency synaptic inputs from the AP to NTS neurons receiving aortic baroreceptor afferent input as well as to neurons receiving sensory input conveyed by the vagus nerve.

The physiological relevance of NMDA receptors in AP transmission to baroreceptor neurons may reside in two characteristics of the receptors that allow added signal processing capacity beyond that provided by non-NMDA receptors. First, NMDA receptor channel conductance is maximal when the cell is depolarized, suggesting that NMDA receptor contribution to AP synaptic transmission may be enhanced as the neurons are further depolarized by increasing frequencies of baroreceptor input. Second, the slowly developing, prolonged depolarization mediated by NMDA receptors lengthens the time during which subthreshold or otherwise ineffective baroreceptor signals can be integrated.

One impetus to focus on the role of glutamate in the AP-NTS pathway was our previous data from voltage-clamp experiments in the rat brain stem slice. In that study, when the membrane potentials of NTS neurons were clamped at voltages positive to −40 mV, low-frequency (0.2 Hz) AP stimulation evoked slowly developing NMDA EPSC; these were in addition to the fast non-NMDA receptor EPSC detectable at membrane potentials of −100 to +80 mV in the same neuron. However, when the cells were tested at −60 mV, under current clamp conditions, only non-NMDA receptors were required for AP stimulation to generate action potentials in the NTS neurons (4). The findings showed that at least when the membrane potential of an NTS neuron is at −60 mV in a brain stem slice, low-frequency AP synaptic transmission, which is generation of postsynaptic action potentials, is mediated by activation of non-NMDA receptors.

The whole cell recordings using the voltage-clamp technique in the slice allowed for the unequivocal identification of non-NMDA and NMDA receptor-mediated currents on a single neuron in the AP-NTS synaptic pathway. However, in the slice preparation, the afferent fibers from sources outside the slice are severed, removing synaptic inputs from other CNS areas. In the intact animal these synaptic inputs are
preserved. Thus, in the in vivo experiments, a higher level of synaptic input from outside the AP-NTS axis may have resulted in the AP inputs being tested on neurons having slightly more positive membrane potentials compared with neurons in the slice. Subtle depolarization of the membrane potential may have lessened the Mg$^{2+}$ block of the NMDA receptor channel (28), thereby optimizing the contribution of NMDA receptors to AP-NTS transmission. Consistent with that premise, in the whole animal blockade of either non-NMDA or NMDA receptors resulted in a trend, albeit not statistically significant, toward a decrease in the baseline activity of the aortic baroreceptor neurons by 33 and 46%, respectively, suggesting a tonic glutamatergic input depolarizing the cells.

The trend for NBQX and AP5 to decrease the baseline activity of the aortic baroreceptor neurons in the current study raises the possibility that this decrease explained the antagonist effects on the synaptically evoked responses of the cells. This seems unlikely, since there was no significant correlation between NBQX-evoked effects on spontaneous activity and the magnitude of the NBQX blockade of AP-evoked responses ($R = 0.2$, $P = 0.65$); there also was no correlation between AP5 effects on spontaneous activity and AP-evoked responses ($R = 0.4$, $P = 0.23$). Furthermore, in the vagal afferent neurons, NBQX, which exhibited the largest effect observed with an individual antagonist in blocking AP synaptic transmission (63%), only decreased spontaneous activity by 20%.
Although not the main focus of this study, the data also add to the considerable evidence that both non-NMDA and NMDA receptors participate in baroreceptor signaling in the NTS (12, 13, 20, 21, 30). Of related interest, data obtained from studies examining sensory afferent transmission in NTS slices have led to the proposal that whether the neuron is second- or higher-order in sensory afferent pathways may correlate with the contribution of non-NMDA vs. NMDA receptors in synaptic transmission (1). Intracellular recordings made in NTS neurons at resting membrane potentials have shown that blockade of non-NMDA receptors decreased solitary tract-evoked EPSP amplitude by >85%, whereas blockade of NMDA receptors had relatively little effect, decreasing the amplitude by <20% (2). However, in a voltage-clamp study in neurons in the same NTS region that met the presumptive criteria for monosynaptic activation, when the membrane potential was held at voltages positive to -45 mV or when Mg²⁺ was reduced to nominal amounts, low-frequency (0.2 or 0.5 Hz) solitary tract stimulation evoked NMDA-receptor-mediated EPSC (3); these were in addition to non-NMDA receptor-mediated EPSC, which were detectable at voltages from -90 to +60 mV. Together these findings suggest that in vitro whereas both non-NMDA and NMDA receptors are synaptically activated on presumptive second-order neurons in the NTS during low-frequency stimulation of sensory afferent fibers in the solitary tract, non-NMDA receptors are dominant in mediating changes in postsynaptic membrane potentials associated with synaptic transmission at these synapses. The reasoning has been extended to suggest that whereas NMDA receptors probably do not mediate synaptic transmission to second-order neurons, they may mediate sensory afferent transmission via polysynaptic pathways to higher-order neurons (1).

In the majority of neurons in the present study, both AP and the ADN inputs were conveyed over polysynaptic pathways, supporting the reasoning that the contribution of NMDA receptors may be more prominent in synaptic transmission at NTS neurons via polysynaptic pathways. However, the presumptive nature of the criteria for distinguishing monosynaptic activated neurons must be acknowledged. Miles (24) used two criteria: 1) the ability of the cell to discharge an action potential to each of two stimuli, and 2) a minimum onset latency of <0.5 ms for solitary tract excitatory postsynaptic potentials in a medullary slice. Scheuer and Mifflin (31) recently reported that in second-order NTS neurons identified anatomically by anterograde tracing of the vagus nerve, the ability of the labeled NTS cells to follow two stimuli separated by 5 ms was more reliable in differentiating monosynaptically from polysynaptically activated cells than was the use of minimum latency variability. However, the above criteria, used for either solitary tract- or peripheral sensory afferent fiber-evoked responses, may not be as reliable for differentiating cells monosynaptically activated versus polysynaptically activated by AP stimulation. We recently showed in vitro that NTS cells failed to reliably discharge an action potential in response to each of two AP stimuli separated by 5 ms, despite a relatively consistent onset latency (<1 ms variability) (4). The inability of the NTS neurons to follow both AP stimuli in vitro and in vivo may be due not to the presence of more than one synapse in the pathway but to factors such as conduction failure at branch points, a possibility consistent with the morphology of the AP neurons described as small and multipolar (25).

Of consideration also is whether the excitatory input from the AP to NTS units arose from the depolarization of constituent cell bodies or dendrites or of axons coursing through the AP. In two previous studies (5, 38), we have shown that AP injections of kainic acid abolished the ability of AP neurons to evoke action potentials in NTS neurons. We further showed that while the kainic acid blockade of AP synaptic transmission to an NTS neuron was impaired, the same neuron could still be synaptically activated by either ADN or vagus nerve stimulation. From these previous data suggesting that kainic acid can transiently produce a depolarization blockade of somatodendritic but not axonal membranes (7), we interpreted the results to suggest that electrical stimulation in the AP largely activates cell bodies or dendrites and not axons coursing through the structure. This assumption is further supported by anatomical studies suggesting that the AP contains cell bodies for the most part, with very few fibers coursing through the structure (25).

Whereas the current findings suggest that glutamate is the primary neurotransmitter acting at both non-NMDA and NMDA receptors in the AP-NTS-ADN pathway, they do not address the potential contributions of neuromodulators. One likely neuromodulator in the AP-NTS synaptic pathway is norepinephrine, which has been shown by Hasser and Bishop (14) to contribute to AP augmentation of baroreflex function in the intact rabbit.

In summary, the data support the hypothesis that the aortic baroreflex afferent pathway contains NTS neurons strategically placed to also receive synaptic signals from the AP. The findings further suggest a neural network in which AP modulation of baroreceptor signaling may occur at higher-order NTS neurons in the aortic baroreflex pathway. The convergence of glutamatergic inputs activating both non-NMDA and NMDA receptors from the AP and aortic baroreceptors on these NTS neurons may represent a pivotal site in the central network whereby activation of AP neurons augments baroreflex function.

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