Am J Physiol Heart Circ Physiol 284: H884–H891, 2003;
10.1152/ajpheart.00601.2002.

Activity-dependent role of NMDA receptors in transmission of cardiac mechanoreceptor input to the NTS

J. L. SEAGARD, C. DEAN, AND F. A. HOPP
Zablocki Department of Veterans Affairs Medical Center and Department of Anesthesiology, Medical College of Wisconsin, Milwaukee, Wisconsin 53295

Submitted 15 July 2002; accepted in final form 12 November 2002

Seagard, J. L., C. Dean, and F. A. Hopp. Activity-dependent role of NMDA receptors in transmission of cardiac mechanoreceptor input to the NTS. *Am J Physiol Heart Circ Physiol* 284: H884–H891, 2003; 10.1152/ajpheart.00601.2002.—Evidence suggests that transmission of barosensitive input from arterial baroreceptors and cardiac mechanoreceptors at nucleus tractus solitarius (NTS) neurons involves non-*N*-methyl-D-aspartate (NMDA) glutamate receptors, but there is a possibility that the contribution of NMDA receptors might increase during periods of increased afferent input, when enhanced neuronal depolarization could increase the activation of NMDA receptors by removal of a Mg^{2+} block. Thus the effects of NMDA on cardiac mechanoreceptor-modulated NTS neuronal discharges were examined at different levels of arterial pressure used to change cardiac mechanoreceptor afferent input. To determine whether the response was specific to NMDA, (±)-α-amino-3-hydroxy-5-methylisoxazole-4-proprionic acid (AMPA) was also administered at different levels of neuronal discharge. In anesthetized dogs, neuronal activity was recorded from the NTS while NMDA or AMPA was picoejected at high versus low arterial stimulating pressures. NMDA, but not AMPA, produced a significantly greater discharge of mechanoreceptor-driven NTS neurons at higher versus lower levels of stimulating pressure. These data suggest that the role played by NMDA receptors is greater during periods of enhanced neuronal depolarization, which could be produced by increases in afferent barosensitive input.

medulla; nucleus tractus solitarius; AMPA; baroreceptors

MANY STUDIES have pointed to glutamate as the primary neurotransmitter for cardiovascular receptor inputs, including baroreceptor (2, 10, 21, 23) and cardiac receptor (19, 23) afferent input to neurons in the nucleus tractus solitarius (NTS), but the contribution of glutamate receptor subtypes to the transmission of this afferent input has not been conclusively determined. Evidence indicates that non-*N*-methyl-D-aspartate (NMDA) receptors may be the primary ionotropic receptor subtype involved in transmission to neurons that directly receive afferent baroreceptor activity (22), although additional evidence also supports a role for NMDA receptors (5, 6, 21). Studies from this laboratory have found that both NMDA and non-NMDA receptor antagonists alter discharges of both baroreceptor-modulated (16, 18) and cardiac mechanoreceptor-modulated (17) NTS neurons, suggesting that each type of ionotropic glutamate receptor may contribute to excitation of "baroreceptive" neurons, or neurons that receive barosensitive, pressure-related information from peripheral receptors. Whereas both glutamate receptor subtypes were found to be involved in the activation of these neurons, blockade of non-NMDA receptors eliminated activity in the majority of neurons, suggesting a greater role for this glutamate receptor subtype. However, the possibility exists that increased excitation of NTS baroreceptive neurons, induced by increases in afferent input from peripheral barosensitive receptors, will enhance the central contribution of NMDA receptors due to removal of a Mg^{2+} block of the channels with neuronal depolarization. This role for NMDA receptors has been described for other neural pathways (4, 20). Thus the role of NMDA receptors may be greater during periods of increased afferent input, as would occur during increases in blood pressure (BP), because some degree of neuronal depolarization could increase the availability of NMDA receptors. Therefore, the present study was conducted to examine whether the amount of NMDA-induced excitation of cardiac mechanoreceptor-modulated NTS neurons, activated by pressure-sensitive input from cardiac mechanoreceptors, was dependent on the level of afferent mechanoreceptor input. Results from this study suggest that NMDA receptors contribute more to the discharge of cardiac mechanoreceptor-modulated neurons during periods of increased pressure, suggesting that there is an activity-dependent role for NMDA receptors in the transmission of mechanosensitive input.

MATERIALS AND METHODS

General preparation. The discharge of cardiac mechanoreceptor-modulated neurons in the NTS was studied in anesthetized 12- to 15-kg mongrel dogs (initial dose of 50 mg/kg α-chloralose and 500 mg/kg urethane, supplemental continuous infusion of 250 mg α-chloralose + 2.5 g urethane/h iv). All experimental procedures followed were approved by the Animal Care and Use Committees of the Medical College of

Address for reprint requests and other correspondence: J. L. Seagard, Research Service 151, Zablocki VA Medical Center, 5000 W. National Ave., Milwaukee, WI 53295 (E-mail: jseagard@mcw.edu).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

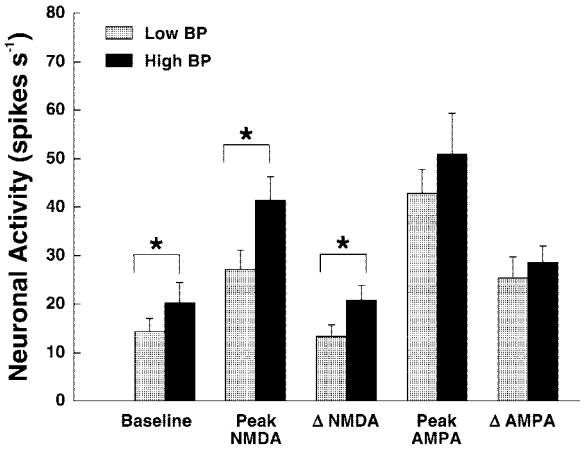

Fig. 1. Summed data for the effects of *N*-methyl-D-aspartate (NMDA) or (±)-α-amino-3-hydroxy-5-methylisoxazole-4-proprionic acid (AMPA) on cardiac mechanoreceptor-modulated neuronal discharge at different levels of arterial pressure. The bar graph demonstrates the effects of micropressure ejection (picoejection) of NMDA or AMPA on discharge of nucleus tractus solitarius (NTS) neurons receiving cardiac mechanoreceptor input at a high [high blood pressure (BP), 135–145 mmHg] versus low (low BP, 85–95 mmHg) level of stimulating pressure. The baroreceptive nature of the neurons is demonstrated by a significant increase in baseline discharge at the high BP level of stimulation versus low BP. Picoejection of NMDA resulted in a significantly greater peak level of discharge (peak NMDA) at high versus low BP. This degree of increase resulted in a significantly greater magnitude of the activity evoked by NMDA (ΔNMDA = peak − baseline activity) at high versus low BP. Unlike NMDA, the peak response (peak AMPA) and magnitude of evoked activity (ΔAMPA) to picoejection of AMPA was not significantly different at high versus low BP. *Significant difference between values for low versus high BP with $P < 0.05$.

Wisconsin and the Zablocki Department of Veterans Affairs Medical Center. The left femoral artery and vein were cannulated to permit measurement of arterial BP and infusion of anesthetic, respectively. Arterial blood gases were measured using an ABL 30 Radiometer Blood Gas Analyzer (Copenhagen, Denmark) and kept within normal ranges (P_{CO_2} 35–45 mmHg, $P_{O_2} > 100$ mmHg, pH 7.37–7.42) by adjustment of ventilation and infusion of bicarbonate. Arterial BP was measured via the catheter in the left femoral artery, which was connected to a Statham pressure transducer and a Grass model 7D polygraph (Quincy, MA). The carotid sinus and aortic depressor nerves were sectioned bilaterally to eliminate arterial baroreceptor afferent input, because the study was focused on examination of the responses of a specific population of neurons that received cardiac mechanoreceptor inputs.

After nerve isolation, the animal was placed in a head holder (Kopf; Tujunga, CA) for stereotaxic placement of a four-barrel micropipette for neuronal recording and pressure ejection. With the animal in the stereotaxic frame, an occipital craniotomy was performed, the dura was opened, and the dorsal portion of the medulla was exposed by lifting the vermis cerebelli. The animals were given a bilateral pneumothorax to reduce brain movement associated with changes in thoracic pressure.

Central neuronal activity was recorded using the multibarrel glass micropipette (total tip diameter 10–30 μm), with one barrel for neuronal recording containing a fine (7 μm) carbon filament connected via a high-impedance preamplifier (gain = 1,000; 0.1–10 kHz bandpass) and filter/amplifier (fourth-order Butterworth; 100 Hz–3 kHz bandpass) to a Vetter model 3000A PCM Recording Adapter (Rebersburgh, PA) and videocassette recorder for later data analysis. The remaining three barrels were filled with vehicle [artificial cerebrospinal fluid (aCSF)] and glutamate receptor agonists, as explained below, for picoejection (pressure ejection) onto recorded neurons using a system designed and constructed in the laboratory. The volumes of ejected solutions were measured visually by determining the change in meniscus height in each barrel using a ×50 monocular microscope with a calibrated graticule (7 nl/division). With the use of the obex as a zero reference, penetrations were randomly made into the left or right NTS from 1.0 mm caudal to 2.0 mm rostral to the obex, 0.0–2.0 mm lateral to the midline, and from the surface to 2.0 mm deep. The obex was described as the point where the central canal opens into the fourth ventricle, and this anatomic landmark is visible on the dorsal surface of the medulla. An incision was made in the pia, through which the electrode was inserted and advanced slowly using a hydraulic microdrive. Extracellular unit activity was recorded to

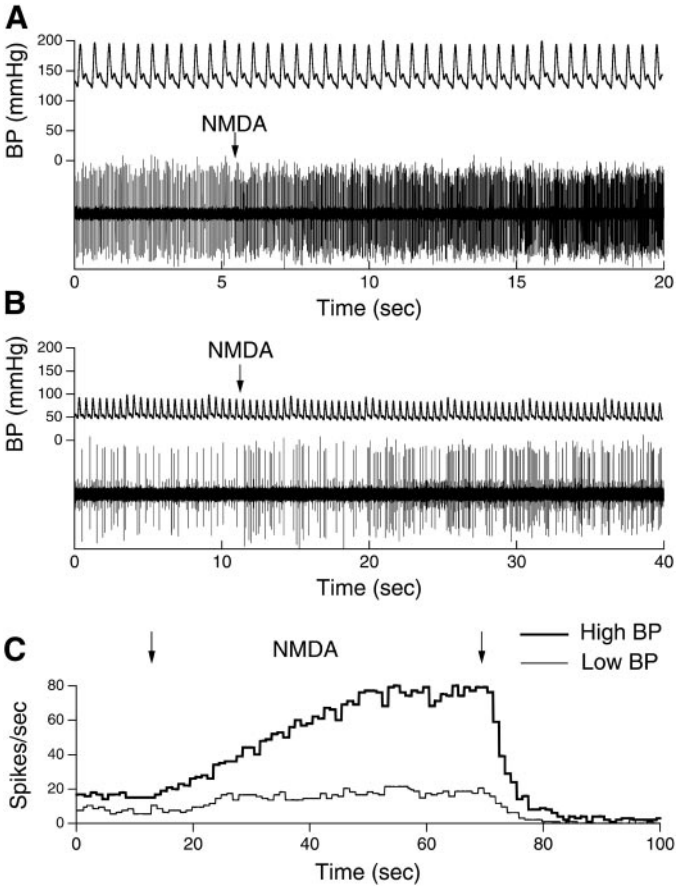

Fig. 2. Example of a cardiac mechanoreceptor-modulated NTS neuron that had a differential response to NMDA. Examples of raw neuronal activity (*A* and *B*) and averaged neuronal response (*C*) of a cardiac mechanoreceptor-modulated neuron to picoejection of NMDA are shown. The neuron had a higher rate of baseline discharge at high (*A*) versus low (*B*) stimulating levels of BP, as is also shown in the averaged neuronal activity traces in *C*. The time scale of *A* was expanded to permit better discrimination of the unit discharge. The increase in baseline neuronal activity was accompanied by an enhanced response to picoejection of NMDA (arrows) at the higher versus lower level of BP. Despite the increase in baseline activity, the increase in peak activity at high BP was sufficient to result in a greater magnitude of evoked activity to NMDA administration (peak − baseline activity) at high versus low BP (*C*).

identify neurons that displayed pulse-synchronous discharge, which correlated with the rising phase of aortic pressure and increased during elevations in arterial BP. These neurons have been previously shown to arise from cardiac mechanoreceptors with left vagal afferent pathways (17). Raw central neuronal activity and arterial BP were recorded on tape for later analysis. Unit activity was also directed to a time/amplitude window discriminator, which allowed spikes to be selected based on amplitude combined with shape. The discriminator uses an electronic threshold to trigger off the amplitude of the spike and a window through which the falling edge of the potential must fall. While all attempts were made to record from single neuronal recordings, the combination of criteria from the discriminator allowed selection of discharge from a single neuron in small multineuronal recordings if other action potentials were present. While it was possible in most experiments to obtain a clear single unit, this careful use of the discriminator ensured that only the single unit of interest was counted, even if recruitment of additional units was observed. If it was not possible to discriminate a single unit, even during high-frequency responses, the neuron was not studied. The discriminator generated a standard pulse for each spike that matched the prescribed preset windows. The pulse output of the discriminator was then fed into a digital counter/timer whose analog output was proportional to the number of spikes per unit time. These signals were displayed on-line on the Grass polygraph to monitor activity during the experiment.

Experimental protocol. To regulate and alter BP, animals were given hexamethonium (20 mg/kg iv), followed by a slow infusion of phenylephrine (1.0 mg/100 ml iv) to maintain BP at a mean pressure of 135–145 mmHg to provide an adequate level of stimulation of cardiopulmonary receptors at physiological levels of pressure. In addition, small increases in the phenylephrine infusion rate were used to elevate BP slightly to test for pressure-sensitive modulation of recorded neurons. The presence of hexamethonium prevented any baroreflex-induced changes in heart rate that would normally occur with changes in BP. With the pressure controlled, the NTS was randomly explored using the multibarrel electrode until activity from a single dorsal medullary neuron that displayed a distinct cardiac rhythm was obtained. The pulse-synchronous nature of the discharge was determined by comparing neuronal discharge with the BP pulse. Small changes in BP were produced by changes in the infusion rate of phenylephrine to confirm the pressure-related sensitivity of the afferent-evoked discharge of the NTS neuron. Pressures were reestablished at control levels, and, after a control period of recording, 15 nl aCSF was picoejected onto the neuron to test for vehicle and ejection movement effects on the discharge of the NTS neuron. If any vehicle effects were noted, the experiment was stopped until a new vehicle with no effects was made and tested. The drugs were then remixed in the new vehicle, which had no effects on neuronal activity.

The following protocol was utilized to determine whether the role of NMDA receptors was a function of the level of ongoing activity of single NTS neurons receiving cardiac mechanoreceptor input. BP was set randomly at a low (80–90 mmHg) or high (135–145 mmHg) mean BP, and baseline neuronal discharge at this level of afferent stimulation was recorded over a 20-s period. NMDA (NMDA receptor agonist, 100 μM) was then picoejected (7–15 nl total volume) until a plateau in the increased discharge produced in response to activation of NMDA receptors on the recorded neuron was obtained and recorded. After recovery from the picoejection, the stimulating pressure was set to the remaining high or low level, after which baseline activity at this new pressure was recorded. Picoejection of NMDA was then repeated at the same rate and dose to establish the plateau firing rate at the new pressure, which was recorded for later analysis. In eight of the neurons tested for NMDA, the effects of the non-NMDA receptor agonist [(±)-α-amino-3-hydroxy-5-methylisoxazole-4-proprionic acid (AMPA)] were also examined at the two levels of neuronal activation. AMPA (3.75 μM, 7–15 nl total volume) was picoejected onto the neuron in a manner similar to NMDA, with the order of administration of glutamate agonists randomized. Care was taken to allow the neuron to return to the baseline level of discharge before the second agonist was tested. Baseline neuronal activity and peak levels of activity induced by NMDA or AMPA administration

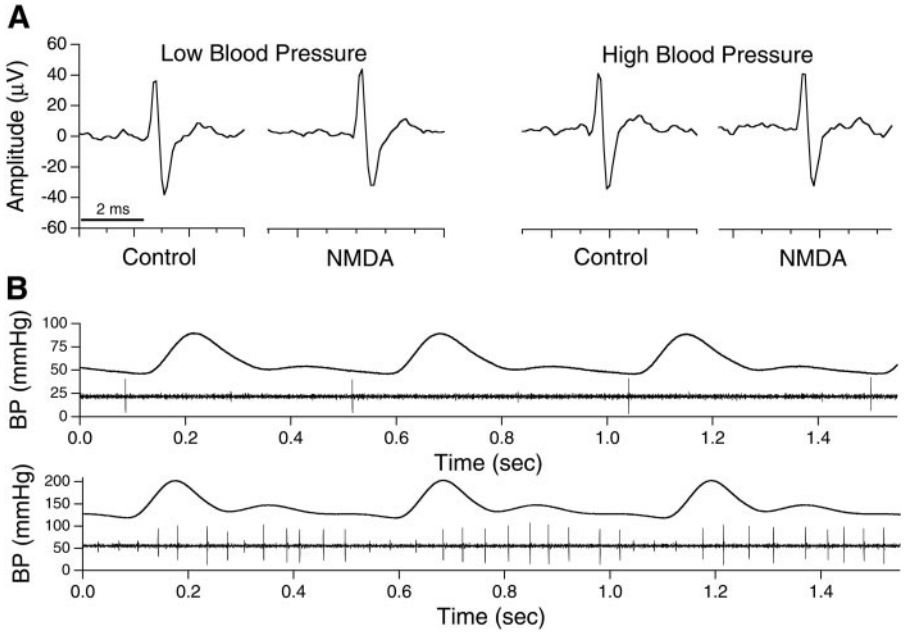

Fig. 3. Pulse-synchronous firing pattern and action potential characterization of the cardiac mechanoreceptor-modulated NTS neuron from Fig. 2. *A*: action potential of the neuron from Fig. 2 at different stimulating BP before and after exposure to NMDA. Care was taken when the spikes were analyzed to ensure that the same spike was counted, based on its amplitude and distinctive shape. As can be seen in *A*, changing BP and administering NMDA did not significantly change the shape or amplitude of the action potential. *B*: pulse-synchronous firing of the neuron studied in Fig. 2. Discharge of the neuron is shown at both the low (*top*) and high (*bottom*) stimulating pressures to show pulse synchronicity as well as the pressure-sensitive nature of the discharge. As can be seen, the number of spikes per pulse increased at the higher pressure. Similar responses were obtained for all neurons studied, although the number of spikes per pulse varied among neurons.

were determined for the two different levels of arterial-stimulating BP.

To ensure that any enhanced responses of the neurons to administration of NMDA was due to increased availability of the NMDA receptors versus altered release of glutamate or other modulators at the higher pressure, a limited additional protocol was performed on three pulse-synchronous neurons. As described for the pressure stimulation protocols, a baroreceptive neuron that responded to pressure activation of cardiac receptors was identified. The stimulation pressure was then lowered to a level below the threshold pressure needed to activate the neuron to prevent physiological changes in peripheral receptor input. AMPA (3.75 μM) was then picoejected at a slow constant rate (20–40 fmol/min) to establish a steady-state level of neuronal excitation via activation of non-NMDA receptors. Once activity had plateaued, NMDA (100 μM) was simultaneously picoejected at 1.2 pmol/min, a rate shown to be effective in activation of NMDA receptors. This slow rate of ejection was continued until a new plateau level for nerve activity was established. Frequency of picoejection of AMPA was then increased (40–60 fmol/min) to establish a new, higher steady-state of neuronal excitation. NMDA was again simultaneously picoejected onto the neuron at the same rate and dose as the first trial to examine the effects of excitation of NMDA receptors at this new level of neuronal activation. Because AMPA only activates non-NMDA receptors, any response to activation of the NMDA receptors at a higher level of AMPA administration should be due to increased availability of NMDA receptors and not to changes in glutamate or neuromodulator release.

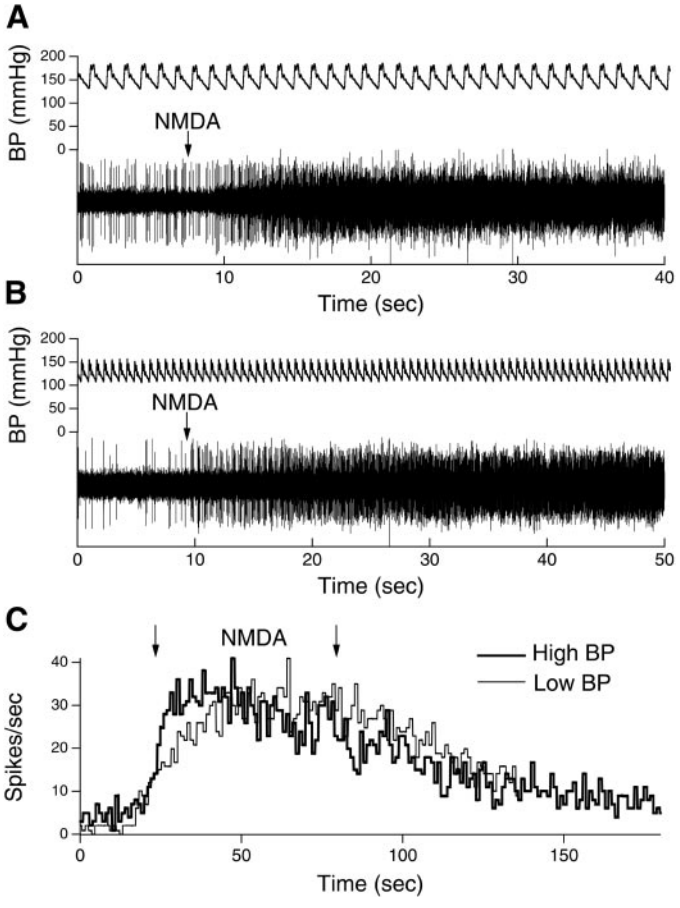

Fig. 4. Example of a cardiac mechanoreceptor-modulated NTS neuron that does not have a differential response to NMDA. Examples of raw neuronal activity (*A* and *B*) and averaged neuronal response (*C*) of a cardiac mechanoreceptor-modulated neuron that responded to picoejection of NMDA (arrows) but did not have a differential response at high (high BP) versus low (low BP) levels of stimulating pressure (*C*) are shown. The neuron had a slightly higher rate of baseline discharge at high (*A*) versus low (*B*) stimulating levels of BP, as is also shown in the averaged neuronal activity traces in *C*. However, the peak response and magnitude of the evoked activity to NMDA administration (peak − baseline activity) at high versus low BP were not significantly different (*C*).

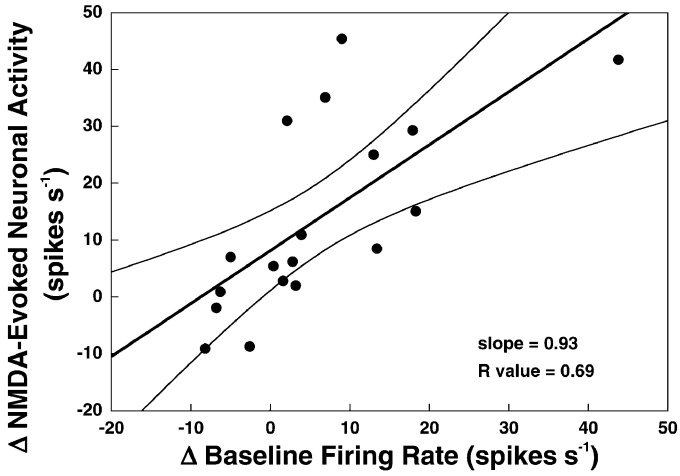

Fig. 5. This graph shows the linear regression of differences in the baseline firing rate versus differences in NMDA-evoked activity at high versus low stimulating pressures for 18 neurons (●). Regression analysis yielded a R value of 0.69 and a slope of 0.93, indicating a direct relationship between the change obtained in baseline firing rate and the magnitude of the response to administration NMDA. This suggests that increases in baseline firing induced by higher stimulating arterial pressure (increased barosensitive afferent input) results in increased responses to activation of NMDA receptors. Conversely, decreases in baseline pressure also result in decreases in magnitude of the response to NMDA administration. Thin lines represent 95% confidence intervals for the regression.

Data analysis. Analysis of recorded unit neuronal activity was accomplished off-line from recorded data using a Hewlett-Packard 310 computer equipped with a 16-channel Infotek 12-bit analog-to-digital converter. Unit activity was directed through a window discriminator whose output was then fed into a digital counter/timer that was sampled at a frequency of 20 Hz. Averaged spikes per second were obtained for a 20-s baseline control period before each picoejection administration of NMDA or AMPA. Peak unit discharge rates in response to administration of either NMDA or AMPA were determined for both high and low levels of stimulating BP. Given the change in baseline discharge that occurred with a change in arterial pressure, a key measure of the effects of either glutamate agonist was the magnitude of the agonist-evoked activity, or the difference in neuronal discharge from baseline to peak response to NMDA or AMPA administration at each pressure. This magnitude of evoked activity as well as baseline and peak levels of activity for AMPA or NMDA administration were compared for high versus low arterial pressures using a one-way ANOVA, with significance set at $P < 0.05$.

RESULTS

Eighteen neurons identified as cardiac mechanoreceptor modulated by their pulse-synchronous and pres-

sure-sensitive discharges were examined to determine the response to picoejection of NMDA at high versus low stimulation pressures. The summed data show that increases in arterial pressure were found to produce significant increases in baseline discharge rates of the cardiac mechanoreceptor-modulated NTS neurons, verifying the pressure sensitivity of the afferent input and ensuring that there was increased afferent input and thus greater NTS neuronal activation at the higher pressures (baseline, Fig. 1). Picoejection of NMDA induced a significantly greater increase in peak discharge at the high versus low BP (peak NMDA, Fig. 1). The magnitude of the NMDA-evoked difference in firing rate from baseline to peak discharge rate was also significantly greater at the higher versus lower pressure (ΔNMDA, Fig. 1). However, there was a range of both pressure sensitivities and responses to administration of NMDA. An increase in the magnitude of the NMDA-evoked response at the high stimulating BP was obtained in 14 of 18 neurons studied. Two of the neurons did not have any differential response to NMDA, and two neurons actually had a decrease in the magnitude of the NMDA-evoked activity at the higher stimulating pressure. The raw discharge and averaged activity of a neuron in response to picoejection of NMDA at high (*A*) versus low BP (*B*) is shown in Fig. 2. The traces reflect the increase in the baseline neuronal discharge rate at the higher pressure and the enhanced response to picoejection of NMDA at this level of neuronal activation. Some variation in spike amplitude due to respiration is evident in the raw activity traces. The complete averaged discharge response of this neuron that had an increase in baseline activity, and a significantly greater evoked activity in response to NMDA in response to the higher BP is shown in Fig. 2*C*. The increase in baseline discharge was associated with an increased peak discharge rate and a greater magnitude in the NMDA-evoked activity at the higher stimulating BP. Figure 3 demonstrates the pulse-synchronous and pressure-sensitive nature of the discharge of this neuron (*B*) and the stable shape of the action potential at different stimulating BP (*A*) before and after administration of NMDA, necessary to ensure accurate analysis of neuronal firing rates. Figure 4 shows raw and averaged neuronal activity for a neuron that had a small increase in baseline discharge but no difference in the NMDA-evoked response at the high versus low stimulating pressures.

To determine whether there was a relationship between the amount of change in the baseline firing rate and the magnitude of the response to NMDA administration at low versus high stimulating BP, the values for the change in baseline firing rate from low to high pressure for each neuron were plotted versus the change in the magnitude of the NMDA-evoked responses for low to high BP (Fig. 5). Linear regression was used to examine the relationship between these parameters. As can be seen, there was a correlation between the baseline firing rate and the peak increase in response to NMDA picoejection ($R = 0.69$). This suggests that there is a direct relationship between the change in baseline firing, or ongoing discharge, with the magnitude of the response to administration of NMDA. Thus greater increases in the baseline discharge rate will result in greater increases in discharge in response to NMDA, and vice versa.

Unlike NMDA, there was no significant difference in the peak rates of firing in response to AMPA administration (peak AMPA, Fig. 1), and thus no significant difference in the magnitude of the evoked discharge between high and low stimulation pressures (ΔAMPA, Fig. 1). For four of eight neurons tested, AMPA induced a slightly greater magnitude of evoked activity at the high versus low BP. No difference in AMPA-evoked activity was seen in the remaining four neurons. An

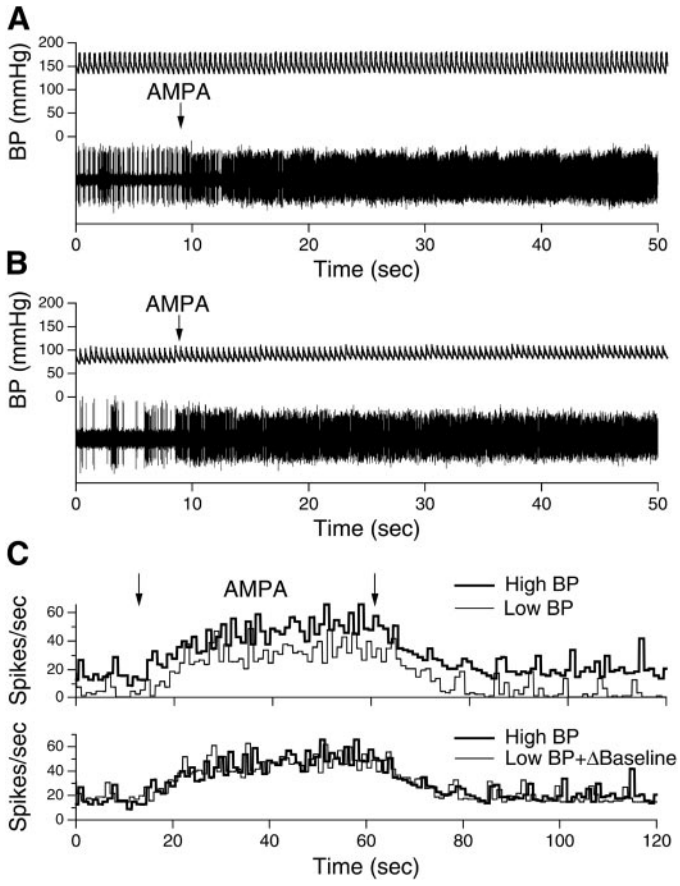

Fig. 6. Effects of AMPA on discharge of a cardiac mechanoreceptor-modulated NTS neuron at two stimulating pressures. Examples of raw neuronal activity (*A* and *B*) and averaged neuronal response (*C*) of a cardiac mechanoreceptor-modulated neuron to picoejection of AMPA are shown. The neuron had a higher rate of baseline discharge at high (*A*) versus low (*B*) stimulating levels of BP, as is also shown in the averaged neuronal activity traces in *C*. Superimposed on the pulse-synchronous discharge of the cardiac mechanoreceptor-modulated discharge of this neuron are respiratory-related bursts of activity. The neuron had a greater peak response to AMPA administration (between arrows) at high versus low BP (*C*, *top*). However, the increase in baseline activity accounted for the increase in peak activity at high BP, resulting in no difference in the magnitude of evoked activity to AMPA administration (peak − baseline activity) at high versus low BP. This lack in the difference in magnitude is shown in the traces in *C*, *bottom*, where the average difference in baseline activity at high versus low BP (determined from *C*, *top*) is added to the low BP tracing (low BP + Δbaseline), resulting in an overlapping curve to high BP.

example of a neuron that had an increase in baseline activity in response to the higher stimulating pressure, an increase in peak discharge to AMPA administration, but no difference in the magnitude of the response to AMPA administration (peak minus baseline activity) is shown in Fig. 6. The raw neuronal activity rates in Fig. 6, *A* and *B*, demonstrate the increase in baseline discharge of the neuron to high versus low stimulating pressures (BP) as well as a respiratory-modulated component in the discharge of this neuron. Administration of AMPA produced a significant increase in firing at both stimulating BP, as reflected in the averaged neuronal traces shown in Fig 6*C*, *top*. The elevation in baseline resulted in the elevated peak discharge rate, but the magnitude of the AMPA-evoked discharge was not different for low versus high stimulating pressures. This is demonstrated by the traces shown in Fig. 6*C*, *bottom*, where the difference in baseline activity between that at high stimulating BP versus low BP was added to the curve for the low BP response. As can be seen, this resulted in complete overlap of both curves, demonstrating that AMPA evoked the same amount of activity at the two stimulating pressures. This type of response is different from that seen for most responses to NMDA (Fig. 2), where, despite an increase in baseline activity, the magnitude of the change of NMDA-evoked activity was greater at the high versus low stimulating BP.

In a limited study in three cardiac mechanoreceptor-modulated neurons, baseline activity was increased via picoejection of AMPA rather than by increasing BP. BP was held constant at a low level to eliminate changes in glutamate or neuromodulator release as a variable in these studies. The evoked response of each neuron to picoejection of NMDA was greater at the higher level of AMPA administration than at the lower level. An example of this response is shown in Fig. 7. Administration of AMPA at a low rate (AMPA1) increased baseline activity slightly, but there was no response to subsequent administration of NMDA. However, increasing the rate of AMPA administration (AMPA2) produced a greater increase in firing and subsequent administration of NMDA at the same rate and amount now increased discharge of the neuron. These data suggest that there is an increased availability of NMDA receptors, because identical amounts of NMDA were administered during each trial, eliminating differential availability of neurotransmitter as a possible confounding influence.

DISCUSSION

The results from this study suggest that NMDA receptors play a greater role in central transmission of barosensitive afferent input during periods of elevated BP, when rates of discharge of peripheral barosensitive receptors are greater. This increased level of afferent input produces a greater activation of NTS neurons, which could result in depolarization and loss of the Mg^{2+} blockade of NMDA receptors. Because similar responses to NMDA administration were obtained regardless of whether the NTS neuron was activated by increasing levels of afferent input or by increasing amounts of picoejected AMPA to directly activate the non-NMDA receptors, it suggests that the response is due primarily to increased activation of NMDA receptors and not to increased release of neurotransmitters/modulators. Whereas there was some tendency for an activity-dependent increase in neuronal discharge in response to administration of AMPA as well in a few neurons, there was no significant effect in response to AMPA as a group. It is possible that the trend seen for increased discharge to AMPA at high stimulating pressures may be due to greater availability of NMDA receptors at the higher BP, which could have been activated by endogenous release of glutamate. The order of the neurons was not determined in these studies, and, therefore, it is not known whether second- or higher-order neurons were studied. This factor may also be important, based on earlier studies.

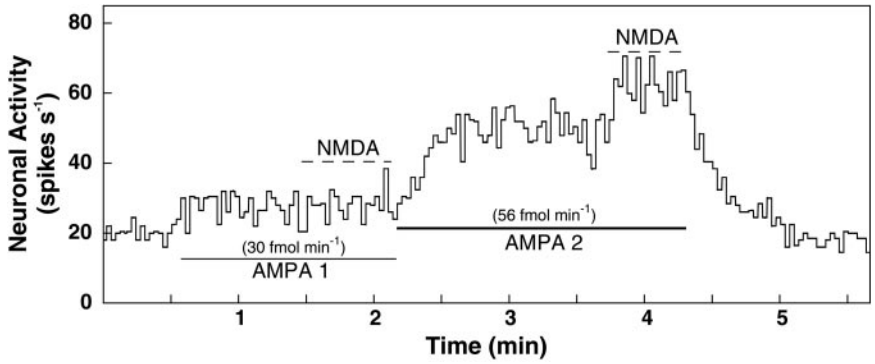

Fig. 7. Effect of NMDA at two levels of NTS neuronal activation produced by direct activation of non-NMDA receptors. Picoejection of AMPA (3.75 μM) at two rates (in fmol/min) was used to dose dependently increase the discharge of a pressure-sensitive cardiac mechanoreceptor-modulated NTS neuron through activation of non-NMDA receptors. Picoejection of NMDA (100 μM) at a constant rate (1.2 pmol/min) was superimposed on the levels of activity produced by AMPA to determine the effects of NMDA receptor activation on neuronal discharge at the two levels of neuronal excitability. The greater response to NMDA at the higher rate of neuronal discharge (greater rate of AMPA administration) suggests that the role of the NMDA receptors was enhanced during the higher level of neuronal activity.

The roles of different glutamate receptors in the transmission of afferent input from barosensitive arterial baroreceptors and cardiac mechanoreceptors are not completely understood. There is evidence from whole animal studies (6, 14, 19, 21, 22) that different types of glutamate receptors activate baroreceptive NTS neurons, although there is some controversy as to the extent of these individual contributions. Other studies from brain slices, which have examined neuronal responses to solitary tract stimulation, have also shown evidence for roles for NMDA receptors, non-NMDA receptors, or both in the activation of NTS neurons by sensory inputs (1–3, 13). These studies have not been able to assign a baroreceptor role for the neurons, because the generalized stimulation of the solitary tract was used to activate the neurons. However, a recent study by Bonham and Chen (5) has found a role for NMDA receptors in the transmission of baroreceptor input to second-order NTS neurons. Studies from this laboratory have found that both NMDA and non-NMDA receptor antagonists alter discharge of both baroreceptor-modulated (16, 18) and cardiac mechanoreceptor-modulated (17) NTS neuronal discharge, suggesting that each type of glutamate ionotropic receptor may contribute to excitation of the neurons. However, in most neurons, blockade of non-NMDA receptors had the greatest attenuating effect on neuronal activity, suggesting a greater role for non-NMDA receptors. Results from the current study suggest that increased excitation of cardiac mechanoreceptor-modulated NTS neurons, which could occur during periods of transient hypertension, could enhance the central contribution of NMDA receptors, a possibility not examined in our earlier studies. The recent study of Bonham and Chen (5) also suggests that the role for NMDA receptors in the transmission of baroreceptive information increases as the neurons become depolarized.

The effect of neuronal depolarization on enhancing the activation of NMDA receptors has been described in other systems. In the hippocampus, high-frequency stimulation has been found to lead to depolarization of the postsynaptic membrane, which removes the Mg^{2+} block for NMDA channels, allowing Ca^{2+} influx. This calcium, acting as a second messenger, is thought to lead to long-term potentiation of discharge of the neuron (4, 20). Conversely, some studies have found a desensitization or adaptation to the continued administration of either NMDA or AMPA (15). Long-term depression via NMDA receptors has been reported after low-frequency stimulation in the hippocampus (8). It is proposed that high-frequency stimulation may lead to higher intracellular Ca^{2+}, which induces potentiation, whereas low intracellular Ca^{2+}, resulting from low-frequency stimulation, may lead to depression. It is therefore possible that the phosphorylation state of the NMDA receptor influences whether hippocampal synapses, and thus NTS synapses, are potentiated or depressed (9).

The possibility that variable levels of neuronal activation may result in activity-dependent excitation of NMDA receptors was the focus of this study. Long-term potentiation, long-term depression, accommodation, and other nonlinear responses to depolarization have been reported for NTS neurons thought to receive barosensitive inputs (7, 11, 12). The role of NMDA receptors in initiation of these responses has not been well defined, and the extracellular responses of the neurons to different levels of physiological activation by pressure stimulation of afferent inputs has not been described. We did not observe any decaying responses or adaptation to administration of either NMDA or AMPA in this study. However, our administration of either agent was restricted to ~3 min, which might not be sufficient to induce the short-term depression reported for some of these neurons. We did see a potentiation of firing to the administration of NMDA in neurons with enhanced degrees of excitation at the high stimulating BP or level of AMPA administration, suggesting that activation of these receptors may contribute to the short-term potentiation reported by others. In conclusion, we found evidence for an activity-dependent role for NMDA receptors in the excitation of cardiac mechanoreceptor-modulated NTS neurons. The importance of the role of this finding during physiological regulation of BP, particularly during hypertension, remains to be determined.

The authors acknowledge the excellent technical and histological assistance of Claudia A. Hermes and Angela R. Cowan.

This research was supported by Veterans Affairs Medical Research Funds, National Heart, Lung, and Blood Institute Grant HL-55490, and American Heart Association Grant-In-Aid 0150518.

REFERENCES

1. **Andresen MC and Yang M.** Non-NMDA receptors mediate sensory afferent synaptic transmission in medial nucleus tractus solitarius. *Am J Physiol Heart Circ Physiol* 259: H1307–H1311, 1990.
2. **Andresen MC and Yang M.** Excitatory amino acid receptors and afferent synaptic transmission in the nucleus tractus solitarius. In: *Nucleus of the Solitary Tract*, edited by Barraco IRA. Boca Raton, FL: CRC, 1993, p. 187–192.
3. **Aylwin ML, Horowitz JM, and Bonham AC.** NMDA receptors contribute to primary visceral afferent transmission in the nucleus of the solitary tract. *J Neurophysiol* 77: 2539–2548, 1997.
4. **Bashir Z, Alford S, Davies S, Randall A, and Collingridge G.** Long-term potentiation of NMDA receptor-mediated synaptic transmission in the hippocampus. *Nature* 349: 156–158, 1991.
5. **Bonham AC and Chen CY.** Glutamatergic neural transmission in the nucleus tractus solitarius: *N*-methyl-D-asparate receptors. *Clin Exp Pharmacol Physiol* 29: 497–502, 2002.
6. **Chen CY and Bonham AC.** Non-NMDA and NMDA receptors transmit area postrema input to aortic baroreceptor neurons in NTS. *Am J Physiol Heart Circ Physiol* 275: H1695–H1702, 1998.
7. **Felder RB and Mifflin SW.** Baroreceptor and chemoreceptor afferent processing in the solitary tract nucleus. In: *Nucleus of the Solitary Tract*, edited by Barraco IRA. Boca Raton, FL: CRC, 1993, p. 169–186.
8. **Kirkwood A, Dudek S, Gold J, Aizenman C, and Bear M.** Common forms of synaptic plasticity in the hippocampus and neocortex in vitro. *Science* 260: 1518–1521, 1993.
9. **Mammen AT and Huganir RL.** Regulation of NMDA receptors by protein phosphorylation. In: *The Inotropic Glutamate Receptors*, edited by Monaghan DT and Wenthold RJ. Totowa, NJ: Humana, 1997, p. 135–148.

10. **Meeley MP, Underwood MD, Talman WT, and Reis DJ.** Content and in vitro release of endogenous amino acids in the area of the nucleus of the solitary tract of the rat. *J Neurochem* 53: 1807–1817, 1989.
11. **Mifflin SW.** Short-term potentiation of carotid sinus nerve inputs to neurons in the nucleus of the solitary tract. *Respir Physiol* 110: 229–236, 1997.
12. **Mifflin SW and Felder RB.** Synaptic mechanisms regulating cardiovascular afferent inputs to solitary tract nucleus. *Am J Physiol Heart Circ Physiol* 259: H653–H661, 1990.
13. **Miller BD and Felder RD.** Excitatory amino acid receptors intrinsic to synaptic transmission in nucleus tractus solitarii. *Brain Res* 456: 333–343, 1988.
14. **Ohta H and Talman WT.** Both NMDA and non-NMDA receptors in the NTS participate in the baroreceptor reflex in rats. *Am J Physiol Regul Integr Comp Physiol* 267: R1065–R1070, 1994.
15. **Otis TS, Zhang S, and Trussel LO.** Direct measurement of AMPA receptor desensitization induced by glutamatergic synaptic transmission. *J Neurosci* 16: 7496–7504, 1996.
16. **Seagard JL, Dean C, and Hopp FA.** Glutamate receptor subtypes involved in transmission of baroreceptor input to NTS neurons. *Soc Neurosci Abstr* 23: 722, 1997.
17. **Seagard JL, Dean C, and Hopp FA.** Role of glutamate receptors in transmission of vagal cardiac input to neurons in the nucleus tractus solitarius. *J Physiol* 520: 243–253, 1999.
18. **Seagard JL, Dean C, and Hopp FA.** Neurochemical transmission of baroreceptor input in the NTS. *Brain Res Bull* 51: 111–118, 2000.
19. **Wilson CG, Zhang Z, and Bonham AC.** Non-NMDA receptors transmit cardiopulmonary C fibre input in nucleus tractus solitarii in rats. *J Physiol* 493: 773–785, 1996.
20. **Xie X, Berger T, and Barrionuevo G.** Isolated NMDA receptor-mediated synaptic responses express both LTP and LTD. *J Neurophysiol* 67: 1009–1013, 1992.
21. **Zhang J and Mifflin SW.** Influences of excitatory amino acid receptor agonists on nucleus of the solitary tract neurons receiving aortic depressor nerve inputs. *J Pharmacol Exp Ther* 282: 639–647, 1997.
22. **Zhang J and Mifflin SW.** Differential roles for NMDA and non-NMDA receptor subtypes in baroreceptor afferent integration in the nucleus of the solitary tract of the rat. *J Physiol* 511: 733–745, 1998.
23. **Zhang W and Mifflin SW.** Excitatory amino-acid receptors contribute to carotid sinus and vagus nerve evoked excitation of neurons in the nucleus of the tractus solitarius. *J Auton Nerv Syst* 55: 50–56, 1995.